Isolation, Crystallisation, and Synthesis of the Dimethyl Ester of Porphyrin *a*, the Iron-free Prosthetic Group of Cytochrome *c* Oxidase[1,2]†

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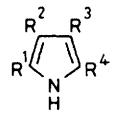
The structure of haem *a*, the prosthetic group of cytochrome *c* oxidase, has been confirmed by unambiguous synthesis of the dimethyl ester (**2b**) of its iron-free derivative, porphyrin *a*. The latter has been isolated in homogeneous state from beef heart and has been crystallised for the first time. The identity of the natural and synthetic samples of porphyrin *a* dimethyl ester was established by full spectroscopic and h.p.l.c. comparison. The reliability of these comparisons was strengthened by the synthesis of a closely related isomer (**10a**) of porphyrin *a* (**2b**) and a demonstration that the two isomers could readily be distinguished.

Cytochrome *c* oxidase (E.C. 1.9.3.1.) is the terminal enzyme of the respiratory chain and its prosthetic group, haem *a*, has attracted the efforts of many chemists since it was isolated in 1951 by Warburg.[3] Removal of the iron from haem *a* yields porphyrin *a* and this too has been extensively studied; the results obtained have been reviewed.[4-9] Even though it is almost certain that the samples of porphyrin *a* used in the classical structural work were not homogeneous because of the instability of the pigment and its tight association with lipids (see later), remarkable progress was made.

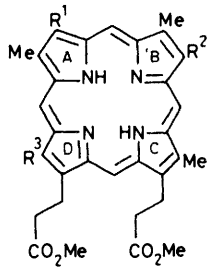
The presence of formyl and vinyl substituents in conjugation with the macrocycle was established at an early stage and Lemberg[10] proposed that these groups occupied opposite pyrrolic rings in the macrocycle to account for the characteristic electronic absorption spectrum of porphyrin *a* (oxo-rhodo type[11]). Furthermore, the formation of the pyrrole tricarboxylic acid (**1a**) by permanganate oxidation of porphyrin *a* nitrile, prepared by the dehydration of the oxime derivative of porphyrin *a*, established the location of the formyl group on a pyrrole ring bearing a propionic acid side chain.[12] In addition, heating haem *a* with resorcinol yielded, after the removal of iron and methylation of the acidic side chains, cytodeuteroporphyrin dimethyl ester (**2a**), the structure of which was established by synthesis.[5] Vinyl and formyl groups are known to be removed by molten resorcinol and the third labile substituent cleaved by this treatment was shown to be an α-hydroxyalkyl side chain.[13] The carbon skeleton of this latter substituent was established by Lynen *et al.*,[14] although they were in error regarding its degree of saturation. This was corrected by a penetrating ^{1}H n.m.r. study of haem *a* by Caughey *et al.*[15] who showed that the side chain contained three double bonds and suggested the *trans-trans* configuration. Taken together, these data led to the proposal of structure (**2b**) for porphyrin *a* dimethyl ester.

Isolation of Crystalline Porphyrin a *Dimethyl Ester.*—Two preparations of porphyrin *a* were made from beef heart and both were converted into the corresponding dimethyl ester. One involved the acidic extraction process[16] which directly afforded crude porphyrin *a*. Basic extraction[15] was used for the second preparation which gave haem *a* as its dipyridyl complex. This was then demetallated with acidic iron(II) sulphate before esterification. Both samples of porphyrin *a* dimethyl ester were amorphous (as in earlier work) and both were apparently homogeneous by t.l.c. in a range of solvents. However, both were in fact seriously heterogeneous as shown by h.p.l.c. (Figure) and extensive chromatography, followed by pre-



(**1**)

	R^1	R^2	R^3	R^4
a;	CO_2H	$CH_2CH_2CO_2H$	CN	CO_2H
b;	Me	Me	CO_2Et	H
c;	$AcOCH_2$	Me	CH_2CH_2OAc	CO_2CH_2Ph



(**2**)

	R^1	R^2	R^2
a;	H	H	H
b;	–CH(OH)CH_2-farnesyl*	$CH{=}CH_2$	CHO
c;	–CH(OAc)CH_2-farnesyl*	$CH{=}CH_2$	CHO
d;	CO_2Me	$CH{=}CH_2$	CHO
e;	–CH(OH)CH_2-hexahydrofarnesyl	$CH{=}CH_2$	CHO
f;	–CH(OH)CH_2-farnesyl*	$CH{=}CH_2$	Me
g;	CO_2H	$CH{=}CH_2$	CHO
h;	COCl	$CH{=}CH_2$	CHO
i;	–C(O)CH(CO_2Me)-farnesyl*	$CH{=}CH_2$	CHO
j;	–C(O)CH_2-farnesyl*	$CH{=}CH_2$	CHO
k;	–CH(OH)CH_2-farnesyl*	$CH{=}CH_2$	CH_2OH

* *trans-trans*-Farnesyl side chain.

† This paper is regarded as Part 56 in P.S.C.'s series on the Chemistry of Pyrrolic Compounds and as Part 24 in A.R.B.'s series on Porphyrins and Related Macrocycles.

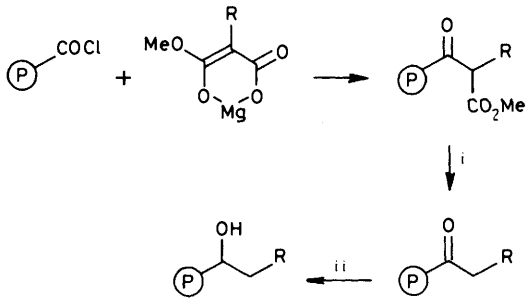
Figure. Crude porphyrin *a* dimethyl ester derived from haem *a* dipyridyl complex (see text) fractionated by h.p.l.c. on two $10\mu C_{18}$ Bondapak columns in series with degassed acetonitrile as solvent. Flow rate 2.5 ml min^{-1} at *ca.* 2 500 lb in^{-2} with a u.v.-visible analyser set at 412 nm. I = Injection point, peak a is porphyrin *a* dimethyl ester, peak b is the porphyrin of molecular weight 862 (see text)

parative h.p.l.c., were required to afford pure porphyrin *a* dimethyl ester. This then crystallised.*

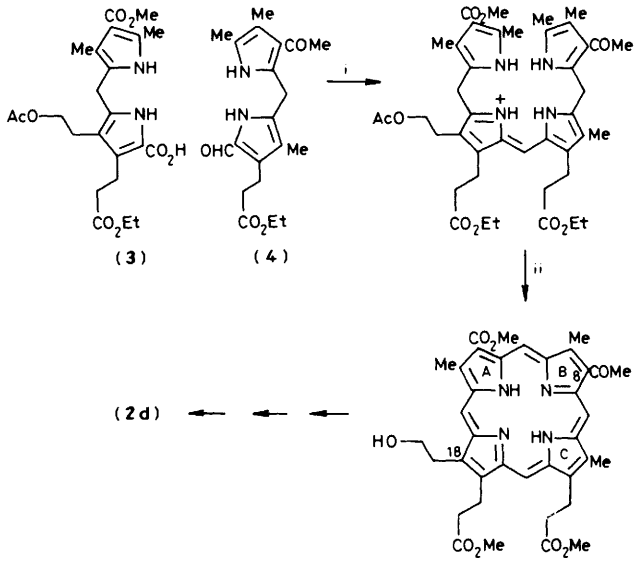
During this phase of our studies, an important observation was made which simplified all subsequent work. Porphyrin *a* dimethyl ester was found to be extremely photolabile and small quantities were totally destroyed by handling in light. It was necessary to work in dimmed light with careful protection of flasks, columns *etc.* by aluminium foil or black cloth; sub-milligram quantities of porphyrin *a* dimethyl ester then survived.

Pure porphyrin *a* dimethyl ester showed M^+ 826.4757 by FD-ms which rigorously established the molecular size and composition as $C_{51}H_{62}N_4O_6$, thus supporting structure (**2b**). The 270 MHz ^1H n.m.r. spectra were also in agreement with structure (**2b**) and the corresponding *O*-acetate (**2c**), obtained by simple acetylation, showed the expected shifts relative to the parent alcohol. Finally, Jones oxidation and, separately, borohydride reduction of porphyrin *a* ester (**2b**) were carried out to afford, respectively, the corresponding ketone (**2j**) and diol (**2k**); each product gave a single peak (>95% pure) by h.p.l.c. so giving further confirmation of the homogeneity of the isolated porphyrin *a* ester (**2b**). However, for none of these materials was it possible by n.m.r. alone to deduce with certainty the stereochemistry of the farnesyl (3,7,11-trimethyldodeca-2,6,10-trienyl) side chain. Confirmation of the *trans-trans* configuration of structure (**2b**) was just one of the benefits expected from a total synthesis of porphyrin *a* dimethyl ester.

* Much more work is needed on the second porphyrin which accompanies porphyrin *a* dimethyl ester (Figure). The molecular weight of this substance, by field desorption mass spectroscopy (FD-ms), is 862, *i.e.* 36 units higher than porphyrin *a* ester.



Scheme 1. (P) = Porphyrin macrocycle, R = farnesyl or tetrahydrofarnesyl. *Reagents:* i. LiI, pyridine; ii, $NaBH_4$

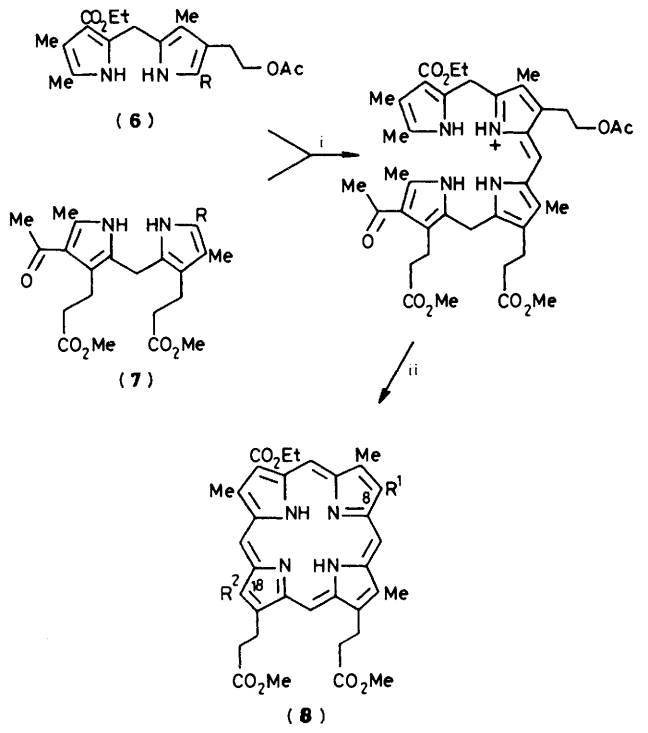


Scheme 2. *Reagents:* i, H^+; ii, $Cu(OAc)_2$ then H_2SO_4 then MeOH–H^+

Synthesis of Porphyrin a *Dimethyl Ester.*—In two earlier papers, a strategy for the synthesis of porphyrin *a* was outlined. The first[17] described the preparation of the intermediate (**2d**) which provides the macrocycle for porphyrin *a*; the second[18] established that the required terpenoid side chain could be introduced by condensation of a porphyrin acid chloride with magnesium complexes of substituted malonates (Scheme 1). These methods led to the synthesis of hexahydroporphyrin *a* dimethyl ester (**2e**)[18] and of a compound (**2f**) of possible significance for the biosynthesis of porphyrin *a*.[19]

The published synthesis[17] of porphyrin (**2d**) involved the condensation of the methylenedipyrroles (**3**) and (**4**) followed by the oxidative cyclisation of the resultant bilene-*b* to give the porphyrin (**5**) in an overall yield of 33% from the methylenedipyrroles [the East–West approach (Scheme 2)]. The initial procedure[17] for the conversion of the product (**5**) into the porphyrin (**2d**) gave only a 10% yield, but this has subsequently been raised[20,21] to 43%.

In the present work, an improved method based upon a North–South approach was used for the synthesis of porphyrin (**2d**). Condensation of the known pyrroles (**1b**)[22] and (**1c**)[23] gave the methylenedipyrrole (**6a**) which was hydrogenolysed to give the acid (**6b**). The bilene-*b* derived from the condensation of this acid with the formylmethylenedipyrrole (**7a**)[19] was



(6) **a**; R = CO_2CH_2Ph (8) **a**; R^1 = CH_2CH_2OH, R^2 = MeCO
b; R = CO_2H **b**; R^1 = CH_2CH_2OH, R^2 = MeCH(OH)
(7) **a**; R = CHO **c**; R^1 = CH_2CH_2Cl, R^2 = $CH{=}CH_2$
b; R = CO_2H **d**; R^1 = CH_2CH_2Cl, R^2 = CHO

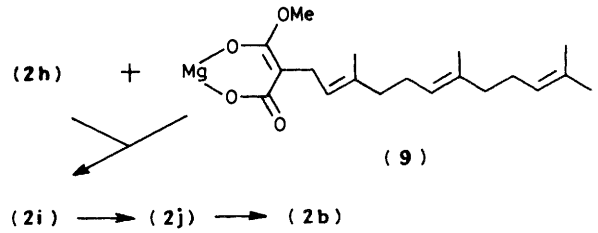
Scheme 3. *Reagents:* i, H^+; ii, $Cu(OAc)_2$ then H_2SO_4 then MeOH–H^+

cyclised by treatment with copper(II) acetate to yield the porphyrin (**8a**), after demetallation of the initially formed chelate and methanolysis of the acetate ester (Scheme 3). Sodium borohydride reduction of the ketone (**8a**) furnished the porphyrin diol (**8b**) which was treated with dimethylformamide and benzoyl chloride. As expected,[24] this reagent not only dehydrated the 1-hydroxyethyl group at position 18, but also transformed the 2-hydroxyethyl substituent at position 8 into the 2-chloroethyl derivative to form the porphyrin (**8c**). The reaction of the vinylporphyrin (**8c**) with osmium tetraoxide and oxidation of the intermediate diol with sodium periodate produced the formylporphyrin (**8d**). This product was protected as its acetal with ethanediol and then all the ester groups were hydrolysed and a vinyl group was generated at position 8 using sodium hydroxide. Cold methanolic sulphuric acid selectively esterified the propionic acids to give the porphyrin acid (**2g**); diazomethane converted this into the ester (**2d**), identical in all respects with the product obtained earlier.[17]

This North–South approach has three advantages over the earlier one.[17] (*a*) The starting materials are more accessible; (*b*) the conversion (**8a**)→(**2g**) involves fewer steps and a higher yield (50%), and the product (**2g**) is the acid required for the attachment of the terpenoid side chain (the East–West strategy furnished the ester); and (*c*) the yield of the porphyrin (**8a**) from the methylenedipyrroles (**6b**) and (**7a**) was unusually high (40%).

At the stage of the acid (**2g**) in the earlier work,[18] the formyl group was protected, but this turned out to be disadvantageous. Subsequently it was found that the steps of the formation of the acid chloride (**2h**) followed by the attachment of the terpenoid side chain could be carried out under appropriate conditions without protection of the formyl group and this sequence was adopted here.

The acid chloride (**2h**) reacted with the magnesium chelate (**9**)



Scheme 4.

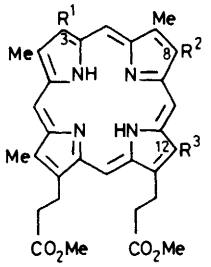
generated from monomethyl *trans,trans*-farnesylmalonate and isopropylmagnesium bromide (see Scheme 4) to give the porphyrin β-oxoester (**2i**); from this step onward, it was necessary to carry out all operations with protection against light. The β-oxoester (**2i**) was cleaved using lithium iodide to afford the ketone (**2j**) the formyl group of which was protected as its dimethyl acetal. Reduction of this derivative with sodium borohydride followed by hydrolysis of the dimethyl acetal and brief treatment with ethereal diazomethane to reverse any ester hydrolysis yielded (*R,S*)-porphyrin *a* dimethyl ester (**2b**) as a crystalline product.

It was clear from the difficulties experienced in separating the pure natural porphyrin *a* dimethyl ester from the accompanying pigments that a combination of powerful techniques would have to be used in order to demonstrate beyond question that the natural and synthetic materials were structurally identical.* The first step was to show that the synthetic sample was >99% pure and that it was inseparable from natural porphyrin *a* dimethyl ester by h.p.l.c., even after five recycles through the most efficient column with the best solvent system. Also, the u.v.-visible and n.m.r. spectra of the synthetic and natural samples were superimposable. The identity test by n.m.r. was made still more stringent by mixing the synthetic and natural samples (*ca.* 3:4 parts) and measuring the ^1H n.m.r. spectrum after addition of a shift reagent [Eu([$^2H_9$]-fod)$_3$]. The signals shifted in the expected way and, specifically, no new signals separated from singlets or double doublets which at the outset were clearly observable.

Chemical transformations of the synthetic and natural samples were also carried out. They were *O*-acetylated to yield two samples of the porphyrin (**2c**) which gave identical u.v.-visible and n.m.r. spectra, and a mixture of the samples showed one peak after five recycles on h.p.l.c. Also, the ketone (**2j**) prepared above by oxidation of the natural material was shown by rigorous comparison to be identical with the synthetic intermediate (**2j**). Finally, iron was reinserted into natural porphyrin *a* ester to yield the ester of haem *a* [Fe^{III} complex of (**2b**)] and this was fused with resorcinol. This yielded cytodeuteroporphyrin (**2a**) which gave u.v.-visible and mass spectra identical with those from a synthetic sample of this porphyrin kindly provided by Dr. S. F. MacDonald.[5] A mixture of these two samples of porphyrin (**2a**) ran as one peak on h.p.l.c. after five recycles. The synthetic sample of porphyrin *a* ester (**2b**) was taken through the same steps to afford cytodeuteroporphyrin (**2a**), again shown as above to be identical with the earlier samples of this material.

These comparisons allow great confidence in the identity of the synthetic and natural samples of porphyrin *a* ester. We felt that the evidence could be made overwhelming by demonstrating that porphyrin *a* ester could be distinguished

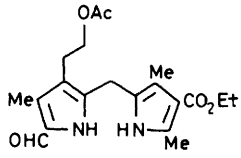
* The stereochemistry at the hydroxy-bearing carbon of structure (**2b**) is not being considered at this stage. Presumably natural porphyrin *a* is one enantiomer whereas the synthetic sample is racemic; however, this causes no problems for the methods used to compare the synthetic and natural samples.



(10)

	R^1	R^2	R^3
a;	$CH=CH_2$	$-CH(OH)CH_2$-farnesyl*	CHO
b;	CH_2CH_2OH	CO_2Et	COMe
c;	$CH=CH_2$	CO_2H	CHO
d;	$CH=CH_2$	CO_2Me	CHO
e;	CH_2CH_2Cl	CO_2Et	$CH=CH_2$
f;	CH_2CH_2Cl	CO_2Et	CHO
g;	$CH=CH_2$	$-C(O)CH_2$-farnesyl*	CHO
h;	$CH=CH_2$	$-CH(OAc)CH_2$-farnesyl*	CHO

* *trans-trans*-Farnesyl side chain.



(11)

from an isomer purposely chosen to be extremely similar. The synthesis of that isomer is now described.

Synthesis of Isoporphyrin a *Dimethyl Ester* (**10a**).—The route to this porphyrin commenced with the condensation of the known methylenedipyrroles (**7b**)[19] and (**11**)[21] to give an intermediate bilene-*b* which was cyclised with copper(II) acetate to construct the porphyrin macrocycle. Removal of the copper from the initially formed chelate and methanolysis of the side-chain esters furnished the product (**10b**). Modification of the side chains at positions 3 and 12 was carried out as above for porphyrin *a* dimethyl ester, to yield the porphyrin acid (**10c**) from which the characterisable methyl ester (**10d**) was prepared by reaction with diazomethane.

The acid chloride derived from acid (**10c**) was converted *via* (**10g**) into isoporphyrin *a* dimethyl ester (**10a**) by following the chemistry already described for the preparation of porphyrin *a* dimethyl ester and summarised in Scheme 1 (R = farnesyl).

The power of the methods used to check the identity of natural and synthetic samples of porphyrin *a* dimethyl ester were then tested on the foregoing isomeric system as follows: (*a*) the ^1H n.m.r. spectra of porphyrin *a* ester (**2b**) and isoporphyrin *a* ester (**10a**) were obviously different; (*b*) a mixture of these two isomeric esters was shown to be resolved by h.p.l.c., almost complete separation being achieved after one recycle; and (*c*) the *O*-acetate of isoporphyrin *a* ester (**10h**) was readily separable from the *O*-acetate of porphyrin *a* ester (**2c**) by one passage through the h.p.l.c. column. Thus, the methods used above in the crucial comparison of synthetic and natural samples are reliable.

It follows from the sum of experiments here reported that the structure (**2b**) is now firmly established for porphyrin *a* dimethyl ester; porphyrin *a* is the corresponding dicarboxylic acid and hence the constitution of haem *a*, the iron complex of this latter macrocycle and the prosthetic group of cytochrome *c* oxidase, is rigorously established.

Experimental

General Directions.—M.p.s are uncorrected and were determined on a Kofler micro-melting-point apparatus. Unless otherwise stated, electronic spectra were recorded in chloroform, freshly distilled from anhydrous potassium carbonate using a Varian Techtron Model 635 or Unicam SP800 spectrophotometer. ^1H N.m.r. spectra of porphyrins were obtained at 80 or 100 MHz (JEOL JMN-4H-100S, JEOL JMN FX-100, Varian CFT-20 and HA-100 spectrometers), or at 400 MHz with a Bruker WH-400 instrument. The spectra of simpler molecules were normally recorded on a Varian A60 spectrometer. Resonances are quoted on the δ scale relative to tetramethylsilane (δ = 0) as internal standard. Peter Spence Grade H and Woelm neutral alumina and Merck Kieselgel H type 60 were used for column chromatography. Unless otherwise stated, column chromatography of porphyrins was carried out using 'ethanol-free' chloroform[19] as eluant. Dry methylene dichloride, dioxane, and tetrahydrofuran were obtained by slow percolation through an alumina column. Pyridine was dried over potassium hydroxide for several days and distilled before use. Light petroleum refers to the fraction with b.p. 60—80 °C, ether refers to diethyl ether, and acetic acid is glacial (17M). Solutions in water-immiscible solvents which had been in contact with water were dried over anhydrous sodium sulphate prior to evaporation. Solvents were usually removed at <40 °C in a Büchi rotary evaporator at *ca.* 30 Torr.

It is essential that all operations on porphyrins carrying the farnesyl side chain be carried out with careful protection against light.

Isolation of Crystalline Porphyrin a *Dimethyl Ester* (**2b**).—(*a*) *By basic extraction.* Crude haem *a* dipyridine complex (178 mg containing *ca.* 18.4 mg haem *a*) was isolated from fresh bovine heart muscle (1 kg) using pyridine–chloroform by the method of Caughey *et al.*[15] with protection against light at all stages (and for the subsequent steps). A solution of all of this crude complex in glacial acetic acid [180 ml, freshly distilled from iron(II) sulphate] was purged with carbon dioxide for 10 min and treated with a solution of iron(II) sulphate heptahydrate (330 mg) in concentrated 'AnalaR' hydrochloric acid (4 ml). The stream of carbon dioxide was continued for 5 min and then the mixture was shaken with diethyl ether (700 ml; distilled from $FeSO_4$) and aqueous 12.2M-sodium acetate (450 ml). The ethereal layer was washed with water (4 × 500 ml) and dilute aqueous sodium hydrogen carbonate solution adjusted to pH 6 (1 × 500 ml), and evaporated to give crude porphyrin *a* (47 mg containing, by u.v.-visible spectroscopy, 9.04 mg of porphyrin *a*). $\lambda_{max.}$(ether) 412, 512, 557, 581, and 642 nm; ratio band III/IV = 1.6.

All of this fraction was applied in ether (20 ml) to a CF11 cellulose column (350 g, 150 × 4 cm) and after being washed with ether–light petroleum (1:1; 50 ml) the column was eluted with light petroleum (800 ml) to remove lipids. Elution with ether gave the porphyrins in a series of fractions and those showing an absorption spectrum with a ratio of bands III/IV of >2.0 were combined and evaporated to a dark residue (14.8 mg containing *ca.* 3.06 mg of porphyrin *a*). This in acetone (5 ml) and ether (25 ml) was treated with an excess of distilled ethereal diazomethane at 18 °C. After 3 min, the solution was purged with nitrogen for 30 min and then evaporated. This dimethyl ester was chromatographed on neutral alumina (6 g; Grade III), first eluting with benzene, which was used until three column volumes had passed, then with chloroform, which gave the fraction containing porphyrin *a* dimethyl ester (2.86 mg).

This fraction was further purified by preparative h.p.l.c. using two C-18 Bondapak analytical columns connected in sequence with degassed acetonitrile as solvent. The slightly faster running main fraction (see Figure) yielded pure *porphyrin* a *dimethyl ester* (1.01 mg) which crystallised from ether–hexane, m.p. 128—130 °C (Found: C, 74.0; H, 7.6; N, 6.6%; *m/z* 826.4757. $C_{51}H_{62}N_4O_6$ requires C, 74.05; H, 7.6; N, 6.8%; *m/z* 826.4670); $\lambda_{max.}$(ether) 412, 517, 560, 584, and 647 nm; ratio bands III/IV = 2.28; $\nu_{max.}$(CH_2Cl_2) 1 655, 1 735, 2 915, 3 303, and 3 490 cm^{-1}; δ (400 MHz; CD_2Cl_2) 11.41 (1 H, s, CHO), 10.67, 10.40, 10.01, 9.78 (each 1 H, s, methines), 8.28 (1 H, dd, C*H*=CH_2), 6.37 and 6.24 (each 1 H, dd CH=CH_2), *ca.* 5.12 (3 H, m, side chain C*H*=C), *ca.* 4.3 (4 H, m, $CH_2CH_2CO_2Me$), 3.73, 3.66, 3.63, 3.61 (15 H, s, 2 × CO_2Me and 3 × ring Me), *ca.* 3.1—3.2 and 2.3—2.7 (8 H, m, side chain CH_2), 1.63, 1.61 (each 3 H, s, side chain Me), and 1.56 (6 H, s, side chain Me).

(*b*) *By acidic extraction.* The crude porphyrin *a* obtained by the published method [16] from bovine heart muscle was used. It was converted into the dimethyl ester and chromatographed as above on alumina to afford partly purified porphyrin *a* dimethyl ester (60 mg). Part of this (6 mg) was fractionated as in section (*a*) by preparative h.p.l.c., the sample being added to the columns in methylene dichloride (total 0.5 ml; *ca.* 150 μl for each injection). Two main bands were separated and the one eluted first afforded pure porphyrin *a* dimethyl ester (2.11 mg) which crystallised from ether–hexane, m.p. 128—130 °C. A larger repeat h.p.l.c. run (11 mg of above partly purified ester) gave further pure porphyrin *a* dimethyl ester (6.37 mg). This product was identical by full spectroscopic comparison [u.v.-visible, i.r., n.m.r., field desorption mass spectra (FD-ms)] with the material from method (*a*) above.

O-*Acetylporphyrin* a *Dimethyl Ester* (**2c**).—Porphyrin *a* dimethyl ester (1.51 mg) in dry pyridine (3 ml) and 'AnalaR' acetic anhydride (0.6 ml) was kept at 23 °C for 50 h. The residue from evaporation of the solution was partitioned between ether (20 ml) and distilled water (30 ml) and the product in the ether was chromatographed on neutral alumina (5 g; Grade III) in benzene. After two column volumes of benzene had been passed through, elution with chloroform gave O-*acetylporphyrin* a *dimethyl ester* (1.26 mg) as a tight band shown by h.p.l.c. on C_{18} Bondapak in acetonitrile to be >95% pure (Found: *m/z* 868.4772. $C_{53}H_{64}N_4O_7$ requires *m/z* 868.4775); $\lambda_{max.}$(ether) 413, 515, 558, 583, and 646 nm; ratio bands III/IV 2.06; $\nu_{max.}$(CH_2Cl_2) 1 665, 1 733, 2 845, 2 920, and 3 300 cm^{-1}; δ (3.64 mM in CD_2Cl_2) (well resolved signals) 11.48 (1 H, s, CHO), 10.90, 10.34, 10.16, and 10.05 (each 1 H, s, 4 methines), 8.10 (1 H, dd, C*H*=CH_2), 7.34 (1 H, t, C*H*OAc), 6.21 (2 H, m, CH=CH_2), 4.66 and 4.40 (2 × 2 H, t, $CH_2CH_2CO_2Me$), 3.79 and 3.60 (s, 15 H, CO_2*Me*, and ring Me), 3.30 (4 H, m, $CH_2CH_2CO_2Me$), 2.26 (3 H, s, CH_3CO), 1.95, 1.58, and 1.49 (12 H, s, Me of side chain), and −3.55 (2 H, s, NH).

Porphyrin a *Ketone Dimethyl Ester* (**2j**).—A solution of porphyrin *a* dimethyl ester (1.25 mg) in acetone (5 ml) was purged with nitrogen for 5 min and then treated with 8M-chromic acid (Jones reagent; [25] 0.02 ml) for 35 s. The mixture was then immediately partitioned between water (20 ml) and ether (20 ml) and the organic phase was washed with water (3 × 25 ml). The product from the ether was chromatographed on neutral alumina (10 g; Grade III) first in benzene and then with chloroform which eluted the *ketone* (0.87 mg). This was >95% homogeneous as shown by h.p.l.c. on 2 × 10μ Porasil CN columns in series using hexane–toluene–acetonitrile (74:20:6) (Found: *m/z* 824.4491. $C_{51}H_{60}N_4O_6$ requires *m/z* 824.4512); $\lambda_{max.}$($CHCl_3$) 428, 522, 561, 589, and 645 nm; ratio bands III/IV 0.99; δ (1.27 mM in CD_2Cl_2) (well resolved signals) 11.49 (1 H, s, CHO), 10.94, 10.48, 10.15, and 10.02 (each 1 H, s, methines), 8.10 (1 H, dd, C*H*=CH_2), 6.24 (2 H, m, CH=CH_2), 4.69 and 4.37 (2 × 2 H, t, $CH_2CH_2CO_2Me$), 3.89 (3 H, s, ring A Me), 3.59 (12 H, s, CO_2Me, and ring Me), 3.35 and 3.25 (2 × 2 H, t, $CH_2CH_2CO_2Me$), 2.89 (2 H, t, $COCH_2$-side chain), and 2.03, 1.69, and 1.48 (12 H, Me of side chain).

Porphyrin a *Diol Dimethyl Ester* (**2k**).—A solution of porphyrin *a* dimethyl ester (2.61 mg) in ether (30 ml) was stirred for 24 h with sodium borohydride (30 mg) and then washed successively with 0.1M-hydrochloric acid (10 ml) and water (3 × 10 ml). The product from the ether was chromatographed on neutral alumina (10 g; Grade III) in chloroform–methanol (19:1) to give the *diol* (1.95 mg). H.p.l.c. under the conditions used for the ketone above showed this product to be >98% pure (Found: *m/z* 828. $C_{51}H_{64}N_4O_6$ requires *m/z* 828); $\lambda_{max.}$(ether) 399, 500, 534, 571, and 624 nm; ratio bands III/IV 0.72; $\nu_{max.}$(CH_2Cl_2) 1 725, 2 850, 2 910, and 3 200—3 400 cm^{-1}; δ (2.62 mM in CD_2Cl_2) (well resolved signals) 10.49, 10.27, 10.24, and 10.17 (4 × 1 H, s, methines), 8.27 (1 H, dd, C*H*=CH_2), 6.22 (2 H, m, CH=CH_2), 6.03 (2 H, s, CH_2OH), 4.41 (4 H, t, $CH_2CH_2CO_2Me$), 3.64, 3.61, and 3.51 (15 H, CO_2Me, ring Me), 3.36 (4 H, t, $CH_2CH_2CO_2Me$), and 1.99, 1.52, and 1.47 (12 H, Me of side chain).

Synthesis of Porphyrin a *Dimethyl Ester*

Benzyl 4-(2-*Acetoxyethyl*)-3′-*ethoxycarbonyl*-3,4′,5′-*trimethyl*-2,2′-*methylenedipyrrole*-5-*carboxylate* (**6a**).—A mixture of ethyl 2,3-dimethylpyrrole-4-carboxylate [22] (1.67 g), benzyl 3-(2-acetoxyethyl)-5-acetoxymethyl-4-methylpyrrole-2-carboxylate [23] (3.73 g) and acetic acid (20 ml) was heated on a steam-bath under nitrogen for 1 h. The solvent was evaporated, the last traces of acetic acid being removed by distillation with toluene. Recrystallisation of the residue from ether–light petroleum gave the *methylenedipyrrole* (4.5 g), m.p. 122—123 °C (Found: C, 67.3; H, 6.6; N, 5.7. $C_{27}H_{32}N_2O_6$ requires C, 67.5; H, 6.7; N, 5.8%); δ ($CDCl_3$, 100 MHz) 1.29 (3 H, t, *J* 7.5 Hz, $CO_2CH_2CH_3$), 1.94, 1.99, 2.03, 2.10 (12 H, 3 × ring Me, $OCOCH_3$), 3.04 (2 H, t, *J* 7.5 Hz, CH_2CH_2OAc), 4.15 (6 H, m, CH_2CH_2OAc, $CO_2CH_2CH_3$, methylene protons), 5.20 (2 H, CH_2Ph), 7.31 (6 H, $CH_2C_6H_5$), and 8.29 and 9.81 (each 1 H, NH).

Dimethyl 18-*Acetyl*-3-*ethoxycarbonyl*-8-(2-*hydroxyethyl*)-2,7,12-*trimethylporphyrin*-13,17-*dipropionate* (**8a**).—A solution of 4-(2-acetoxyethyl)-3′-ethoxycarbonyl-3,4′,5′-trimethyl-2,2′-methylenedipyrrole-5-carboxylic acid (1.3 g, from the foregoing benzyl ester by hydrogenolysis) in trifluoroacetic acid (15 ml) was stirred at room temperature for 3 min. 4′-Acetyl-3,3′-bis(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-methylenedipyrrole-5-carbaldehyde [19] (1.38 g) was then added in one portion; the mixture was stirred in an ice-bath for 5 min, and diluted by the slow addition of methanol (25 ml). This solution was kept for 20 min and then poured into warm (50 °C) acetic acid–methanol (1:1; 1.4 l) containing copper(II) acetate (13.7 g) and anhydrous sodium acetate (6.8 g). The mixture was heated overnight on a steam-bath to form the porphyrin macrocycle, then water was added and the product isolated by repeated chloroform extraction. The combined chloroform extracts were washed with water and the porphyrin copper chelate chromatographed on silica (50 g) using chloroform.

The dry copper complex was mixed with trifluoroacetic acid (5 ml), sulphuric acid (18M; 50 ml) was added, and the mixture was shaken for 10 min after which it was cooled in an ice-bath and diluted carefully with cold methanol (1 l). The solution was kept at room temperature overnight, diluted with ice–water (1 l) and shaken for 5 min to hydrolyse any acetal formed from the

acetyl function. The porphyrin was transferred into chloroform and purified by chromatography on silica (50 g) with commercial chloroform to yield the *title porphyrin* (942 mg), m.p. 242—243 °C from methylene dichloride–light petroleum (Found: C, 66.5; H, 6.1; N, 8.1. $C_{38}H_{42}N_4O_8$ requires C, 66.8; H, 6.2; N, 8.2%); $\lambda_{max.}$(log ε) 420 (5.22), 514 (4.15), 549 (3.87), 584 (3.81), and 636 (3.47) nm; δ ($CDCl_3$–CF_3CO_2D, 10:1; 100 MHz), 1.77 (3 H, t, *J* 7.5 Hz, $CO_2CH_2CH_3$), *ca.* 3.2 (4 H, m, 2 × $CH_2CH_2CO_2Me$), 3.40 (3 H, $COCH_3$), 3.50, 3.57 (12 H, 2 × ring Me, 2 × CO_2CH_3), 3.93 (3 H, C2 Me), *ca.* 4.25 (6 H, m, 2 × $CH_2CH_2CO_2Me$, CH_2CH_2OH), 4.70 (2 H, br t, CH_2CH_2OH), 4.88 (2 H, q, *J* 7.5 Hz, $CO_2CH_2CH_3$), and 10.55, 11.11, 11.15, 11.43 (each 1 H, methine protons).

Dimethyl 8-(2-*Chloroethyl*)-3-*ethoxycarbonyl*-2,7,12-*trimethyl*-18-*vinylporphyrin*-13,17-*dipropionate* (**8c**).—Sodium borohydride (300 mg) in absolute ethanol (30 ml) was added to a solution of the foregoing porphyrin (500 mg) in chloroform (150 ml) and the solution was heated under reflux for 10 min. The cooled mixture was shaken with ice-cold hydrochloric acid (0.05M; 500 ml) until decomposition of the excess of borohydride was complete; aqueous ammonia (15M; 3 ml) was added and the mixture was extracted with chloroform. The extracts were washed with water and the hydroxyethyl derivative (430 mg) purified by chromatography on silica (30 g) using commercial chloroform.

This porphyrin (430 mg) in dimethylformamide (86 ml) containing benzoyl chloride (4.3 ml) was heated on a steam-bath for 75 min. The cooled mixture was diluted with cold aqueous triethylamine (2.2%; 400 ml), and the precipitated porphyrin was kept at 5 °C for 1 h before it was collected on Kieselguhr and washed with water. The porphyrin was recovered into chloroform and the resulting residue was dissolved in methanolic sulphuric acid (3%; 250 ml) and kept at 5 °C overnight. The product, isolated as usual, was chromatographed on silica (30 g) in light petroleum–chloroform (1:1) and recrystallised from methylene dichloride–light petroleum to give the *vinylporphyrin* (400 mg), m.p. 197—198 °C (Found: C, 66.8; H, 5.9; N, 8.2. $C_{38}H_{41}ClN_4O_6$ requires C, 66.6; H, 6.0; N, 8.2%); $\lambda_{max.}$(log ε) 413 (5.28), 510 (4.16), 547 (4.01), 580 (3.87), and 634 (3.57) nm; δ (CF_3CO_2D; 100 MHz) 1.92 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 3.24 (4 H, m, 2 × $CH_2CH_2CO_2Me$), 3.74, 3.77, 3.79, 3.80 (12 H, 2 × ring Me, 2 × CO_2CH_3), 4.08 (3 H, 2-Me), 4.27 (2 H, t, CH_2CH_2Cl), 4.67 (6 H, m, 2 × $CH_2CH_2CO_2Me$, CH_2CH_2Cl), 5.10 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 6.50 (2 H, m, $CH{=}CH_2$), 8.25 (1 H, m, $CH{=}CH_2$), and 10.93, 11.15, 11.16, 11.77 (each 1 H, methine protons).

Dimethyl 8-(2-*Chloroethyl*)-3-*ethoxycarbonyl*-18-*formyl*-2,7,12-*trimethylporphyrin*-13,17-*dipropionate* (**8d**).—To a stirred solution of the foregoing vinylporphyrin (780 mg) in dry pyridine (90 ml) at room temperature was added dropwise osmium tetraoxide in pyridine (2%). When all the vinylporphyrin had reacted (t.l.c.), aqueous sodium sulphite (6%; 50 ml) was added and the mixture stirred at 80—85 °C for 30 min. The hot mixture was filtered through Kieselguhr and the pad washed with hot aqueous pyridine (50%; 30 ml). The filtrate was diluted with water (600 ml), partly* neutralised with hydrochloric acid, and extracted with chloroform (1 × 200 ml; 4 × 50 ml).† The residue from chloroform was treated in pyridine (100 ml) with aqueous sodium periodate (5%; 50 ml) at 60 °C for 10 min. Water (600 ml) was added, the mixture partly neutralised with hydrochloric acid (to avoid dication formation) and the product isolated by chloroform extraction (1 × 200 ml; 4 × 50 ml). The combined extracts were washed with aqueous acetic acid (2%; 500 ml) the aqueous phase back-extracted with chloroform (4 × 50 ml) and the residue from the combined organic solutions kept in methanolic sulphuric acid (5%; 400 ml) for 16 h. Water and aqueous ammonia were added and the product returned to chloroform (1 × 200 ml; 3 × 50 ml). The extract was concentrated (to 200 ml) and shaken well with hydrochloric acid (10M; 50 ml) to decompose any acetal. Brine (600 ml) and aqueous ammonia were added, the organic layer was separated and the aqueous phase extracted with chloroform (3 × 50 ml). The residue from the combined chloroform extracts was chromatographed on silica (30 g) to give the *formylporphyrin* (642 mg), m.p. 246—247 °C, from chloroform–methanol containing one drop of triethylamine (Found: C, 64.8; H, 5.7; N, 8.2. $C_{37}H_{39}ClN_4O_7$ requires C, 64.7; H, 5.7; N, 8.1%); $\lambda_{max.}$(log ε) 429 (5.24), 520 (4.14), 558 (4.03), 588 (3.88), and 645 (3.45) nm; δ (CF_3CO_2D) 1.93 (3 H, t, *J* 7.0 Hz, $CO_2CH_2CH_3$), 3.35 (4 H, m, 2 × $CH_2CH_2CO_2CH_3$), 3.73, 3.75, 3.77, 3.81 (12 H, 2 × ring Me, 2 × CO_2CH_3), 4.11 (3 H, 2-Me), 4.26 (2 H, br t, CH_2CH_2Cl), 4.65 (4 H, br m, 13-$CH_2CH_2CO_2Me$, CH_2CH_2Cl), 5.15 (4 H, m, 17-$CH_2CH_2CO_2Me$, $CO_2CH_2CH_3$), and 10.90, 11.32, 11.70, 11.73, 11.77 (each 1 H, methine protons, CHO).

Additional product was obtained by stripping the chromatographic column with chloroform–methanol (1:1) to give material which was esterified with diazomethane and combined with mother-liquors from the main product and minor fractions from the first column. Rechromatography of this material (10 g; silica) gave the formylporphyrin (34 mg).

Dimethyl 3-*Carboxy*-18-*formyl*-2,7,12-*trimethyl*-8-*vinylporphyrin*-13,17-*dipropionate* (**2g**).—A mixture of the foregoing aldehyde (640 mg), toluene-*p*-sulphonic acid (300 mg), ethanediol (3 g), and chloroform (150 ml) was boiled gently for 10 min. Aqueous ammonia (2 ml) was added and the mixture shaken with water (800 ml). The water layer was extracted with chloroform (3 × 50 ml) and these washings and the main chloroform extract were evaporated. A solution of the dried (70 °C for 1 h) residue in pyridine (240 ml) was refluxed under nitrogen for 5 min, diluted carefully with water (40 ml) and, after a further 5 min under reflux, aqueous sodium hydroxide (3.5%, 50 ml) was added. The mixture was then heated for 2.5 h, aqueous acetic acid (30%; 50 ml) was added followed by more water (300 ml) and the mixture concentrated (to 100 ml). The porphyrin triacid was collected on Kieselguhr, the pad washed with water and dried (110 °C), and the porphyrin recovered by dissolution in methanolic sulphuric acid (3%; 400 ml). The filtered solution was kept at room temperature overnight, water (400 ml) was added, and the product extracted into chloroform (1 × 200 ml; 3 × 30 ml). Triethylamine (2 ml) was added to the combined extract which was washed with brine (700 ml) and filtered through Kieselguhr. The solution was then shaken with hydrochloric acid (6M; 50 ml) for 1 min and sufficient dilute aqueous ammonia was added to adjust the pH to *ca.* 2. Methanol (200 ml) was introduced, the mixture shaken again, and the chloroform layer separated; the aqueous phase was extracted with chloroform (3 × 30 ml). The combined chloroform solutions were again treated with triethylamine (1 ml), washed with brine (500 ml), and run through a small cellulose column. Elution was continued with chloroform and the eluted product was finally dissolved in a hot mixture of chloroform (30 ml) containing triethylamine (3 ml) and acetic acid (6 ml) was added whereupon the porphyrin acid (**2g**) (487 mg) separated, m.p. >330 °C; δ ($CDCl_3$ + trace CF_3CO_2D; 400 MHz) 3.16 (2 H, t), 3.30 (2 H, t) (2 × $CH_2CH_2CO_2CH_3$), 3.50 (3 H) and 3.57

* It is important to avoid forming the porphyrin dication which does not dissolve readily in chloroform.

† A quantity of porphyrin remained in the aqueous layer so sodium periodate in water (10%; 5 ml) was added to convert the glycol into aldehyde. This was extracted into chloroform (1 × 100 ml; 3 × 50 ml) after the addition of acetic acid and was combined at the appropriate stage with the main chloroform extract.

(3 H) (2 × $CH_2CH_2CO_2CH_3$), 3.59 (3 H), 3.71 (3 H) (2 × ring Me), 4.03 (3 H, 2-Me), 4.41 (2 H, t) and 4.79 (2 H, t) (2 × $CH_2CH_2CO_2Me$), 6.31 (1 H, d) and 6.48 (1 H, d) ($CH{=}CH_2$), 8.09 (1 H, dd, $CH{=}CH_2$), and 10.49, 11.15, 11.49, 11.55, 11.59 (each 1 H, 4 × methine protons; CHO). The acid was used without further purification; for characterisation it was converted into the trimethyl ester (**2d**) with ethereal diazomethane. This product was identical (t.l.c., m.p., mixed m.p., n.m.r.) with the material obtained earlier.[17]

Dimethyl 18-Formyl-2,7,12-trimethyl-3-[(4E,8E)-5,9,13-trimethyltetradeca-4,8,12-trienoyl]-8-vinylporphyrin-13,17-dipropionate (*Porphyrin* a *Ketone Dimethyl Ester*) (**2j**).—A solution of isopropylmagnesium bromide [prepared under nitrogen from isopropyl bromide (0.82 g) and magnesium (200 mg) in anhydrous tetrahydrofuran (7 ml)] was added *via* a syringe to a stirred solution (5 °C) of monomethyl (*E,E*)-farnesylmalonate[19] (1.07 g) in anhydrous tetrahydrofuran (2 ml) under nitrogen. The mixture was then heated under reflux for 10 min and cooled.

A mixture of oxalyl chloride (0.7 g) and dimethyl 3-carboxy-18-formyl-2,7,12-trimethyl-8-vinylporphyrin-13,17-dipropionate (100 mg) was warmed at 60 °C until a sample, when treated with methanol, indicated that acid chloride formation was complete (t.l.c.; *ca.* 45 min). An excess of oxalyl chloride was removed at room temperature (first at 30 Torr, finally at 0.5 Torr) and the residue treated with the solution of magnesium chelate prepared above. **All operations beyond this point were carried out with maximum exclusion of light.** The mixture was swirled until all the porphyrin had dissolved whereupon it was heated on the steam-bath for 1 min, cooled (5 °C), treated with trifluoroacetic acid (2 ml) and partitioned between chloroform (100 ml) and dilute brine (700 ml). The aqueous layer was separated and extracted with chloroform (3 × 30 ml). The combined organic solutions were washed with dilute brine (700 ml), the solvent removed, and the residue was dried azeotropically by evaporation of benzene (2 × 100 ml) followed by light petroleum (200 ml) and was then treated with fresh light petroleum (100 ml) and Kieselguhr. The mixture was swirled while it was cooled in a dry ice–acetone bath and then the solids were collected, washed with cold (0 °C) light petroleum (75 ml) and the porphyrin was dissolved in chloroform. The light petroleum filtrate was diluted with an equal volume of ether and washed with aqueous potassium hydroxide solution (0.7%; 30 ml) and dilute brine (2 × 400 ml) and evaporated. The residue was combined with that from the earlier solution in chloroform and chromatographed on silica (10 g) to yield the β-oxoester (**2i**) in two fractions, more and less polar. The former had the expected visible spectrum whereas that of the latter suggested that the formyl group had been affected, possibly by the formation of some unstable adduct. Fortunately, the less polar product was slowly converted into the more polar one during work-up.

The residues from both fractions were refluxed under nitrogen in dry pyridine (15 ml) containing lithium iodide (400 mg) for 60 h. Acetic acid (1 ml) was added to the cooled mixture which was partitioned between dilute brine (300 ml) and chloroform (50 ml). The aqueous phase was separated and extracted with chloroform (2 × 25 ml) and pyridine–chloroform (1:4; 2 × 20 ml). The organic extracts were combined and evaporated, the last traces of pyridine being removed by distillation with toluene. The residue was partly dissolved in chloroform–methanol (2:1; 150 ml) and treated with ethereal diazomethane; when dissolution was complete it was evaporated and the product chromatographed on silica (10 g) to give the *ketone* (53 mg). For analysis, this was rechromatographed (2 ×) and finally recrystallised from methylene dichloride–light petroleum to give a product, m.p. 114—116 °C, which resolidified and melted again at 120—122 °C (Found: C, 73.9; H, 7.5; N, 6.8. $C_{51}H_{60}N_4O_6$ requires C, 74.2; H, 7.3; N, 6.8%); $\lambda_{max.}$($CHCl_3$) (log ε) 432 (5.17), 523 (4.11), 562 (4.11), 591.5 (3.91), and 649 nm (3.49); δ ($CDCl_3$; 100 MHz) 1.58, 1.59, 1.65, 1.75 (each 3 H, 4 × side chain Me), *ca.* 2.1 (8 H, m, 4 × side chain CH_2), *ca.* 2.9 (8 H, m, $COCH_2CH_2CH{=}$, 2 × $CH_2CH_2CO_2Me$), 3.32, 3.37, 3.38 (9 H, 3 × ring Me), 3.51, 3.56 (6 H, 2 × CO_2CH_3), *ca.* 4.1 (4 H, br m, 2 × $CH_2CH_2CO_2Me$), 5.15 (2 H, br m, 2 × side chain CH=), 5.50 (1 H, br m, side chain CH=), 6.25 (2 H, m, $CH{=}CH_2$), 8.00 (1 H, m, $CH{=}CH_2$), 8.90, 9.26, 9.60, 9.80 (each 1 H, methine protons), and 10.96 (1 H, CHO).

Dimethyl 18-Formyl-2,7,12-trimethyl-3[(4E,8E)-1-hydroxy-5,9,13-trimethyltetradeca-4,8,12-trienyl]-8-vinylporphyrin-13,17-dipropionate (*Porphyrin* a *Dimethyl Ester*) (**2b**).—A mixture of chloroform (10 ml), methanol (5 ml), toluene-*p*-sulphonic acid (18 mg) and porphyrin *a* ketone dimethyl ester (19 mg) was allowed to stand at room temperature for 5 min and then treated with a solution of sodium borohydride (50 mg) in absolute ethanol (3.5 ml). After an additional 5 min at room temperature, more chloroform (50 ml) was added and the mixture shaken with hydrochloric acid (0.01M; 800 ml) to decompose the excess of borohydride; the aqueous phase was then separated and extracted with chloroform (3 × 30 ml). All chloroform solutions were combined and shaken vigorously for 1 min with hydrochloric acid (6M; 50 ml) to hydrolyse the acetal function. Aqueous ammonia (0.7M; 600 ml) was added, the aqueous phase extracted with chloroform (2 × 30 ml), and the combined extracts were treated with diazomethane. The product was purified by chromatography on silica and by h.p.l.c. (C_{18} Bondapak, acetonitrile) to give *porphyrin* a *dimethyl ester* (14 mg), m.p. 124—125 °C from methylene dichloride–light petroleum (Found: C, 74.1; H, 7.5; N, 6.9%; *m/z* 826.4666. $C_{51}H_{62}N_4O_6$ requires C, 74.1; H, 7.6; N, 6.8%; *m/z* 826.4669); $\lambda_{max.}$(log ε) 417 (5.24), 520 (3.95), 562 (4.31), 585 (4.10), and 645 nm (3.28); δ (400 MHz; CD_2Cl_2) 11.37 (1 H, s, CHO), 10.59, 10.36, 9.96, 9.62 (each1 H, s, methines), 8.26 (1 H, dd, $CH{=}CH_2$), 6.39 and 6.24 (each 1 H, dd, $CH{=}CH_2$), *ca.* 5.10 (3 H, m, side-chain CH=C), *ca.* 4.2—4.4 (4 H, m, $CH_2CH_2CO_2Me$), 3.71, 3.64, 3.63, 3.61, 3.59 (each 3 H, s, 2 × CO_2Me and 3 × ring Me), 3.12—3.24 and 2.31—2.73 (8 H, m, side-chain CH_2), 1.60 (3 H), 1.57 (3 H), and 1.55 (6 H) (s, side-chain Me).

Comparison of Synthetic and Natural Samples of Porphyrin a *Dimethyl Ester* (**2b**) *and its O-Acetyl Derivative* (**2c**).—(*a*) *Spectroscopy and h.p.l.c.* Synthetic and natural samples of porphyrin *a* dimethyl ester were compared separately by h.p.l.c. using two C_{18} Bondapak analytical columns in series with acetonitrile at 2.5 ml min^{-1}; they showed identical retention times. A 1:1 mixture of the two samples was run on the same columns this time with acetonitrile–water (9:1) and after five recycles a single symmetrical peak was still observed. The two samples were also compared by u.v.-visible i.r., n.m.r., and mass spectroscopy and gave identical spectra in each case.

The n.m.r. comparison by stepwise addition of shift reagent [Eu($[^2H_9]$-fod)$_3$] was carried out in CD_2Cl_2 at 270 MHz. The signals were shifted in the expected way and, importantly, those which were well separated throughout from other signals (from CHO, methines, and $CH{=}CH_2$) did not show any appearance of new signals.

The natural and synthetic *O*-acetyl derivatives were compared in the same ways by all the above methods, except the n.m.r. shift study, and were identical.

(*b*) *Fusion with resorcinol.* Natural porphyrin *a* dimethyl ester (0.74 mg) in dimethylformamide (3 ml) was heated under reflux for 1 min, then treated with iron(II) chloride (1.03 mg) and heated for 30 min. The cooled mixture was partitioned between ether (30 ml) and water (25 ml), the organic layer was washed with water (3 × 25 ml) and evaporated to give haem *a* dimethyl

ester (0.69 mg); h.p.l.c. on C_{18} Bondapak in methanol showed >95% purity.

A mixture of this product with resorcinol (500 mg) was heated at 205 °C for 20 min and the cooled melt in ether (50 ml) was washed with 1% hydrochloric acid (2 × 25 ml) and water (3 × 30 ml). The product from the ether in glacial acetic acid (10 ml) was purged with carbon dioxide for 10 min and treated with iron(II) sulphate heptahydrate (13 mg) in 'AnalaR' concentrated hydrochloric acid (0.4 ml). After carbon dioxide had been passed through for 5 min, the solution was partitioned between ether (30 ml) and 12.2M-aqueous sodium acetate (25 ml) and the water-washed ether layer was evaporated. The residue by p.l.c. on silica using dichloromethane–ether (9:1) gave cytodeuteroporphyrin dimethyl ester (**2a**) (0.086 mg) (Found: *m/z* 524.2391. $C_{31}H_{32}N_4O_4$ requires *m/z* 524.2422). H.p.l.c. on 2 × CN Bondapak analytical columns in series with hexane–toluene–acetonitrile (74:20:6) at 1 ml min^{-1} showed >97% purity. Co-injection with a synthetic sample[2] gave one peak after three recycles.

The synthetic porphyrin *a* dimethyl ester was converted as above into the corresponding haem *a* ester (0.205 mg) and was heated with resorcinol (210 mg) and worked up as earlier. The cytodeuteroporphyrin dimethyl ester (0.01 mg) so obtained was identical by h.p.l.c. to the foregoing samples using the same conditions as above.

Synthesis of Isoporphyrin a *Dimethyl Ester*

Dimethyl 12-Acetyl-8-ethoxycarbonyl-3-(2-hydroxyethyl)-2,7,18-trimethylporphyrin-13,17-dipropionate (**10b**).—4′-Acetyl-3,3′-bis(2-methoxycarbonylethyl)-4,5′-dimethyl-2,2′-methylenedipyrrole-5-carboxylic acid[19] (2 g; obtained from the benzyl ester by hydrogenolysis) was condensed with 3-(2-acetoxyethyl)-4′-ethoxycarbonyl-4,3′,5′-trimethyl-2,2′-methylenedipyrrole-5-carbaldehyde[21] (1.73 g) as for compound (**8a**) above. The resultant bilene was cyclised with a mixture of copper(II) acetate (19 g) and sodium acetate (9.5 g) in acetic acid–methanol (1950 ml; 1:1) and the product was worked up as earlier to give the *title porphyrin* (990 mg), m.p. 206—208 °C (from chloroform–methanol) (Found: C, 66.5; H, 6.0; N, 8.3. $C_{38}H_{42}N_4O_8$ requires C, 66.8; H, 6.2; N, 8.2%); $\lambda_{max.}$(log ε) 419.5 (5.29), 512.5 (4.13), 550 (3.96), 584.5 (3.82), and 638 nm (3.65); δ ($CDCl_3$; 100 MHz) 1.77 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 2.97 (4 H, br t, 2 × $CH_2CH_2CO_2Me$), 3.25 (6 H), 3.27 (3 H) and 3.43 (3 H) (3 × ring Me, $COCH_3$), 3.61 (6 H, 2 × CO_2Me), *ca.* 3.8 (2 H, m, $CH_2CH_2CO_2Me$), *ca.* 4.1 (6 H, $CH_2CH_2CO_2Me$, CH_2CH_2OH), 4.84 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), and 9.12 (2 H), 9.34 (1 H), and 10.66 (1 H) (methine protons).

Dimethyl 3-(2-Chloroethyl)-8-ethoxycarbonyl-2,7,18-trimethyl-12-vinylporphyrin-13,17-dipropionate (**10e**).—The foregoing porphyrin (500 mg) was reduced with sodium borohydride (300 mg) as for compound (**8c**) and the resultant 12-(1-hydroxyethyl)porphyrin was dehydrated by being heated with a mixture of dimethylformamide (75 ml) and benzoyl chloride (3.75 ml). Isolation of the product in the usual fashion gave the *porphyrin* (430 mg) from chloroform–methanol, which melted at 170 °C, resolidified and melted again at 195—197 °C (Found: 66.8; H, 6.1; N, 8.3. $C_{38}H_{41}ClN_4O_6$ requires C, 66.6; H, 6.0; N, 8.2%); $\lambda_{max.}$(log ε) 413 (5.28), 510 (4.13), 547 (4.02), 579 (3.87), and 634 nm (3.56); δ ($CDCl_3$) 1.83 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 3.15 (4 H, br t, partly obscured by Me peak, 2 × $CH_2CH_2CO_2Me$), 3.03 (3 H), 3.27 (3 H), and 3.41 (3 H) (ring Me), 3.62 (3 H) and 3.67 (3 H) (2 × CO_2Me), *ca.* 3.7 (4 H, m, partly obscured by Me peaks, CH_2CH_2Cl), 4.02 (2 H, br t), 4.21 (2 H, br t, 2 × $CH_2CH_2CO_2Me$), 4.88 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 6.35 (2 H, m, $CH{=}CH_2$), 8.00 (1 H, m, $CH{=}CH_2$), and 8.76, 9.13, 9.36, 10.77 (each 1 H, methine protons).

Dimethyl 3-(2-Chloroethyl)-8-ethoxycarbonyl-12-formyl-2,7,18-trimethylporphyrin-13,17-dipropionate (**10f**).—Oxidation of the foregoing vinylporphyrin (200 mg) by successive treatment with osmium tetraoxide in pyridine and sodium periodate, as outlined for the porphyrin (**8d**), yielded the *formylporphyrin* (143 mg), m.p. 241—242 °C (from chloroform–methanol containing a drop of triethylamine) (Found: C, 64.9; H, 5.9; N, 7.9. $C_{37}H_{39}ClN_4O_7$ requires C, 64.7; H, 5.7; N, 8.1%); $\lambda_{max.}$(log ε) 426 (5.30), 518 (4.08), 559 (4.05), 589 (3.87), and 645 nm (3.73); δ ($CDCl_3$; 100 MHz) 1.92 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), *ca.* 3.0 (4 H, m, 2 × $CH_2CH_2CO_2Me$), 3.32, 3.45, 3.52 (each 3 H, 3 × ring Me), 3.49 (3 H) and 3.59 (3 H) (2 × CO_2Me), *ca.* 4.0 (8 H, m, 2 × $CH_2CH_2CO_2Me$, CH_2CH_2Cl) 4.97 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 8.98, 9.06, 9.38, 10.55 (each 1 H, methine protons), and 11.09 (1 H, CHO).

Dimethyl 8-Carboxy-12-formyl-2,7,18-trimethyl-3-vinylporphyrin-13,17-dipropionate (**10c**).—The foregoing aldehyde (230 mg) as its acetal with ethanediol was refluxed with sodium hydroxide in aqueous pyridine as described for compound (**2g**) above. After treatment with methanolic sulphuric acid, the vinylporphyrin acid (178 mg) was obtained by acidification (acetic acid) of a solution of the product in chloroform–methanol–triethylamine, m.p. >330 °C; δ (CF_3CO_2D; 100 MHz) 3.27, 3.40 (4 H, overlapping t, 2 × $CH_2CH_2CO_2Me$), 3.73 (3 H), 3.77 (3 H), 3.79 (6 H), and 4.10 (3 H) (3 × ring Me, 2 × CO_2CH_3), 4.68 (2 H, br t, 17-$CH_2CH_2CO_2Me$), 5.07 (2 H, br t, 13-$CH_2CH_2CO_2Me$), 6.50 (2 H, m, $CH{=}CH_2$), 8.22 (1 H, m, $CH{=}CH_2$), 10.98, 11.08, 11.34, 11.75 (each 1 H, methine protons), and 12.36 (1 H, CHO).

This acid was not easy to purify (owing to insolubility) but the material obtained above was sufficiently pure for subsequent operations. For characterisation, it was converted as described below into the methyl ester.

Dimethyl 12-Formyl-8-methoxycarbonyl-2,7,18-trimethyl-3-vinylporphyrin-13,17-dipropionate (**10d**).—A solution of the foregoing acid (50 mg) in dioxane was esterified with ethereal diazomethane. The product was chromatographed on silica and then recrystallised from chloroform–methanol to give the *methyl ester* (40 mg), m.p. 219—221 °C (Found: C, 67.7; H, 5.5; N, 8.5. $C_{36}H_{36}N_4O_7$ requires C, 67.9; H, 5.7; N, 8.8%); $\lambda_{max.}$(log ε) 428 (5.28), 521.5 (4.08), 563 (4.10), 592.5 (3.88), and 649 nm (3.73); δ ($CDCl_3$; 100 MHz) 2.78 (3 H, ring Me), 2.95 (4 H, t, 2 × $CH_2CH_2CO_2Me$), 3.08 (3 H) and 3.21 (3 H) (2 × ring Me), 3.53 (3 H) and 3.61 (3 H) (2 × $CH_2CH_2CO_2CH_3$), 3.90, 4.03 (4 H, overlapping t, 2 × $CH_2CH_2CO_2Me$), 4.34 (3 H, ring CO_2Me), 5.80 (2 H, m, $CH{=}CH_2$), 7.15 (1 H, m, $CH{=}CH_2$), 8.03, 8.75, 8.81, 9.84 (each 1 H, methine protons), and 10.97 (1 H, CHO).

Dimethyl 12-Formyl-2,17,18-trimethyl-8-[(4E,8E)-5,9,13-trimethyltetradeca-4,8,12-trienoyl]-3-vinylporphyrin-13,17-dipropionate (**10g**).—The acid chloride obtained from the acid (**10c**) (100 mg) was treated with the magnesium chelate derived from monomethyl (*E,E*)-farnesylmalonate[19] (1.07 g) all as described for synthesis of the isomer (**2j**). **In the absence of direct light,** the sequence was continued as already described for the isomeric system and the intermediate β-oxoester was cleaved with lithium iodide in pyridine to yield the *ketone* (30 mg), after purification by chromatography on silica (10 g), m.p. 93—95 °C (Found: C, 74.4; H, 7.4; N, 6.8. $C_{51}H_{60}N_4O_6$ requires C, 74.2; H, 7.3; N, 6.8%); $\lambda_{max.}$($CHCl_3$) (log ε) 428.5 (5.24), 522 (4.07), 563 (4.11), 592 (3.82), and 649 nm (3.71); δ ($CDCl_3$; 100 MHz) 1.55 (6 H), 1.60 (3 H), and 1.71 (3 H) (4 × side chain Me), 2.00 (8 H, m, 4 × side

chain CH_2), 2.80 (2 H, m, $COCH_2CH_2CH$=), 3.00 (6 H, m, 2 × $CH_2CH_2CO_2Me$, $COCH_2CH_2CH$=), 3.24 (6 H) and 3.28 (3 H) (3 × ring Me), 3.52 (3 H) and 3.57 (3 H) (2 × CO_2Me), 3.97 (2 H, br t, 17-$CH_2CH_2CO_2Me$), 4.21 (2 H, br t, 13-$CH_2CH_2CO_2Me$), 5.10 (2 H, m, 2 × side chain =CH), 5.43 (1 H, br t, side chain =CH), 6.05 (2 H, m, $CH=CH_2$), 7.60 (1 H, m, $CH=CH_2$), 8.88 (1 H), 9.07 (2 H), and 10.17 (1 H) (methine protons), and 11.25 (1 H, CHO).

Dimethyl 12-Formyl-8-[(4E,8E)-1-hydroxy-5,9,13-trimethyltetradeca-4,8,12-trienyl]-2,17,18-trimethyl-3-vinylporphyrin-13,17-dipropionate (*Isoporphyrin* a *Dimethyl Ester*) (**10a**).—The foregoing aldehyde (**10g**) (42 mg) was protected as its dimethyl acetal, reduced with sodium borohydride, and deprotected as described for porphyrin *a* dimethyl ester (**2b**). The alcohol was purified by chromatography on silica (8 g) and recrystallisation from methylene dichloride–light petroleum to give *isoporphyrin* a *dimethyl ester* (32 mg), m.p. 145—148 °C (from ether–hexane); the crystals had m.p. 157—158 °C (Found: C, 74.2, 74.1; H, 7.7, 7.6; N, 6.8, 6.8. $C_{51}H_{62}N_4O_6$ requires C, 74.1; H, 7.5; N, 6.8%); $\lambda_{max.}$(log ε) 417 (5.27), 519 (3.95), 563 (4.32), 584 (4.10), and 644 nm (3.33); δ ($CDCl_3$; 100 MHz) 1.43 (3 H), 1.52 (6 H), and 1.59 (3 H) (4 × side chain Me), 1.95 (8 H, m, 4 × side chain CH_2), 2.20 (2 H, m, $CHOHCH_2$), 2.94 (2 H, t) and 3.00 (2 H, t) (2 × $CH_2CH_2CO_2Me$), 3.33 (3 H), 3.42 (3 H), and 3.48 (3 H) (3 × ring Me), 3.54 (3 H) and 3.58 (3 H) (2 × CO_2Me), 4.02 (4 H, br m, 2 × $CH_2CH_2CO_2Me$), 5.1 (3 H, m, side chain CH=), 6.16 (3 H, m, $CHOHCH_2CH_2$, $CH=CH_2$), 7.98 (1 H, m, $CH=CH_2$), 9.00, 9.31, 9.55, 10.34 (each 1 H, methine protons), and 11.14 (1 H, CHO).

Comparison of the Dimethyl Esters of Porphyrin a (**2b**) *and Isoporphyrin* a (**10a**).—(*a*) A mixture of the two esters (**2b**) and (**10a**) was completely separated after one recycle through two μCN Bondapak columns at 1 ml min^{-1} with hexane–toluene–acetonitrile (200:50:7).

(*b*) Isoporphyrin *a* ester (0.43 mg) in pyridine (0.4 ml) and acetic anhydride (0.2 ml) was kept at 23 °C for 48 h and the solution was then evaporated. The residue was chromatographed first in benzene and then with chloroform on neutral alumina (5 g, Grade III). The porphyrin fraction on evaporation afforded O-*acetyl isoporphyrin* a *dimethyl ester* (0.28 mg) (Found: *m/z* 868.4781. $C_{53}H_{64}N_4O_7$ requires *m/z* 868.4775); $\lambda_{max.}$(ether) 411, 513, 556, 581, and 642 nm; ratio bands III/IV 2.01; $\nu_{max.}$(CH_2Cl_2) 1 663, 1 725, 2 910, and 3 300 cm^{-1}.

This product was mixed with *O*-acetylporphyrin *a* dimethyl ester (**2c**) and the two were completely separated by one passage using exactly the conditions given under section (*a*).

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