Aust. J. Chem., 1981, **34**, 871–83

The Chemistry of Pyrrolic Compounds. XLVII* The Synthesis of a Possible Biogenetic Precursor of Haem *a* and its Relationship to the Prosthetic Group of Myeloperoxidase and to Cryptoporphyrin *a*

Peter S. Clezy and Christopher J. R. Fookes

Department of Organic Chemistry, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033.

Abstract

The synthesis of 18-demethyl-18-formylprotoporphyrin dimethyl ester is described and the role of this compound as an intermediate in the biosynthesis of haem *a* is considered. A possible relationship between a compound of this structure and the prosthetic group of myeloperoxidase is discussed. The cryptoporphyrin *a* fraction of ox heart extracts has been reexamined and shown to consist of a mixture of *Spirographis* porphyrin and its isomer which presumably arise from the *in vitro* oxidation of the vinyl groups in protoporphyrin.

Introduction

In their study of porphyrin *a* (1a),† the iron-free prosthetic group of cytochrome oxidase, Lemberg and his colleagues mainly obtained their material from fresh ox hearts and during these extractions a minor porphyrin fraction was isolated which was called cryptoporphyrin *a*.[1-3] Unlike porphyrin *a* itself, cryptoporphyrin *a* was readily obtained crystalline; it was shown to contain a formyl substituent and a carbon–carbon double bond in conjugation with the porphyrin macrocycle.[3] The electronic spectrum of cryptoporphyrin *a* was reported to resemble that of *Spirographis* porphyrin (2a) although the two porphyrins appeared to differ in melting point and molecular weight as judged by specific extinction coefficient measurements.[3]

Now it seemed likely that cryptoporphyrin *a* was related to porphyrin *a* and therefore a possible structure for the dimethyl ester of this species was the divinylformylporphyrin (2b). This compound might be expected to have an electronic spectrum similar to that given by *Spirographis* porphyrin and could possibly be involved in the biogenesis of porphyrin *a* itself. In addition, a recent report by Wu and Schultz[4] has suggested (2b) as a possible structure for a derivative of the prosthetic group of the enzyme myeloperoxidase. Thus for various reasons we had an interest in the porphyrin (2b) and, in this present paper, its synthesis is described and its properties are discussed in relation to both the prosthetic group of myeloperoxidase and to the question of cryptoporphyrin *a*.

* Part XLVI, *Aust. J. Chem.*, 1980, **33**, 1095.

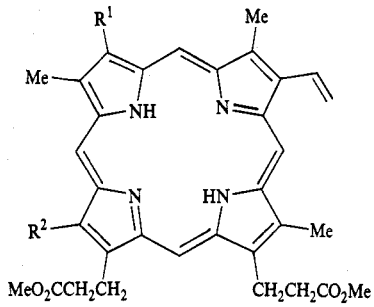
† Represented as the dimethyl ester although the free acid is the naturally occurring species.

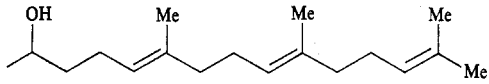
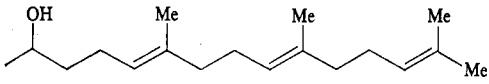
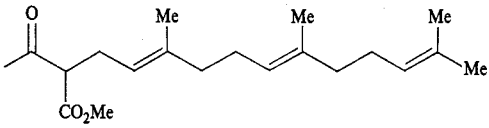
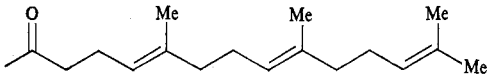
[1] Lemberg, R., *Nature (London)*, 1953, **172**, 619.

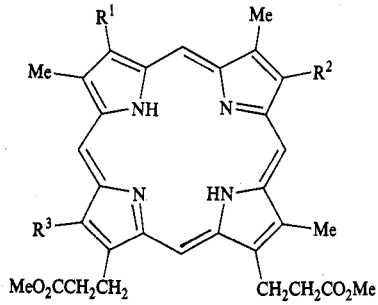
[2] Lemberg, R., and Parker, J., *Aust. J. Exp. Biol. Med. Sci.*, 1955, **33**, 483.

[3] Parker, M. J., *Biochim. Biophys. Acta*, 1959, **35**, 496.

[4] Wu, N. C., and Schultz, J., *FEBS Lett.*, 1975, **60**, 141; *Biochim. Biophys. Acta*, 1978, **536**, 341.



	R^1	R^2
(1a)		CHO
(1b)		Me
(1c)		Me
(1d)		Me



	R^1	R^2	R^3
(2a)	CHO	$CH{=}CH_2$	Me
(2b)	$CH{=}CH_2$	$CH{=}CH_2$	CHO
(2c)	CH_2CH_2OH	CH_2CH_2OH	Ac
(2d)	CH_2CH_2Cl	CH_2CH_2Cl	$CH{=}CH_2$
(2e)	CH_2CH_2Cl	CH_2CH_2Cl	CHO
(2f)	$CH{=}CH_2$	CHO	Me
(2g)	CO_2Me	$CH{=}CH_2$	Me
(2h)	CO_2H	$CH{=}CH_2$	Me

Results and Discussion

Synthesis and Properties of 18-Demethyl-18-formylprotoporphyrin (2b)

The plan of synthesis of (2b) was based on earlier sequences developed in this Laboratory for the preparation of porphyrins containing both formyl and vinyl substituents.[5] In these cases the formyl group was obtained by oxidative cleavage of a vinyl substituent and, therefore, it was necessary to construct a porphyrin in which two distinct types of vinyl precursors existed. Such a porphyrin allows the required degree of selectivity to introduce the ethylenic group destined for conversion into the aldehyde before the construction of the ultimate vinyl group or groups. With this strategy established the porphyrin (2c) became the initial objective for the preparation of (2b) and it seemed that this target porphyrin could be obtained by the oxidative cyclization of a bilene derived from the dipyrrylmethanes (3a) and (3b).

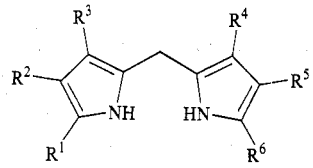
The starting point for the preparation of (3a) was the pyrrole (4a) which was obtained by a Knorr condensation between t-butyl hydroximinoacetoacetate and 3-acetyl-4-oxopentyl acetate[6] in the presence of zinc powder. Treatment of (4a) in turn with sulfuryl chloride, aqueous sodium acetate and iodine in aqueous potassium iodide solution gave the iodopyrrole (4b) which was hydrogenolysed over palladium to furnish the α-free pyrrole (4c). Condensation of (4c) with the α-acetoxymethylpyrrole (4d)[7] yielded the 5-benzyloxycarbonyl-5′-t-butoxycarbonyldipyrrylmethane (3c) from which the required dipyrrylmethanecarboxylic acid (3a) was obtained by hydrogenolysis. Like so many members of this class of mixed ester the dipyrrylmethane (3c) was not obtained crystalline[8,9] but its structure and homogeneity were checked by ^{1}H n.m.r. spectroscopy.

For the preparation of the formyldipyrrylmethane (3b) the pyrrole (4e)[10,11] was hydrogenolysed over palladium-on-charcoal and the resultant acid (4f) heated under reflux in methanol containing *p*-toluenesulfonic acid to give the α-free pyrrole (4g). Condensation of the acetoxymethylpyrrole (4h)[12] with (4g) yielded the dipyrrylmethane (3d) which was hydrogenolysed and the resultant acid treated with trifluoroacetic acid and triethyl orthoformate to give the required formyl derivative (3b).

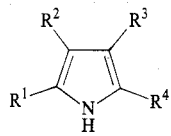
A solution of the formyldipyrrylmethane (3b) and the dipyrrylmethanecarboxylic acid (3a) in methylene dichloride was diluted with methanol containing *p*-toluenesulfonic acid and the mixture stirred until bilene formation was complete as indicated by the intensity of the band at 505 nm reaching a maximum. This intermediate was then treated with trifluoroacetic acid and triethyl orthoformate[13] to introduce the formyl equivalent required for cyclization; this was effected by heating overnight with copper(II) acetate in methanol/acetic acid. The porphyrin copper chelate was converted by sulfuric acid treatment into the metal-free derivative which was isolated

[5] Clezy, P. S., and Diakiw, V., *Aust. J. Chem.*, 1975, **28**, 2703.
[6] Clezy, P. S., and Fookes, C. J. R., *Aust. J. Chem.*, 1977, **30**, 609.
[7] Clezy, P. S., Fookes, C. J. R., and Hai, T. T., *Aust. J. Chem.*, 1978, **31**, 365.
[8] Cavaleiro, J. A. S., Rocha Gonsalves, A. M. d'A., Kenner, G. W., and Smith, K. M., *J. Chem. Soc., Perkin Trans. 1*, 1973, 2471.
[9] Clezy, P. S., Hai, T. T., and Gupta, P. C., *Aust. J. Chem.*, 1976, **29**, 393.
[10] Valasinas, A., and Frydman, B., *J. Org. Chem.*, 1976, **41**, 2991.
[11] Kenner, G. W., Rimmer, J., Smith, K. M., and Unsworth, J. F., *J. Chem. Soc., Perkins Trans. 1*, 1977, 332.
[12] Johnson, A. W., Kay, I. T., Markham, E., Price, R., and Shaw, K. B., *J. Chem. Soc.*, 1959, 3416.
[13] Clezy, P. S., and Fookes, C. J. R., *Aust. J. Chem.*, 1974, **27**, 371.

as the dihydroxyethyl compound (2c) in 27% overall yield from the dipyrrylmethanes. Sodium borohydride reduced the acetyl function in (2c) to give a 1′-hydroxyethyl substituent and reaction of this derivative with dimethylformamide containing benzoyl chloride resulted in the formation of the product (2d) by dehydration of the secondary alcohol and generation of 2′-chloroethyl side chains from the primary alcohols.[14] Oxidation of (2d) with osmium tetroxide followed by cleavage of the glycol with periodate afforded the formylporphyrin (2e) which, with the aldehyde protected by acetal formation, was heated with sodium hydroxide in aqueous pyridine. As a result the divinylporphyrin (2b) was obtained after removal of the protecting acetal group and the re-esterification of the side-chain esters.



	R^1	R^2	R^3	R^4	R^5	R^6
(3a)	Bu^tO_2C	Me	CH_2CH_2OAc	Me	CH_2CH_2OAc	CO_2H
(3b)	Me	Ac	$CH_2CH_2CO_2Me$	$CH_2CH_2CO_2Me$	Me	CHO
(3c)	Bu^tO_2C	Me	CH_2CH_2OAc	Me	CH_2CH_2OAc	CO_2CH_2Ph
(3d)	Me	Ac	$CH_2CH_2CO_2Me$	$CH_2CH_2CO_2Me$	Me	CO_2CH_2Ph



	R^1	R^2	R^3	R^4
(4a)	Bu^tO_2C	Me	CH_2CH_2OAc	Me
(4b)	Bu^tO_2C	Me	CH_2CH_2OAc	I
(4c)	Bu^tO_2C	Me	CH_2CH_2OAc	H
(4d)	$PhCH_2O_2C$	CH_2CH_2OAc	Me	CH_2OAc
(4e)	$PhCH_2O_2C$	$CH_2CH_2CO_2Et$	Ac	Me
(4f)	HO_2C	$CH_2CH_2CO_2Et$	Ac	Me
(4g)	H	$CH_2CH_2CO_2Me$	Ac	Me
(4h)	$PhCH_2O_2C$	Me	$CH_2CH_2CO_2Me$	CH_2OAc

The electronic spectrum of (2b) had an oxo-rhodo absorption pattern[15] and in this differed from cryptoporphyrin *a* which was reported[3] to exhibit a rhodotype spectrum.[15] In addition, the absorption maxima in (2b) were at longer wavelengths than those found in cryptoporphyrin *a* and the two porphyrins had quite different melting point characteristics.

[14] Clezy, P. S., Fookes, C. J. R., and Sternhell, S., *Aust. J. Chem.*, 1978, **31**, 639.
[15] Smith, K. M., 'Porphyrins and Metalloporphyrins' p. 21 (Elsevier: Amsterdam 1975).

On the other hand, the porphyrin (2b) gave an iron chelate which furnished a pyridine haemochrome derivative with a single maximum in the visible at 586 nm. A species with similar spectroscopic characteristics is obtained when pyridine is added to an aqueous solution of reduced myeloperoxidase and the present work would appear to strengthen the proposal made by Wu and Schultz[4] that a structural entity such as (2b) is involved in the prosthetic group of myeloperoxidase. However, it should be pointed out that evidence for the presence of a formyl group in the haem unit of this enzyme is not convincing. Lemberg and Falk[16] have studied the iron chelates of a number of formylporphyrins and shown that they react readily with hydroxylamine, as evidenced by a bathochromic shift in the pyridine haemochrome absorption; yet this reagent produced no spectroscopic change in the case of the pyridine haemochrome of myeloperoxidase.[17] Moreover, Wu and Schultz[4] have cleaved the prosthetic group of myeloperoxidase from the apoprotein by treatment with sodium methoxide and obtained a product with a pyridine haemochrome absorption at 562 nm. These workers suggest that the shift from 586 nm for myeloperoxidase is due to hemiacetal formation but such a derivative is unlikely to survive the acid conditions which pertain during the isolation of the prosthetic group. It would seem more likely that the prosthetic group contains an alkali-labile function, as was suggested by Nichol *et al.*[18]

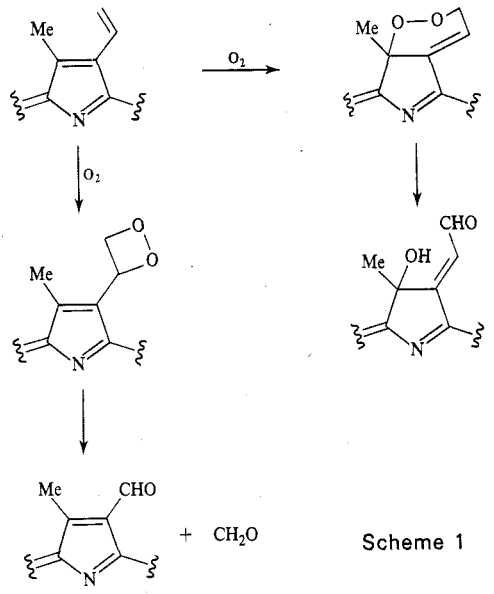
A Reexamination of Cryptoporphyrin a

The obvious difference between the properties of the porphyrin (2b) and those reported for cryptoporphyrin *a* has led us to reinvestigate this latter species and we have, therefore, repeated the extraction of ox heart as described by Parker.[3] In this process the mince is first washed free of a large portion of the haemoglobin present and then de-fatted by successive treatment with aqueous acetone and ether. Haematins are extracted with acidified aqueous acetone and the chelating iron removed by the ferrous sulfate method of Morell and Stewart.[19] An ethereal solution of the crude porphyrins obtained in this manner was extracted initially with 3% hydrochloric acid to remove most of the protoporphyrin; cryptoporphyrin *a* was isolated by extraction with 8% hydrochloric acid which left porphyrin *a* in the ethereal phase.

Parker obtained a yield of 2–3 mg of cryptoporphyrin *a* per kg of fresh mince but the present study produced a much lower porphyrin level in this fraction (*c.* 0·1–0·2 mg/kg). Careful chromatography of the cryptoporphyrin fraction obtained from 10 kg of mince separated it, after methylation, into two components which have been identified by comparison with authentic material as *Spirographis* porphyrin (2a) and its isomer (2f). Horsey and Whitten[20] have shown that the vinyl groups in protoporphyrin can undergo photooxidation in two distinct ways as summarized in Scheme 1. Cycloaddition of oxygen to a diene unit of the vinylporphyrin leads to the hydroxy aldehyde in a sequence previously investigated by Inhoffen and his group,[21] while direct addition of oxygen to the vinyl double bond leads to the

[16] Lemberg, R., and Falk, J. E., *Biochem. J.*, 1951, **49**, 674.
[17] Newton, N., Morell, D. B., Clarke, L., and Clezy, P. S., *Biochim. Biophys. Acta*, 1965, **96**, 476.
[18] Nichol, A. W., Morell, D. B., and Thomson, J., *Biochem. Biophys. Res. Commun.*, 1969, **36**, 576.
[19] Morell, D. B., and Stewart, M., *Aust. J. Exp. Biol. Med. Sci.*, 1956, **34**, 211.
[20] Horsey, B. E., and Whitten, D. G., *J. Am. Chem. Soc.*, 1978, **100**, 1293.
[21] Inhoffen, H. H., Brockmann, H., and Bliesener, K.-M., *Justus Liebigs Ann. Chem.*, 1969, **730**, 173.

formylporphyrin by way of an unstable dioxetane. Presumably, the cryptoporphyrin *a* fraction of porphyrin *a* has its origin in the second of these photooxidations in which protoporphyrin is converted into *Spirographis* porphyrin and its isomer during the isolation procedure.



Scheme 1

Biosynthesis of Porphyrin a

If, as seems likely, protoporphyrin is a biogenetic precursor of porphyrin *a* two main transformations need to be effected to achieve this conversion. The methyl group at C 18 in protoporphyrin has to be oxidized to a formyl substituent and the vinyl group at C 3 requires elaboration to give the terpenoid side chain which is so characteristic of the porphyrin *a* molecule. If the sequence, in fact, takes place in this order then the porphyrin (2b) is a precursor of porphyrin *a*; if the reverse order is followed then the porphyrin (1b) lies between protoporphyrin and porphyrin *a* and it seemed a worthwhile objective to complete this area of work by synthesizing this possible biogenetic precursor.

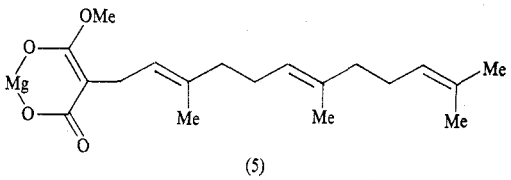
The porphyrin (2g) was already available from our earlier studies[22] and from the oxidation of *Spirographis* porphyrin[6] (2a); alkaline hydrolysis of (2g) followed by methylation of the side-chain esters provided the porphyrin acid (2h). This compound was treated with oxalyl chloride to give the porphyrin acid chloride and this derivative condensed with the magnesium complex (5)[23] which had been obtained from methyl hydrogen farnesylmalonate and isopropylmagnesium bromide. The resultant β-keto ester (1c) was treated with lithium iodide in refluxing pyridine to give the ketone (1d) which was reduced with sodium borohydride to furnish the alcohol (1b).

[22] Clezy, P. S., and Diakiw, V., *Aust. J. Chem.*, 1975, **28**, 1589.
[23] Thompson, M., Barrett, J., McDonald, E., Battersby, A. R., Fookes, C. J. R., Chaudhry, I. A., Clezy, P. S., and Morris, H. R., *J. Chem. Soc., Chem. Commun.*, 1977, 278.

Experimental

(*a*) General

Melting points were uncorrected and were determined with the aid of a Kofler micro melting point apparatus. Analyses were carried out by Mr J. Sussman of the University of New South Wales. Porphyrin ^{1}H n.m.r. spectra were measured at 100 MHz on a Varian HA-100 or XL-100 instrument while the spectra of simpler molecules were determined at 60 MHz with the aid of a Varian A-60 spectrometer. Electronic spectra were obtained in chloroform freshly distilled from anhydrous potassium carbonate by using a Varian Techtron 635 instrument. Chromatographic separations were carried out on Merck Kieselgel 60H; 'ethanol-free' chloroform[24] was used as the eluting solvent unless stated otherwise. Solutions in water immiscible solvents were dried over sodium sulfate prior to evaporation which was normally carried out at reduced pressure (*c.* 30 Torr) in a Büchi rotary evaporator. Acetic acid was glacial (17 M); light petroleum referred to the petroleum fractions of b.p. 60–80°. Pyridine was dried over potassium hydroxide and was freshly distilled before use. Methylene dichloride and tetrahydrofuran were allowed to percolate slowly through alumina (Peter Spence Grade H) before being used.



(5)

(*b*) Dimethyl (*E,E*)-Farnesylmalonate

Triphenylphosphine (14·4 g) was added to a stirred solution of (*E,E*)-farnesol[25] (11·1 g) and carbon tetrabromide (18·2 g) in dry acetonitrile (50 ml) at a rate which maintained the temperature of the mixture at 25 ± 1°. The solution was kept at this temperature for 30 min, the solvent removed and light petroleum (400 ml) added slowly to the vigorously stirred residual oil. The mixture was stored at 5° for 30 min, filtered, and the residue of triphenylphosphine oxide washed well with light petroleum. The filtrate and washings were combined and the solvent removed to give farnesyl bromide (14·2 g) which was used without further purification.

Dimethyl malonate (6·6 g) was added slowly with stirring to anhydrous methanol (50 ml) containing sodium methoxide (from 1·15 g sodium). The clear solution was refluxed for 5 min, cooled, and the above farnesyl bromide added with stirring at a rate which maintained a gentle reflux. When the addition was complete the mixture was refluxed for 1 h after which the solvent was removed and the residue partitioned between ether (400 ml) and water (200 ml). The ethereal phase was washed with brine (2 × 100 ml) and the residue remaining after removal of ether was distilled to give the *malonate* (9·3 g, 55·3% from farnesol) as a light-coloured oil, b.p. 146–149°/0·2 Torr (Found: C, 71·2; H, 9·6. $C_{20}H_{32}O_4$ requires C, 71·4; H, 9·6%). δ ($CDCl_3$, 60 MHz): 1·60, 1·65 (12H, 4 × CH_3), 1·99, 2·02 (8H, 4 × CH_2), 2·60 [2H, m, $CH_2CH(CO_2Me)_2$], 3·30 [1H, m, $CH_2CH(CO_2Me)_2$], 3·72 (6H, 2 × CO_2CH_3), 5·10 (3H, broad m, 3 × CH=).

(*c*) Monomethyl (*E,E*)-Farnesylmalonate

Potassium hydroxide (1·5 g) dissolved in methanol (15 ml) was added to a stirred solution of dimethyl (*E,E*)-farnesylmalonate (9·0 g) in methanol (75 ml). The solution was left at room temperature overnight, concentrated (to 20 ml) and ether (40 ml) and water (30 ml) added. The ethereal phase was washed with water (2 × 10 ml) and the combined aqueous solutions were cooled (ice bath) and acidified with hydrochloric acid (10 M; 3 ml). Isolation of the product was achieved by ether extraction (2 × 40 ml). These extracts were washed with brine (4 × 10 ml) and the solvent removed to give the mono ester (7·4 g) as a pale yellow oil which was dried by distillation of its solution in benzene and then kept at 50° under reduced pressure until its mass was constant. δ ($CDCl_3$, 100

[24] Clezy, P. S., and Fookes, C. J. R., *Aust. J. Chem.*, 1977, **30**, 217.
[25] Bates, R. B., Gale, D. M., and Gruner, B. J., *J. Org. Chem.*, 1963, **28**, 1086.

MHz): 1·61, 1·65, 1·67 (12H, 4 × CH_3), 2·01 (8H, 4 × CH_2), 2·62 [2H, m, $CH_2CH(CO_2H)CO_2Me$], 3·41 [1H, m, $CH_2CH(CO_2H)CO_2Me$], 3·75 (3H, CO_2CH_3), 5·08 (3H, broad m, 3 × =CH), 9·00 (1H, CO_2H).

(*d*) Pyrroles

(i) *t-Butyl 4-(2-Acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylate (4a)*

A stirred solution of t-butyl acetoacetate (55 g) in acetic acid (100 ml) was treated below 30° by the dropwise addition of sodium nitrite (23 g) in water (35 ml), and left to stand overnight.

A solution of 3-acetyl-4-oxopentyl acetate[6] (62 g) in acetic acid (115 ml) was heated to 80° when a mixture of zinc dust (55 g) and sodium acetate (55 g) was added portionwise while the above hydroximino derivative was added dropwise. The reaction mixture was stirred vigorously during the addition which was regulated so that zinc was always in excess and the temperature remained between 90° and 100°. When the addition was complete the mixture was boiled gently for 15 min, cooled and diluted with ice-cold water (2 l.). The precipitated oil solidified upon sitrring and was collected after 2 h and washed well with water to give the *pyrrole* (32·3 g) as colourless prisms, m.p. 92–93°, from aqueous ethanol (Found: C, 63·8; H, 8·2; N, 4·7. $C_{15}H_{23}NO_4$ requires C, 64·0; H, 8·2; N, 5·0%). δ ($CDCl_3$): 1·58 [9H, $C(CH_3)_3$], 2·05 (3H, $OCOH_3$), 2·25 (3H) 2·28 (3H) (ring methyls), 2·71 (2H, t, CH_2CH_2OAc), 4·11 (2H, t, CH_2CH_2OAc), *c.* 9·3 (1H, broad, NH).

(ii) *t-Butyl 4-(2-Acetoxyethyl)-5-iodo-3-methylpyrrole-2-carboxylate (4b)*

Freshly distilled sulfuryl chloride (21·5 ml) was added over 15 min to a vigorously stirred mixture of t-butyl 4-(2-acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylate (21·3 g), anhydrous potassium carbonate (25 g) and dry ether (500 ml). The mixture was stirred overnight at room temperature and filtered; the solvent was removed. Fresh ether (100 ml) was added to the residue and then boiled off; this process was repeated a second time. Acetone (100 ml) was added to the oil, followed by a hot aqueous solution of sodium acetate (25%; 400 ml) and the mixture stirred vigorously while being heated to boiling in an open flask for 30 min to remove the acetone. At this stage the mixture began to darken, so it was immediately cooled and the product extracted into ether (2 × 300 ml). The ethereal extract was washed with water (200 ml) and the carboxylic acid extracted with excess aqueous sodium carbonate (1 M). The aqueous extract was warmed and aerated to remove residual ether before it was cooled and carefully acidified with acetic acid. The precipitate was stirred until it solidified, whereupon it was collected, washed well with water and dried (70°), to give the acid (12·5 g) as colourless prisms, m.p. 146–148°.

This acid (12·5 g) was added to a solution of sodium hydrogen carbonate (10·4 g) in water (70 ml) followed immediately by carbon tetrachloride (70 ml) and the mixture stirred and heated under reflux. Iodine (11·8 g) dissolved in water (70 ml) containing potassium iodide (13·9 g) was added over *c.* 3 min and the refluxing continued for 30 min. Sufficient sodium hydrogen sulfite was introduced to decolorize excess iodine and this was followed by additional water (500 ml). The organic phase was separated and the aqueous phase extracted with chloroform (2 × 50 ml). The combined organic extracts were taken to dryness and the residue recrystallized from chloroform/light petroleum to give the *iodopyrrole* (15·0 g) as colourless plates, m.p. 122–123° (Found: C, 42·8; H, 5·1; N, 3·4. $C_{14}H_{20}INO_4$ requires C, 42·8; H, 5·1; N, 3·6%). δ ($CDCl_3$): 1·58 [9H, $C(CH_3)_3$], 2·05 (3H, $OCOCH_3$), 2·32 (3H, ring methyl), 3·21 (2H, t, CH_2CH_2OAc), 4·12 (2H, t, CH_2CH_2OAc), *c.* 9·2 (1H, broad, NH).

(iii) *t-Butyl 4-(2-Acetoxyethyl)-3-methylpyrrole-2-carboxylate (4c)*

A mixture of t-butyl 4-(2-acetoxyethyl)-5-iodo-3-methylpyrrole-2-carboxylate (14·5 g), magnesium (15 g), palladium-on-charcoal (10%; 1·5 g) and methanol (200 ml) was stirred under hydrogen at *c.* 40° until the uptake of gas ceased. The suspension was filtered, water (700 ml) was added to the filtrate which was then extracted with chloroform (4 × 100 ml) and this solution filtered through alumina (Peter Spence Grade H). Concentration of the filtrate gave a colourless oil which rapidly solidified and which was further purified by recrystallization from light petroleum to give the *pyrrole* (9·1 g) as colourless needles, m.p. 73–75° (Found: C, 62·8; H, 8·0; N, 5·1. $C_{14}H_{21}NO_4$ requires C, 62·9; H, 7·9; N, 5·2%). δ ($CDCl_3$): 1·58 [9H, $C(CH_3)_3$], 2·05 (3H, $OCOCH_3$), 2·28 (3H, ring methyl), 2·75 (2H, t, CH_2CH_2OAc), 4·19 (2H, t, CH_2CH_2OAc), 6·69 (1H, d, *J* 3·0 Hz, aromatic proton), *c.* 9·4 (1H, broad, NH).

(iv) *Methyl 3-(4-Acetyl-5-methylpyrrol-3-yl)propanoate (4g) (with Dr T. T. Hai)*

A solution of benzyl 4-acetyl-3-(2-ethoxycarbonylethyl)-5-methylpyrrole-2-carboxylate[10,11] (10 g) in methanol (300 ml) containing triethylamine (5 drops) was shaken with hydrogen in the presence of palladium-on-charcoal (10%; 800 mg) until the uptake of gas ceased. The catalyst was removed by filtration and the filtrate evaporated to dryness to give the acid (7·15 g) which was used without further purification.

A solution of this acid (7·15 g) in methanol (120 ml) containing *p*-toluenesulfonic acid (22·5 g) was refluxed until the evolution of gas ceased (35 min). The cooled mixture was diluted with ice-water and the product isolated by chloroform extraction. Removal of solvent from the combined chloroform extracts left a residue which crystallized from chloroform/light petroleum to give the *α-free pyrrole* (3·3 g), m.p. 102–103° (Found: C, 63·3; H, 7·2; N, 6·3. $C_{11}H_{15}NO_3$ requires C, 63·1; H, 7·2; N, 6·7%). δ ($CDCl_3$, 100 MHz): 2·42 (3H), 2·50 (3H) (ring methyl, $COCH_3$), 2·62 (2H, t, *J* 7·5 Hz, $CH_2CH_2CO_2Me$), 3·03 (2H, t, *J* 7·5 Hz, $CH_2CH_2CO_2Me$), 3·66 (3H, CO_2CH_3), 6·40 (1H, d, *J c.* 3·0 Hz, ring proton), 8·85 (1H, NH).

(*e*) Dipyrrylmethanes

(i) *Benzyl 4′-Acetyl-3,3′-di(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-dipyrrylmethane-5-carboxylate (3d) (with Dr T. T. Hai)*

A mixture of 3-acetyl-4-(2-methoxycarbonylethyl)-2-methylpyrrole (4 g), benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate[12] (7·2 g) and acetic acid (60 ml) was heated on a steam bath under nitrogen for 1 h. The solvent was removed (the final traces by co-distillation with toluene) and the residue recrystallized from ether followed by acetone/light petroleum to give the *dipyrrylmethane* (9·3 g), m.p. 156–157° (Found: C, 66·7; H, 6·6; N, 5·2. $C_{29}H_{34}N_2O_7$ requires C, 66·6; H, 6·6; N, 5·4%). δ ($CDCl_3$, 100 MHz): 2·29 (3H), 2·36 (3H), 2·42 (3H) (2× ring methyl, $COCH_3$), *c.* 2·8 (8H, m, 2× $CH_2CH_2CO_2Me$), 3·50 (3H), 3·65 (3H) (2× CO_2CH_3), 3·87 (2H, methane protons), 5·21 (2H, $CH_2C_6H_5$), 7·32 (5H, $CH_2C_6H_5$), 9·35 (1H), 9·54 (1H) (NH).

(ii) *4′-Acetyl-3,3′-di(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-dipyrrylmethane-5-carbaldehyde (3b) (with Dr T. T .Hai)*

A solution of benzyl 4′-acetyl-3,3′-di(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-dipyrrylmethane-5-carboxylate (6 g) in methanol (300 ml) containing triethylamine (5 drops) was shaken with hydrogen in the presence of palladium-on-charcoal (600 mg; 10%) until the uptake of gas ceased. Dilute aqueous ammonia was added to dissolve the precipitated acid and the mixture filtered to remove the catalyst. The filtrate was diluted with ice-water, acidified with acetic acid, and left at 5° for 1 h. The acid (4·5 g) was collected, dried and used without further purification.

A solution of this acid (4·5 g) in trifluoroacetic acid (45 ml) was stirred at room temperature for 2 min, triethyl orthoformate (15 ml) was added, and the mixture stirred for 5 min. The solution was cooled in an ice bath and ice-cold water added dropwise to precipitate the product. This mixture was kept at 0° for 1 h, the solid was collected and recrystallized from chloroform/methanol to give the *aldehyde* (3·85 g), m.p. 180–181° (Found: C, 63·4; H, 6·8; N, 6·6. $C_{22}H_{28}N_2O_6$ requires C, 63·4; H, 6·8; N, 6·7%). δ ($CDCl_3$, 100 MHz): 2·29 (3H) 2·37 (3H) 2·44 (3H) (2× ring methyl, $COCH_3$), *c.* 2·8 (8H, m, 2× $CH_2CH_2CO_2Me$), 3·69 (3H), 3·71 (3H) (2× CO_2CH_3), 3·94 (2H, methane protons), 9·44 (1H, CHO), 9·77 (1H), 10·33 (1H) (NH).

(iii) *Benzyl 4,3′-Di(2-Acetoxyethyl)-5′-t-butoxycarbonyl-3,4′-dimethyl-2,2′-dipyrrylmethane-5-carboxylate (3c)*

A mixture of t-butyl 4-(2-acetoxyethyl)-3-methylpyrrole-2-carboxylate (1·87 g), benzyl 3-(2-acetoxyethyl)-5-acetoxymethyl-4-methylpyrrole-2-carboxylate[7] (2·61 g) and acetic acid (20 ml) was heated on the steam bath for 3 h. The solution was cooled and the solvent removed, the last traces by co-distillation with toluene. The oily residue was chromatographed on silica (30 g, eluting solvent 'ethanol-free' chloroform/light petroleum, 1:1) to give the dipyrrylmethane (3·1 g) as an oil which was homogeneous by t.l.c. and ^1H n.m.r. spectroscopy. δ ($CDCl_3$): 1·52 [9H, $C(CH_3)_3$], 2·00 (9H, 2× $OCOCH_3$, 1× ring methyl), 2·25 (3H, ring methyl), 2·71 (2H, t), 3·04 (2H, t) (2× CH_2-CH_2OAc), 3·92 (2H, methane protons), 4·05 (2H, t), 4·17 (2H, t) (2× CH_2CH_2OAc), 5·25 (2H, $CH_2C_6H_5$), 7·33 (5H, $CH_2C_6H_5$), 9·30 (1H) 9·73 (1H) (NH).

(*f*) Porphyrins

(i) *Dimethyl 3-Acetyl-8,13-di(2-hydroxyethyl)-7,12,17-trimethylporphyrin-2,18-dipropionate* (*2c*)

A solution of benzyl 4,3′-di(2-acetoxyethyl)-5′-t-butoxycarbonyl-3,4′-dimethyl-2,2′-dipyrrylmethane-5-carboxylate (2·0 g) in ethanol (25 ml) containing triethylamine (2 drops) was stirred at *c.* 40° in contact with palladium-on-charcoal (0·2 g; 10%) in an atmosphere of hydrogen. When the uptake of gas ceased the mixture was filtered through kieselguhr to remove the catalyst and the solvent evaporated from the filtrate to leave the acid as a friable solid (1·70 g) which was used without further purification.

A stirred solution of this acid (1·62 g) and 4′-acetyl-3,3′-di(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-dipyrrylmethane-5-carbaldehyde (1·25 g) in methylene dichloride (180 ml) was treated with *p*-toluenesulfonic acid (2·6 g) in methanol (45 ml) and the stirring continued until bilene formation was complete (absorption at 505 nm reached a maximum; 2·5 h). The solution was then washed with aqueous potassium carbonate (1%; 500 ml) and the aqueous phase re-extracted with fresh chloroform (3 × 100 ml). The combined organic solutions were evaporated below 40° and a freshly prepared solution of triethyl orthoformate (3·5 ml) in trifluoroacetic acid (10·5 ml) was added to the residue. This mixture was stirred until the bilene-*b* had dissolved, the solution was left for 15 min and then added to a hot mixture of copper(II) acetate (10 g), sodium acetate (5 g), acetic acid (500 ml) and methanol (400 ml). Cyclization was allowed to proceed on the steam bath for 20 h when the reaction mixture was worked up by chloroform extraction to give the porphyrin copper complex which was purified by chromatography on silica (40 g; eluting solvent, 1% methanol in chloroform). The chelate was dissolved in trifluoroacetic acid (5 ml) and this solution diluted with sulfuric acid (18 M; 25 ml) to remove the metal; cold methanol (500 ml) was then carefully added and the mixture left at 5° overnight. This solution was diluted with water (3·5 l.), the pH adjusted to *c.* 2 with sodium acetate and the porphyrin collected by extraction with chloroform (1 × 500 ml; 5 × 100 ml). The combined chloroform extracts were washed with water and the product purified by chromatography on silica (30 g; eluting solvent 1·5% methanol in chloroform) and recrystallization from chloroform to give the *title porphyrin* (530 mg) as fine needles, m.p. 259–261° (Found: C, 67·9; H, 6·5; N, 8·6. $C_{37}H_{42}N_4O_7$ requires C, 68·1; H, 6·3; N, 8·5%). λ_{max} (log ε): 410 (5·29), 510 (4·03), 548 (4·14), 586, (3·95), 634 (3·24) nm. δ (CF_3CO_2D): 3·35 (4H, m, 2 × CH_2-CH_2CO_2Me), 3·67 (3H, $COCH_3$), 3·73 (3H), 3·76 (3H) (ring methyl), 3·79 (9H, 1 × ring methyl, 2 × CO_2CH_3), 4·62 (8H, m, 2 × CH_2CH_2OH, 2 × $CH_2CH_2CO_2Me$), *c.* 5·0 (4H, m, 2 × CH_2CH_2OH), 11·02 (1H) 11·05 (1H) 11·33 (1H) 11·36 (1H) (methine protons).

(ii) *Dimethyl 8,13-Di(2-chloroethyl)-7,12,17-trimethyl-3-vinylporphyrin-2,18-dipropionate* (*2d*)

A solution of sodium borohydride (1·0 g) in methanol (70 ml) was added to a solution of dimethyl 3-acetyl-8,13-di(2-hydroxyethyl)-7,12,17-trimethylporphyrin-2,18-dipropionate (450 mg) in chloroform (350 ml) and the mixture was left at room temperature until reduction was complete (*c.* 10 min). The resulting triol was purified by chromatography on silica (10 g, 1–2% methanol in chloroform as eluting solvent) and then heated for 1 h on a steam bath in dimethylformamide (100 ml) containing benzoyl chloride (5 ml). Aqueous triethylamine (6%; 250 ml) was added, the mixture shaken thoroughly and the porphyrin collected on kieselguhr on which it was washed well with water before being redissolved in chloroform.

The product was then purified by chromatography on silica (10 g) and recrystallized from chloroform/methanol to give the *title porphyrin* (387 mg) as flat needles, m.p. 214–216° (Found: C, 65·9; H, 5·9; N, 8·2. $C_{37}H_{40}Cl_2N_4O_4$ requires C, 65·8; H, 6·0; N, 8·3%). λ_{max} (log ε): 405 (5·27), 503 (4·15), 539·5 (4·07), 572 (3·85), 626 (3·58) nm. δ (CF_3CO_2D): 3·26 (4H, t, 2 × $CH_2CH_2CO_2Me$), 3·73 (3H) 3·76 (3H) 3·77 (3H) 3·79 (3H) 3·81 (3H) (3 × ring methyl, 2 × CO_2CH_3), 4·28 (4H, t, 2 × CH_2CH_2Cl), 4·70 (8H, m, 2 × CH_2CH_2Cl, 2 × $CH_2CH_2CO_2Me$), 6·45 (2H, m, $CH{=}CH_2$), 8·25 (1H, m, $CH{=}CH_2$), 11·03 (1H) 11·04 (1H) 11·06 (1H) 11·21 (1H) (methine protons).

(iii) *Dimethyl 8,13-Di(2-chloroethyl)-3-formyl-7,12,17-trimethylporphyrin-2,18-dipropionate* (*2e*)

Osmium tetroxide in pyridine (2%) was added dropwise to a stirred solution of dimethyl 8,13-di(2-chloroethyl)-7,12,17-trimethyl-3-vinylporphyrin-2,18-dipropionate (338 mg) in pyridine (40 ml) until the reaction was complete (t.l.c.). An aqueous solution of sodium sulfite (7·5%; 20 ml) was then added and the mixture heated with vigorous stirring until it boiled. It was maintained at this

temperature for 5 min, filtered through kieselguhr and the residue washed with hot aqueous pyridine (30%; 30 ml). The filtrate was cooled to *c.* 45° and aqueous sodium periodate (10%; 15 ml) was added. When cleavage of the glycol was complete (*c.* 15 min) hydrochloric acid (0·15 M; 700 ml) was added and the product extracted into chloroform (1 × 200 ml; 3 × 100 ml). The organic phase was washed with hydrochloric acid (0·15 M; 700 ml) and the solvent removed; residual pyridine was co-distilled with toluene. A mixture of chloroform (50 ml) and methanol (50 ml) was added to the residue followed by excess ethereal diazomethane. The mixture was shaken vigorously and the volatile components were removed by distillation. Chloroform (50 ml) and more ethereal diazomethane were added and the mixture shaken again. After 10 min the solvent was removed and the product chromatographed on silica (10 g) to give *formylporphyrin* (261 mg) as woolly needles, m.p. 234–237°, from chloroform/methanol (Found: C, 63·6; H, 5·6; N, 8·1. $C_{36}H_{38}Cl_2N_4O_5$ requires C, 63·8; H, 5·6; N, 8·3%). λ_{max} (log ε): 415·5 (5·29), 517 (3·98), 557 (4·39), 580·5 (4·17), 640 (3·43) nm. δ (CF_3CO_2D): 3·27 (2H, t), 3·41 (2H, t) (2 × $CH_2CH_2CO_2Me$), 3·71, 3·75, 3·79 (15H, 3 × ring methyl, 2 × CO_2CH_3), 4·23 (4H, t, 2 × CH_2CH_2Cl), 4·66 (6H, t, 2 × CH_2CH_2Cl, C18 $CH_2CH_2CO_2Me$), 5·07 (2H, t, C2 $CH_2CH_2CO_2Me$), 10·91 (1H) 10·93 (1H) 11·34 (1H) 11·59 (1H) 11·73 (1H) (methine protons, CHO).

(iv) *Dimethyl 3-Formyl-7,12,17-trimethyl-8,13-divinylporphyrin-2,18-dipropionate (2b)*

A mixture of dimethyl 8,13-di(2-chloroethyl)-3-formyl-7,12,17-trimethylporphyrin-2,18-dipropionate (200 mg), *p*-toluenesulfonic acid (100 mg), ethylene glycol (1 g) and chloroform (50 ml) was heated under reflux for 10 min to form the acetal. Aqueous ammonia (1 ml) was added and the mixture shaken with water (300 ml). The aqueous phase was extracted with chloroform (3 × 50 ml) and the combined organic phases were evaporated and thoroughly dried (1 h at 70°). A solution of this residue in pyridine (90 ml) was then refluxed under nitrogen for 5 min and water (15 ml) carefully introduced. After a further 5 min an aqueous solution of sodium hydroxide (3%; 20 ml) was added and the refluxing continued for 3 h. The reaction mixture was then diluted with aqueous acetic acid (25%; 20 ml) followed by water (150 ml) and the volume reduced to *c.* 50 ml. The precipitated product was collected on kieselguhr, washed well with water and dried at 110° before treatment with methanolic sulfuric acid (5%; 200 ml). This mixture was filtered to remove kieselguhr and the filtrate left overnight at 5°. Water (200 ml) was added followed by more water (500 ml) 5 min later and the product isolated by chloroform extraction (1 × 100 ml; 3 × 50 ml). The combined extract was concentrated (to *c.* 50 ml) and shaken for 10 s with hydrochloric acid (10 M; 20 ml) to hydrolyse acetals. Water (800 ml) was introduced and the aqueous phase carefully neutralized with aqueous ammonia before the organic phase was separated; the aqueous phase was extracted with fresh chloroform (3 × 50 ml). The combined chloroform solutions were then briefly treated with ethereal diazomethane and the product purified by chromatography on silica (8 g) to give the *title porphyrin* (151 mg), as woolly needles, m.p. 228–229°, from chloroform/methanol (Found: C, 71·5; H, 5·9; N, 9·2. $C_{36}H_{36}N_4O_5$ requires C, 71·5; H, 6·0; N, 9·3%). λ_{max} (log ε): 420·5 (5·22), 521·5 (3·99), 565 (4·29), 587 (4·11), 647·5 (3·33) nm. δ (CF_3CO_2D): 3·4 (4H, m, 2 × $CH_2CH_2CO_2Me$), 3·73 (6H) 3·77 (3H) 3·79 (3H) 3·82 (3H) (3 × ring methyl, 2 × CO_2CH_3), 4·67 (2H, t, C18 $CH_2CH_2CO_2Me$), 5·08 (2H, t, C2 $CH_2CH_2CO_2Me$), 6·50 (4H, m, 2 × $CH{=}CH_2$), 8·20 (2H, m, $CH{=}CH_2$), 10·93 (2H) 11·33 (1H) 11·62 (1H) 11·75 (1H) (methine protons; CHO).

Pyridine haemochrome: The iron(III) complex of this formylporphyrin was prepared by the procedure described by Morell *et al.*[26] and the spectrum measured in 50% aqueous pyridine, ascorbic acid being used as reducing agent. λ_{max} 586 nm.

(v) *3-Carboxy-8-vinyldeuteroporphyrin Dimethyl Ester (2h)*

A solution of 3-methoxycarbonyl-8-vinyldeuteroporphyrin dimethyl ester[6,22] (140 mg) in pyridine (30 ml) was refluxed under nitrogen for 5 min and then diluted carefully by the addition of water (5 ml). After a further 5 min under reflux an aqueous solution of sodium hydroxide (4%; 5 ml) was added and heating continued for 1 h. Aqueous acetic acid (25%; 5 ml) was then added followed by more water (100 ml) and the mixture concentrated to *c.* 30 ml. The product was collected on kieselguhr, washed with water and dried (110°). Methanolic sulfuric acid (3%; 200 ml) was added, the mixture filtered and the filtrate left at 5° overnight whereupon water (600 ml) was added

[26] Morell, D. B., Barrett, J., and Clezy, P. S., *Biochem. J.*, 1961, **78**, 793.

and the porphyrin extracted into chloroform (1 × 200 ml; 3 × 50 ml). The combined extracts were washed with aqueous methanol (25%; 800 ml), the solvent removed and the product obtained crystalline by the addition of benzene to a boiling solution of the residue in chloroform/methanol to yield the *porphyrin acid* (109 mg) as fine needles, m.p. >300° (Found: C, 69·3; H, 5·8; N, 9·3. $C_{35}H_{36}N_4O_8$ requires C, 69·1; H, 6·0; N, 9·2%). λ_{max} ($CHCl_3$/MeOH, 9:1) (log ε): 410 (5·17), 509·5 (4·03), 548 (3·96), 577·5 (3·79), 633 (3·41) nm.

(vi) *3-[(4E,8E)-5,9,13-Trimethyltetradeca-4,8,12-trienoyl]-8-vinyldeuteroporphyrin Dimethyl Ester (1d)*

A mixture of 3-carboxy-8-vinyldeuteroporphyrin dimethyl ester (100 mg), oxalyl chloride (0·5 g) and methylene dichloride (30 ml) was refluxed for 20 min whereupon most of the solvent was allowed to evaporate. The remainder, with excess oxalyl chloride was removed at room temperature under reduced pressure to give a residue of the porphyrin acid chloride.

Meanwhile a solution of isopropylmagnesium bromide [prepared under nitrogen from isopropyl bromide (0·82 g), magnesium (0·2 g) and tetrahydrofuran (7 ml)] was added by way of a syringe to a stirred mixture of monomethyl (*E,E*)-farnesylmalonate (1·07 g) and tetrahydrofuran (2 ml) which was cooled in an ice bath and maintained under nitrogen. This solution was then refluxed for 10 min, and added while still hot to the porphyrin acid chloride prepared above.* The mixture was shaken vigorously until all the solid had dissolved, the solution refluxed for 1 min and then cooled in an ice bath. Trifluoroacetic acid (1 ml) was added and the mixture partitioned between dilute brine (700 ml) and chloroform (100 ml); fresh chloroform (3 × 50 ml) was used to extract the aqueous phase. The combined extracts were evaporated, the residue dried by distillation with benzene (2 × 100 ml), and light petroleum (200 ml) added. The solvent was evaporated and the residue treated with fresh light petroleum (75 ml) and a little kieselguhr. This mixture was then cooled with shaking in a dry-ice/acetone bath and the solids collected on more kieselguhr which was washed with cold (< 0°) light petroleum (75 ml) and the porphyrin dissolved from the kieselguhr with chloroform. The light petroleum filtrates were diluted with an equal volume of ether and washed with an aqueous solution of potassium carbonate (1·5%; 30 ml) followed by dilute brine (1 × 50 ml; 2 × 20 ml). The solvent was removed and the residue, along with the porphyrin dissolved in chloroform, was chromatographed on silica (10 g) to give the porphyrin β-keto ester (1c) which was immediately dissolved in pyridine (15 ml) and the solution refluxed with lithium iodide (400 mg) under nitrogen for 60 h. The cooled reaction mixture was then treated with acetic acid (1 ml) and partitioned between dilute brine (300 ml) and chloroform (50 ml). The aqueous phase was extracted with chloroform (2 × 25 ml) followed by pyridine/chloroform (1:4; 2 × 20 ml) and the combined organic solutions were evaporated; the last traces of pyridine were removed by co-distillation with toluene (*c.* 100 ml). A suspension of the residue in chloroform/methanol (2:1; 150 ml) was treated with ethereal diazomethane and when methylation was complete volatile components were removed and the residue purified by chromatography (3 ×) on silica (9 g) and recrystallization from chloroform/methanol to give the *ketone* (63 mg) as soft, flat needles, m.p. 135–137° (Found: C, 75·9; H, 7·7; N, 6·9. $C_{51}H_{62}N_4O_5$ requires C, 75·5; H, 7·7; N, 6·9%). λ_{max} (log ε): 413·5 (5·25), 511·5 (4·10), 547 (4·06), 579·5 (3·90), 633 (3·41) nm. δ ($CDCl_3$): 1·56 (6H) 1·64 (3H) 1·71 (3H) (4 × side-chain methyls), 2·05 (8H, m, 4 × side-chain methylenes), 2·80 (2H, m, $COCH_2CH_2$), 3·05 (6H, m, 2 × $CH_2CH_2CO_2Me$, $COCH_2CH_2$), 3·28 (3H) 3·30 (3H) 3·43 (3H) 3·56 (3H) (4 × ring methyls), 3·58 (3H) 3·63 (3H) (2 × $CH_2CH_2CO_2CH_3$), 4·09 (4H, m, 2 × $CH_2CH_2CO_2CH_3$), 5·10 (2H, m, 2 × side-chain methines), 5·42 (1H, t, side-chain methine), 6·20 (2H, m, $CH{=}CH_2$), 8·05 (1H, m, $CH{=}CH_2$), 9·30 (1H) 9·35 (1H) 9·58 (1H) 10·30 (1H) (porphyrin methines).

(vii) *3-[(4E,8E)-1-Hydroxy-5,9,13-trimethyltetradeca-4,8,12-trienyl]-8-vinyldeuteroporphyrin Dimethyl Ester (1b)*

Sodium borohydride (50 mg) dissolved in absolute ethanol (3·5 ml) was added to a solution of 3-[(4*E*,8*E*)-5,9,13-trimethyltetradeca-4,8,12-trienoyl]-8-vinyldeuteroporphyrin dimethyl ester (30 mg) in chloroform (10 ml) containing methanol (5 ml) and *p*-toluenesulfonic acid (20 mg). Reduction was complete after *c.* 10 min at room temperature whereupon the reaction mixture was diluted with methylene dichloride (50 ml) and shaken with dilute hydrochloric acid (0·01 M; 400 ml) until excess borohydride had been decomposed. Aqueous ammonia (15 M; 2 ml) and brine were added and the

* All subsequent steps in the sequence were carried out in the dark.

mixture shaken again. The aqueous layer was re-extracted with methylene dichloride (2 × 20 ml) and the combined organic extracts evaporated to dryness. The residue was purified by chromatography on silica (5 g), with methyl acetate in methylene dichloride (0–10%) as eluent, followed by recrystallization from methylene dichloride/hexane to give the *porphyrin alcohol* (27 mg) as small plates, m.p. 157–159° (Found: C, 75·4; H, 8·0; N, 7·1. $C_{51}H_{64}N_4O_5$ requires C, 75·3; H, 7·9; N, 6·9%). λ_{max} (log ε): 403·5 (5·26), 502 (4·16), 536·5 (4·03), 572 (3·82), 625·5 (3·63) nm. δ ($CDCl_3$): 1·50 (3H) 1·59 (6H) 1·66 (3H) (4 × side-chain methyls), 2·03 (8H, br, 4 × side-chain methylenes), 2·15–2·83 (4H, m, $CHOHCH_2CH_2$); 3·23 (4H, t, 2 × $CH_2CH_2CO_2Me$), 3·50 (3H) 3·52 (6H) 3·59 (3H) (4 × ring methyls), 3·70 (6H, 2 × CO_2CH_3), 4·28 (4H, t, 2 × $CH_2CH_2CO_2Me$), 5·11 (2H, m, 2 × side-chain methines), 5·18 (1H, t, side-chain methine), 6·02 (1H, t, $CHOHCH_2$), 6·25 (2H, m, $CH{=}CH_2$), 8·21 (1H, m, $CH{=}CH_2$), 9·80 (1H) 9·82 (1H) 10·00 (1H) 10·16 (1H) (porphyrin methines).

(g) Investigation of the Cryptoporphyrin *a* Fraction of Ox Heart

Surplus fat was removed from a fresh ox heart (*c.* 900 g) which was then minced and washed in running water until the washings were colourless. The mince was squeezed as dry as possible and soaked in turn, with occasional stirring, in aqueous acetone (80%; 3 l.) and ether (1·5 l.) (30 min in each solvent). It was then left at 5° in a mixture of acetone (2·4 l.), water (540 ml) and hydrochloric acid (60 ml, 10 M). Next day the mince was removed by filtration, washed with a further quantity (500 ml) of the acidic extraction fluid and rejected. The filtrate and washings were diluted with water and the haems transferred into ether. This extract was washed (3 ×) with hydrochloric acid (0·1 M) to remove the majority of the acetone and the solvent evaporated. The fatty residue was dissolved in acetic acid (600 ml) and while a stream of nitrogen was bubbled through this solution a saturated solution of iron(II) sulfate in hydrochloric acid (15 ml; 10 M) was introduced. The mixture was left at room temperature under nitrogen until no further iron complex remained (monitored by hand spectroscope; *c.* 15 min) whereupon the porphyrins were transferred into ether using aqueous sodium acetate to neutralize excess hydrochloric acid. The ethereal solution of the porphyrins (1 l.) was *carefully* washed with water (3 × 500 ml; emulsification occurs very easily) and then with hydrochloric acid (3%; 2 × 500 ml) to remove the majority of the protoporphyrin. Hydrochloric acid (8%; 1 × 250 ml) was used to obtain the cryptoporphyrin *a* fraction leaving the porphyrin *a* in the ethereal solution. The porphyrins in the cryptoporphyrin *a* fraction were returned to ether by neutralizing the hydrochloric acid with solid sodium hydrogen carbonate.

The porphyrins obtained from the cryptoporphyrin *a* fraction of ten similar preparations (*c.* 10 kg of mince) were extracted back into hydrochloric acid (8%) and then returned to ether. This ethereal solution was washed with water and ethereal diazomethane added to esterify the porphyrins which were chromatographed on alumina (20 g; Peter Spence grade H) in ethereal solution. The same solvent eluted protoporphyrin while the cryptoporphyrin *a* fraction was eluted with chloroform/ether (1:3). This latter material was then rechromatographed on silica (8 g); a little protoporphyrin was eluted first followed by the cryptoporphyrin fraction as two components. Mixed fractions were rechromatographed and finally the two individual components were chromatographed separately on silica. The less polar species was recrystallized from chloroform/methanol to give *Spirographis* porphyrin (0·5 mg), m.p. 279–280° (lit.[5] 283–285°) while the more polar component was also recrystallized from the same solvent mixture to give iso*Spirographis* porphyrin (0·5 mg) as fine needles which changed to plates at *c.* 205° and finally melted at 225–227° (lit. 233–235°;[5] 225°;[21,27] 228–230°[28]). The two components were identical (m.m.p.; t.l.c.; ^{1}H n.m.r.; electronic spectrum) with authentic samples of these porphyrins.

Acknowledgment

Financial assistance from the Australian Research Grants Committee is gratefully acknowledged.

Manuscript received 24 November 1980

[27] Sono, M., and Asakura, T., *Biochemistry*, 1974, **13**, 4386.
[28] Games, D. E., Jackson, A. H., and O'Hanlon, P. J., *J. Chem. Soc., Perkin Trans. 1*, 1976, 2501.