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PYRROLES AND RELATED COMPOUNDS—XXXVII[1]

HARDEROPORPHYRIN[2]

ANTHONY H. JACKSON,† GEORGE W. KENNER,* KEVIN M. SMITH
and COLIN J. SUCKLING
The Robert Robinson Laboratories, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, England

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Abstract—Harderoporphyrin, the tricarboxylic acid porphyrin present in the Harderian glands of the rat and hamster is shown to be 4,6,7-tri(2-carboxyethyl)-1,3,5,8-tetramethyl-2-vinylporphin by its ring synthesis via the *b*-oxobilane route. The isomeric 4-vinylporphyrin tricarboxylic acid is also prepared by a similar approach.

Although metal complexes of porphyrins (e.g. haems and chlorophylls) are ubiquitous in Nature, the porphyrins themselves, which are their biosynthetic precursors, are usually found in only small quantities (except in rare disorders of porphyrin metabolism such as the porphyrias). However, the Harderian gland of the rat, an organ found at the rear of the eye, is distinguished by its relatively high content of metal-free porphyrin under normal conditions, as shown dramatically by its brilliant orange fluorescence under UV light.[cf3] The Harderian gland is also a characteristic feature of animals possessing a *membrana nictitans*, or third eyelid, and some (e.g. deer) but not all of these animals have glands containing porphyrin.

The major porphyrin present in the rat Harderian gland has long been recognised[cf3] as protoporphyrin-IX, but more recently, Kennedy showed[4] the presence of a tricarboxylic acid porphyrin which we have subsequently named *harderoporphyrin*.[2] This finding was clearly of considerable interest in relation to porphyrin biosynthesis, because harderoporphyrin is probably derived from a precursor of protoporphyrin-IX. Moreover, evidence has been accumulated to show that the porphyrins are synthesised in the gland itself, but that they do not enter the bloodstream of the animal, nor is haem present in the gland.[5] One reason for the accumulation of metal-free porphyrins, rather than haems, in the Harderian gland is presumably the lack of an iron chelating enzyme; the role of the porphyrins, and indeed, the precise function of the gland itself, is as yet uncertain.[1]

Part XXXII[6] of this Series reported evidence from biological feeding experiments which showed that the tripropionic porphyrinogen which is an intermediate in protoporphyrin-IX metabolism in *Euglena gracilis* is that derived from 4(2-carboxyethyl)-2-vinyldeuteroporphyrin-IX; the same result has also been achieved for avian and mammalian haem metabolism.[7] It was thus important to determine the precise structure of harderoporphyrin, and Dr. Kennedy kindly supplied us with a crude mixture of porphyrin methyl esters which he had obtained by extraction of the glands of normal rats, using methanol containing 5% sulphuric acid. In his earlier experiments, Kennedy had separated the esters of harderoporphyrin and protoporphyrin-IX by chromatography, and shown both by paper and thin layer chromatography that harderoporphyrin was probably a tricarboxylic acid.[2,3] By a combination of chemical and spectrophotometric methods, Kennedy also showed that the porphyrin contained one vinyl group, but no hydroxyl, aldehyde, or ketone functions, and that none of the carboxylic acid groups was attached to the nucleus.[2,3]

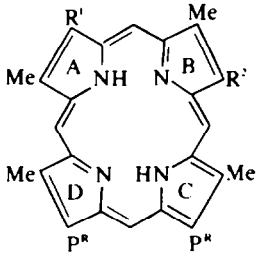
In the present work the mixture of porphyrin esters was hydrolysed to the free acids and the latter were separated by countercurrent distribution between methyl isobutyl ketone–t-butyl alcohol and dilute sulphuric acid.[cf8] Three porphyrinic fractions were obtained and each was re-esterified in methanol–sulphuric acid. The first fraction (64%) was identified as protoporphyrin-IX by comparison with authentic material (countercurrent distribution and mixed m.ps of dimethyl esters); the third fraction (9%) was tentatively identified as coproporphyrin-III by its position in the distribution (insufficient material was available for m.p. comparisons).

The middle fraction (29%) was clearly a tricarboxylic acid porphyrin from its position in the distribution, and was apparently identical with the harderoporphyrin isolated earlier by Kennedy. The mass spectrum of the trimethyl ester showed a parent ion at *m/e* 650 and successive fragmentations corresponding to loss of $CH_2CO_2CH_3$ from three propionic ester side-chains.[cf9] On the basis of this evidence and on the assumption that harderoporphyrin was closely related to protoporphyrin-IX (**1a**), it was assigned structure **1b** or **1c**.

In principle it might have been possible to compare dihydroharderoporphyrin with the known[8] monoethyl-porphyrin tricarboxylic acid (**1d**). However, relatively little harderoporphyrin was available, and bearing in mind the well known tendency of porphyrin esters to exhibit polymorphism, we felt that a rigorous proof of structure required the synthesis of both **1b** and **1c** as their esters (**2b** and **2c**) respectively, and direct comparison of these with the natural material.

The two isomers (**1b** and **1c**) differ only in the interchange of a vinyl group and a propionic acid side-chain in rings A and B and in principle it seemed possible to synthesise the trimethyl esters (**2b** and **2c**) from the same pyrrolic precursors, by a simple extension of the methods we had earlier developed for the synthesis of protoporphyrin-IX dimethyl ester.[10] Thus, the *b*-oxobilane route[10a] could be used for **1b** and the *a*-oxobilane route[10b] for **1c**, and this strategy would minimise the labour of preparing the required synthetic intermediates. Accordingly, for the synthesis of **2b** we attempted the preparation of the pyrromethane amide (**5a**) from the 2-acetoxymethylpyrrole (**3**) and pyrrole amide (**4c**) in acetic acid containing a catalytic quantity of toluene *p*-sulphonic acid hydrate, and a good yield of the required

†Present address: Department of Chemistry, University College, Cardiff, Wales.



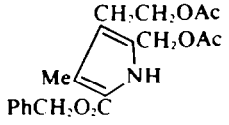
1: R = H 2: R = Me

a: $R^1 = R^2 = V$ **e**: $R^1 = H$; $R^2 = V$
b: $R^1 = V$; $R^2 = P^R$ **f**: $R^1 = CHO$; $R^2 = V$
c: $R^1 = P^R$; $R^2 = V$ **g**: $R^1 = V$; $R^2 = CHO$
d: $R^1 = Et$; $R^2 = P^R$

$P^R = CH_2CH_2CO_2R$ $V = CH{=}CH_2$

immediately with trimethylorthoformate in methylene chloride containing trichloroacetic acid. Addition of pyridine, followed by aerial oxidation then afforded the olive green oxophlorin salt (**9a**), which was not isolated but was converted directly into the violet-red *meso*-acetoxyporphyrin (**10a**) by treatment with acetic anhydride. Hydrogenation of the acetoxyporphyrin followed by reoxidation of the intermediate porphyrinogen with 2,3-dichloro-5,6-dicyanobenzoquinone then afforded the required *meso*-unsubstituted porphyrin (**11a**) in 83% yield from (**10a**).

Transformation of the side-chain acetoxyethyl function into vinyl was effected by the same method[10c] as had been developed in the synthesis of protoporphyrin-IX, i.e. methanolysis of the acetoxyethyl- to the hydroxyethylporphyrin (**12a**), conversion into the chloroethylporphyrin



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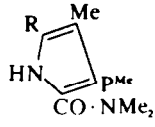
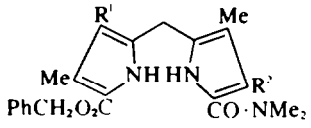


a: R = CO_2CH_2Ph
b: R = I
c: R = H

4



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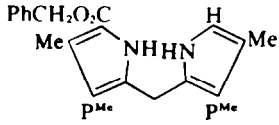

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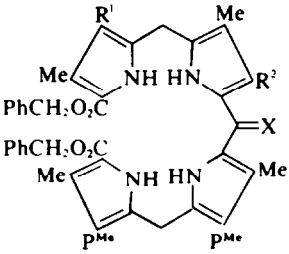
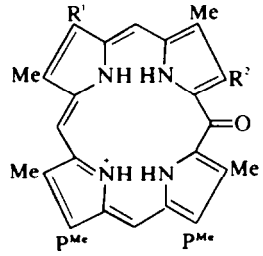




7: X = $\dot{N}Me_2$ 8: X = O 9

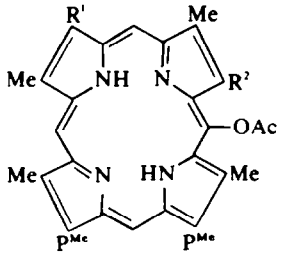
a: $R^1 = CH_2CH_2OAc$; $R^2 = P^{Me}$
b: $R^1 = P^{Me}$; $R^2 = CH_2CH_2OAc$

pyrromethane (**5a**) was obtained. The 2-unsubstituted pyrrole (**4c**) required for this synthesis was prepared from the corresponding benzyl ester (**4a**) by hydrogenolysis of the benzyl group followed by iodinative decarboxylation of the intermediate carboxylic acid and platinum catalysed hydrogenolysis of the iodopyrrole (**4b**).

The phosphoryl chloride complex of the pyrromethane amide (**5a**) was then condensed with the 5-unsubstituted pyrromethane (**6**), and the resultant imine salt intermediate (**7a**) was purified by column chromatography before alkaline hydrolysis to the desired *b*-oxobilane (**8a**). The last compound was not obtained in crystalline form, but it was homogeneous by TLC and its structure was confirmed by NMR and UV spectrometry. Catalytic hydrogenolysis of the benzyl esters gave the corresponding *b*-oxobilane-1′,8′-dicarboxylic acid, which was cyclised (**13a**) with thionyl chloride in dimethylformamide, and elimination of hydrogen chloride from the zinc(II) complex of the latter by treatment with t-butoxide, to give the vinylporphyrin dimethyl ester (**2b**) after methanolsyis.

The synthesis of the isomeric porphyrin (**2c**) by the *a*-oxobilane route, however, ran into unexpected difficulties. The initial pyrroketone (**14a**) was readily prepared by coupling the phosphoryl chloride complex of the pyrrole amide (**4a**) with the 2-unsubstituted pyrrole (**15**) followed by hydrolysis of the intermediate imine salt. However, attempted chlorination of the 5-Me group of this pyrroketone with t-butyl hypochlorite failed to afford a crystalline chloromethyl derivative (**14b**) in spite of repeated attempts; this route was therefore abandoned in favour of the *b*-oxobilane method.

Thus, the pyrrole amide (**17c**) was prepared in a similar



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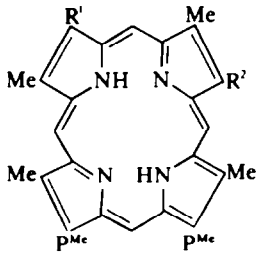
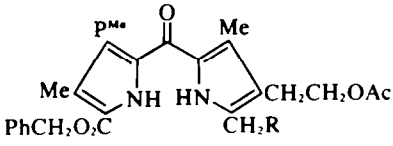
a: $R^1 = CH_2CH_2OAc$; $R^2 = P^{Me}$
b: $R^1 = P^{Me}$; $R^2 = CH_2CH_2OAc$



11a: $R^1 = CH_2CH_2OAc$; $R^2 = P^{Me}$
b: $R^1 = P^{Me}$; $R^2 = CH_2CH_2OAc$
12a: $R^1 = CH_2CH_2OH$; $R^2 = P^{Me}$
b: $R^1 = P^{Me}$; $R^2 = CH_2CH_2OH$
13a: $R^1 = CH_2CH_2Cl$; $R^2 = P^{Me}$
b: $R^1 = P^{Me}$; $R^2 = CH_2CH_2Cl$



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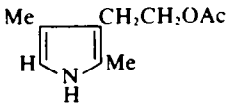
a: R = H
b: R = Cl



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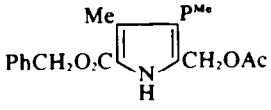

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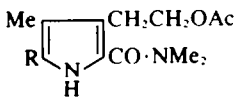

17

a: $R = CO_2CH_2Ph$
b: R = I
c: R = H

manner to its analogue (**4c**) from the benzyl ester (**17a**) via the iodopyrrole (**17b**); when coupled with the acetoxymethylpyrrole (**16**) using MacDonald's conditions[11] it afforded the required pyrromethane amide (**5b**). Thereafter, the synthesis proceeded in an analogous manner to that described above, with the exception that the porphyrinogen obtained by hydrogenation of the *meso*-acetoxyporphyrin (**10b**) was oxidised to the *meso*-unsubstituted porphyrin (**11b**) with air, and gave a rather lower yield than the newer method[12] using DDQ.

At this point we were in a position to compare the two synthetic esters (**2b** and **2c**) with the trimethyl ester of harderoporphyrin, and accordingly, small quantities of all three esters were crystallised to constant m.p. in an identical manner from methylene chloride–light petroleum. The synthetic 2-vinylporphyrin (**2b**; m.p. 177–178°) showed hardly any m.p. depression upon admixture with the ester (m.p. 179°) of the natural product, but admixture of the 4-vinylporphyrin (**2c**; m.p. 176–178°) with the trimethyl ester of the natural product depressed the m.p. to 138–164°. This series of observations therefore appeared to prove that harderoporphyrin is 4-(2-carboxyethyl)-2-vinyl-deuteroporpyrin-IX (**1b**). However, the acceptability of this conclusion was thrown into doubt when syntheses of **2b** and **2c** by a totally different route gave compounds which possessed m.ps somewhat higher than those reported above, viz. **2b** 203–204° and **2c** 216–218°.[6] Since all of the original synthetic samples and all of the natural sample had been exhausted in the present study, complete repetition of the *b*-oxobilane syntheses of **2b** and **2c** gave samples which also possessed the higher m.ps (Experimental). It therefore remained to obtain a fresh sample of the natural product; though we considered the mixed m.p. behaviour with the now exhausted natural sample was virtually definitive, we felt that the existence of even the slightest degree of doubt regarding the identity of harderoporphyrin was intolerable.

Repeated attempts at isolation[13] of a sufficiently large sample of the tripropionic porphyrin from rat or hamster Harderian glands were unsuccessful, even over a period of 4 years. In all cases samples provided[13] to us showed evidence by high performance liquid chromatography (HPLC) of only traces of harderoporphyrin. The dimethyl ester (**2b**) of harderoporphyrin is readily separated[6,14] from the isomer (**2c**) by HPLC, and this indicated that isoharderoporphyrin was not formed. However, in view of the minute amounts of harderoporphyrin obtained we undertook a radioactive dilution analysis using a porphyrin extract obtained from hamster Harderian glands.

Thus, a sample of porphyrin methyl esters extracted from 82 female[15] Hamster glands (total porphyrin content = 6.5 mg by spectrophotometry; sample kindly provided by Dr. J. Mindegaard) was tritiated using hexapyridyl magnesium iodide in pyridine-tritiated water.[6,16] The sample was then re-esterified with 5% sulphuric acid in methanol and diluted with inactive protoporphyrin-IX dimethyl ester (6.7 mg), inactive synthetic **2b** (5.2 mg), and inactive synthetic **2c** (3.3 mg). Chromatography on

alumina efficiently separated the protoporphyrin-IX dimethyl ester from the tripropionic ester mixture; comparison of the molar activities of the two fractions indicated the presence of 3.7% of harderoporphyrin in the original extract from the gland (assuming only protoporphyrin-IX and harderoporphyrin to be present in the extract and also the susceptibility of each to tritiation to be identical). HPLC separation[cf.6] of the tripropionic fraction showed all of the activity to be located in the least mobile (i.e. **2b**) fraction.

The structure of harderoporphyrin is thus clearly defined by our studies as 4,6,7-tri(2-carboxyethyl)-1,3,5,8-tetramethyl-2-vinylporphin (**1b**), and it therefore seems likely that it is formed in Nature by dehydrogenation of an intermediate in the conversion of coproporphyrinogen-III into protoporphyrinogen-IX.

It has also been shown[6,7,17] that a tricarboxylic acid porphyrinogen is found in low concentration during the enzymic conversion of coproporphyrinogen-III into protoporphyrinogen-IX, and that the corresponding porphyrin is identical with harderoporphyrin[6,7] rather than its isomer. Evidence has also been obtained recently from countercurrent distribution and mass spectrometric studies that harderoporphyrin occurs in small amounts in both normal and variegate porphyric bile, and in bone marrow.[18] Thus, in accord with our previous suggestion,[2] it now seems certain that the 2-propionic acid group of coproporphyrinogen-III is preferentially attacked by coproporphyrinogen oxidase, and it is significant in this context that two other biologically important porphyrins, pemptoporphyrin (**1e**) and chlorocruoroporphyrin (**1f**) result from further degradation of the substituent at position-2. Recent studies in Cardiff provide strong support for this view and provide a partial explanation for the apparent specificity of coproporphyrinogen oxidase.[7] Syntheses from protoporphyrin-IX, of harderoporphyrin, pemptoporphyrin, chlorocruoroporphyrin, and their 2(4) isomers are described in the accompanying paper.[1]

EXPERIMENTAL

General conditions were as described in the preceding paper.[1]

Pyrroles

2 - *Iodo* - 4 - (2 - *methoxycarbonylethyl*) - 3 - *methyl* - 5 - *dimethylcarbamoylpyrrole* (**4b**). Benzyl 4 - (2 - methoxycarbonylethyl) - 3 - methyl - 5 - dimethylcarbamoylpyrrole - 2 - carboxylate[8] (8 g) in THF (250 ml) and triethylamine (0.2 ml) was hydrogenated over 10% Pd-C (1 g) during 2.5 hr at room temp. and atmospheric pressure, after which time the uptake of H_2 had ceased. The catalyst was removed by filtration through celite and the filtrate was evaporated to dryness *in vacuo*, the oily residue being taken up in MeOH (67.5 ml). A soln of $NaHCO_3$ (5 g) in water (46 ml) was added and the soln was heated to 70° before addition of a soln of I_2 (5.15 g) and KI (8.75 g) in water (20 ml) and MeOH (60 ml) to the stirred soln during 2 hr. The pyrrole was extracted into dichloromethane after the mixture had cooled and the organic phase (*ca* 150 ml) was washed with water before being dried ($MgSO_4$) and evaporated to dryness. Trituration of the resultant oil with petroleum ether gave the product, which was recrystallised from dichloromethane–light petroleum to give pale yellow prisms (5.4 g; 74%) with m.p. 159–160°. (Found: C, 39.7; H, 4.6; N, 7.4. $C_{12}H_{17}IN_2O_3$ requires: C, 39.6; H, 4.7; N, 7.7%). NMR spectrum, τ 0.38 (1H, s broad) NH, 6.31 (3H, s) OCH_3, 6.90 (6H, s) $N(CH_3)_2$, 6.9–7.5 (4H, m) CH_2CH_2, 8.0 (3H, s) β-CH_3.

4 - (2 - *Acetoxyethyl*) - 2 - *iodo* - 3 - *methyl* - 5 - *dimethylcarbamoylpyrrole*, (**17b**). This compound was prepared in an analogous manner to the pyrrole above, from benzyl 4 - (2 - acetoxyethyl) - 3 - methyl - 5 - dimethylcarbamoylpyrrole - 2 - carboxylate,[10c] in 80% yield, and was crystallised from dichloromethane–light petroleum to give pale yellow prisms, m.p. 133–134°. (Found: C, 39.9; H, 4.7; N, 7.5. $C_{12}H_{17}IN_2O_3$ requires: C, 39.6; H, 4.7; N, 7.7%). NMR spectrum, τ 0.58 (1H, s broad) NH, 5.95, 7.18 (both 2H, t) OCH_2CH_2, 6.93 (6H, s) $N(CH_3)_2$, 7.98 (3H, s) β-CH_3, and 8.0 (3H, s) $COCH_3$.

4 - (2 - *Acetoxyethyl*) - 3,5 - *dimethylpyrrole* - 2 - *carboxylic acid*. Benzyl 4 - (2 - acetoxyethyl) - 3,5 - dimethylpyrrole - 2 - carboxylate[10c] (6 g) in THF (200 ml) and triethylamine (0.2 ml) was hydrogenated over 10% Pd-C (600 mg) at atmospheric pressure and room temp. during 1.5 hr, after which time the uptake of H_2 had ceased. The catalyst was removed by filtration on a bed of celite, and the filtrate was evaporated to dryness *in vacuo* to give colourless crystals (4.2 g; 100%) with m.p. 181° after crystallisation from THF–light petroleum. (Found: C, 58.6; H, 6.6; N, 6.2. $C_{11}H_{15}NO_4$ requires: C, 58.7; H, 6.7; N, 6.2%). NMR spectrum in D_2O/NaOD, τ 6.27, 7.23 (both 2H, t) OCH_2CH_2, 7.55 (3H, s) α-CH_3, 7.69 (3H, s) β-CH_3, 7.86 (3H, s) $COCH_3$.

t - Butyl 5 - *acetoxymethyl* - 4 - (2 - *methoxycarbonylethyl*) - 3 - *methylpyrrole* - 2 - *carboxylate*. t-Butyl 4 - (2 - methoxycarbonylethyl) - 3,5 - dimethylpyrrole - 2 - carboxylate[19] (25 g) in glacial HOAc (500 ml) and Ac_2O (10 ml) was treated, during 2 hr, with small portions of lead tetra-acetate (44 g added in all). The stirred soln was then set aside overnight before dropwise addition of water (1 l.). The ppt was removed by filtration, and washed well with water before crystallisation from light petroleum, giving the required *acetoxymethylpyrrole* as colourless crystals (25.3 g; 98%) with m.p. 79–80°. (Found: C, 60.4; H, 7.5; N, 4.1. $C_{17}H_{25}NO_6$ requires: C, 60.2; H, 7.4; N, 4.1%). NMR spectrum, τ 0.62 (1H, s broad) NH, 4.93 (2H, s) CH_2O, 6.32 (3H, s) OCH_3, 7.0–7.65 (4H, m) CH_2CH_2, 7.74 (3H, s) β-CH_3, 7.95 (3H, s) $COCH_3$, 8.43 (9H, s) $C(CH_3)_3$.

Pyrromethanes

Benzyl 3 - (2 - *acetoxyethyl*) - 4' - (2 - *methoxycarbonylethyl*) - 5' - *dimethylcarbamoyl* - 3',4 - *dimethylpyrromethane* - 5 - *carboxylate* (**5a**). Benzyl 3 - (2 - acetoxyethyl) - 2 - acetoxymethyl - 4 - methylpyrrole - 5 - carboxylate (3.0 g; obtained from the corresponding 2-methylpyrrole with lead tetra-acetate) and 4(2 - methoxycarbonylethyl) - 3 - methyl - 5 - dimethylcarbamoylpyrrole (2.0 g; obtained by hydrogenation over Adams platinum oxide catalyst of 2 - iodo - 4 - (2 - methoxycarbonylethyl) - 3 - methyl - 5 - dimethylcarbamoylpyrrole in MeOH in the presence of NaOAc) were suspended in glacial AcOH (25 ml). After stirring and heating to 50°, toluene *p*-sulphonic acid hydrate (60 mg) was added and the soln was stirred at this temp. during 4 hr. The mixture was poured into water (500 ml) and the product was extracted into dichloromethane (500 ml) which was washed with water (500 ml), dried (Na_2SO_4) and then evaporated to dryness *in vacuo*. The required *pyrromethane* was crystallised from ether–n-hexane to give pale yellow prisms (3.9 g; 88%) with m.p. 158–159°. (Found: C, 65.6; H, 6.7; N, 7.7. $C_{30}H_{37}N_3O_7$ requires: C, 65.3; H, 6.8; N, 7.6%). NMR spectrum, τ 0.02, 0.12 (each 1H, s broad) NH, 2.76 (5H, s), 4.80 (2H, s) $C_6H_5CH_2$, 6.03 (2H, t) OCH_2, 6.26 (2H, s) CH_2, 6.41 (3H, s) OCH_3, 7.0–7.7 (6H, m) CH_2CH_2O and CH_2CH_2CO, 7.08 (6H, s) NMe_2, 7.77, 8.04 (each 3H, s) β-Me and 8.02 (3H, s) COMe.

Benzyl 4' - (2 - *acetoxyethyl*) - 3 - (2 - *methoxycarbonylethyl*) - 3',4 - *dimethyl*-5' - *dimethylcarbamoylpyrromethane* - 5 - *carboxylate* (**5b**). 2 - Iodo - 4 - (2 - acetoxyethyl) - 3 - methyl - 5 - dimethylcarbamoylpyrrole (5.0 g) was hydrogenated over Adams platinum oxide catalyst as described above and the resulting 2-unsubstituted pyrrole (3.4 g; 99%) treated with benzyl 2-acetoxymethyl - 3 - (2 - methoxycarbonylethyl) - 4 - methylpyrrole - 5 - carboxylate (4.90 g) in glacial AcOH (140 ml) containing NaOAc (5.50 g) at 120–130° for 25 min. The mixture was then poured into cold water, extracted with CH_2Cl_2, and the organic phase was then washed with water, dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on alumina (Brockmann Grade III), the pyrromethane being eluted using 10% EtOAc in benzene. Evaporation of the eluates gave a colourless oil which crystallised readily. It was recrystallised from CH_2Cl_2–light petroleum, giving 6.22 g (82%) of colourless needles, m.p. 132°. (Found: C, 65.5; H, 6.7; N, 7.8. $C_{30}H_{37}N_3O_7$ requires: C, 65.3; H, 6.8; N, 7.6%), τ, 0.33, 0.47 (each 1H, br) NH, 2.71 (5H, s), 4.77 (2H,

s) $C_6H_5CH_2$, 5.87 (2H, t) OCH_2, 6.18 (2H, s) $-CH_2-$, 6.38 (3H, s) OMe, 7.00 (6H, s) NMe_2, 7.0–7.8 (6H, m) CH_2CH_2CO and CH_2CH_2O, 7.75, 7.99 (each 3H, s) β-Me and 8.02 (3H, s) COMe.

Pyrroketone

Benzyl 4' - (2 - acetoxyethyl) - 3 - (2 - methoxycarbonylethyl) - 3',4,5' - trimethylpyrroketone - 5 - carboxylate (**14a**). Benzyl 3 - (2 - methoxycarbonylethyl) - 4 - methyl - 5 - dimethylcarbamoylpyrrole - 5 - carboxylate (3.72 g)[9] in dry ethylene dichloride (15 ml) was treated with $POCl_3$ (0.98 ml) and then heated under reflux for 4 hr before being cooled to room temp., placed under an atmosphere of N_2 and treated with 3 - (2 - acetoxyethyl) - 2,4 - dimethylpyrrole (1.81 g)[10] in ethylene dichloride (7 ml) added in two portions over 20 min. The mixture was then heated under reflux for 1.5 hr under N_2 before addition of ethylene dichloride (15 ml) and sodium acetate (9 g) in H_2O (10 ml); this mixture was then heated under reflux with vigorous stirring for a further 1.5 hr. On cooling and standing overnight the pyrroketone separated as a pale brown oil which readily crystallised on trituration with MeOH/ether. It was recrystallised from MeOH to give the pyrroketone (1.6 g; 32%) as pale yellow prisms, m.p. 131°. (Found: C, 66.2; H, 6.6; N, 5.8. $C_{28}H_{32}N_2O_7$ requires: C, 66.1; H, 6.3; N, 5.5%), τ, 0.28, 0.57 (each 1H, br) NH, 2.65 (5H, s), 4.70 (2H, s) $C_6H_5CH_2$, 5.92 (2H, t) OCH_2, 6.41 (3H, s) OMe, 6.7–7.5 (6H, m) CH_2CH_2CO and CH_2CH_2O, 7.67 (3H, s) 5'-Me, 7.98, 7.75 (each 3H, s) β-Me, and 8.04 (3H, s) COMe.

Porphyrins

β - Acetoxy - 2 - (2 - acetoxyethyl) - 4,6,7 - tri - (2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**10a**). Compound **5a** (1.0 g) in $POCl_3$ (10 ml) was heated at 50° for 1.5 hr before evaporation to give an oil; last traces of $POCl_3$ were chased out by evaporation of ethylene dibromide. Compound **6**[20] (obtained by pyrolysis of the corresponding carboxylic acid (940 mg)) and the above $POCl_3$ complex were dissolved in CH_2Cl_2 (12 ml) and heated under a current of N_2 at 40° for 67 hr before evaporation of the solvent and chromatography of the resultant oil on alumina (Grade III, 100 g). The product imine salt was eluted with 80–100% EtOAc in benzene and finally MeOH. The residue from evaporation of the appropriate eluates was dissolved in CH_2Cl_2 (50 ml) and heated under reflux with virogous stirring with Na_2CO_3 (5 g) in H_2O (45 ml) for 5 hr. The organic layer was separated, washed with H_2O (100 ml), dried ($MgSO_4$), and evaporated to dryness to give the crude *b*-oxobilane (**8a**) (1.4 g) (τ, 0.70 (br) NH, 2.70 (10H, s) 4.77 (4H, s) $2\times C_6H_5CH_2$, 6.15 (2H, m) OCH_2, 6.36 (4H, s) methane-H, 6.41, 6.49 (each 3H, s) OMe, 7.0–7.7 (14H, m) CH_2CH_2CO and CH_2CH_2O, 7.74 (6H, s), 7.98, 8.11 (each 3H, s) $4\times\beta$-Me and 8.05 (3H, s) COMe.

The above *b*-oxobilane (1.3 g) in THF (100 ml) and NEt_3 (0.2 ml) was hydrogenated at room temp. and atmospheric pressure over 10% palladised charcoal (200 mg) until uptake had ceased. The catalyst was filtered off on Celite and the filtrates were evaporated to dryness. The residual brown foamed solid in CH_2Cl_2 (272 ml) and trimethylorthoformate (7.2 ml) was treated with 1M trichloroacetic acid in CH_2Cl_2 (49.5 ml) before being stirred in darkness for 3 hr. Pyridine (10 ml) was added and the soln was stirred in the light and open to the atmosphere for 50 hr. The solvents were evaporated and the residual olive green solid was dissolved in pyridine (30 ml) and Ac_2O (10 ml). After 45 min the red soln was evaporated to dryness and the residue was chromatographed on alumina (Grade III) eluting with CH_2Cl_2. The red eluates were evaporated and the residue was recrystallised from CH_2Cl_2–n-hexane to give the *acetoxyporphyrin* (139 mg), m.p. 199–200°. (Found: C, 65.80; H, 6.44; N, 7.34. $C_{42}H_{48}N_4O_{10}$ requires: C, 65.61; H, 6.29; N, 7.29%), τ, (0.1M), 0.09, 0.10, 0.41 (each 1H, s) *meso*-H, 5.30 (2H, t) OCH_2, 5.8 (8H, m) α-CH_2CH_2, 6.20, 6.32, 6.40 (each 3H, s) OMe, 6.42, 6.53, 6.63, 6.71 (each 3H, s) Me, 6.8 (6H, m) β-CH_2CH_2, 7.01 (3H, s) *meso*-$OCOCH_3$, and 8.00 (3H, s) COMe, λ_{max} 403 (ϵ 175,000), 501 (14,900), 532 (5300), 552 (5500) and 622 nm (1400).

β - Acetoxy - 4 - (2 - acetoxyethyl) - 2,6,7 - tri - (2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**10b**). This porphyrin was similarly prepared from compounds **5b** and **6**; the *b*-oxobilane (**8b**) was an intermediate, τ, 0.72 (br) NH, 2.70 (10H, s), 4.77 (4H, s) $2\times C_6H_5CH_2$, 6.10 (2H, m) CH_2O, 6.41, 6.43, 6.54 (each 3H, s) OMe, 6.36 (4H, s) methane-H, 7.0–7.7 (14H, m) CH_2CH_2CO and CH_2CH_2O, 7.74 (6H, s), 7.98, 8.11 (each 3H, s) $4\times$ Me and 8.05 (3H, s) COMe. After hydrogenation and cyclisation of the *b*-oxobilane as described above, the *acetoxyporphyrin* was obtained from CH_2Cl_2–n-hexane, with m.p. 212–213°. (Found: C, 65.66; H, 6.15; N, 7.47. $C_{42}H_{48}N_4O_{10}$ requires: C, 65.61; H, 6.29; N, 7.29%), τ, (0.1M), 0.17, 0.22, 0.51 (each 1H, s) *meso*-H, 5.16 (2H, t) OCH_2, 5.89 (8H, m) α-CH_2CH_2, 6.34 (3H, s) 6.41 (6H, s) OMe, 6.45, 6.54, 6.73, 6.77 (each 3H, s) $4\times$ Me, 6.8 (6H, m) β-CH_2CH_2, 7.06 (3H, s) *meso*-$OCOCH_3$, and 7.80 (3H, s) COMe, λ_{max} 403 (ϵ 174,000), 501 (16,000), 532 (5700), 552 (6400) and 624 nm (1400).

2 - (2 - Acetoxyethyl) - 4,6,7 - tri - (2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**11a**). Porphyrin **10a** (260 mg) in THF (75 ml) and NEt_3 (0.1 ml) was hydrogenated at room temp. and atmospheric pressure over 10% Pd-C (130 mg) until the soln became colourless (6 hr). The catalyst was removed by filtration through Celite and the filtrates were treated with DDQ (285 mg) in benzene (20 ml) before evaporation to dryness. The residue was immediately chromatographed on alumina (Grade III) eluting with CH_2Cl_2. Evaporation of the red eluates and crystallisation of the residue from CH_2Cl_2–n-hexane gave the *porphyrin* (200 mg, 83%), m.p. 155–156°. (Found: C, 67.45; H, 6.64; N, 7.87. $C_{40}H_{46}N_4O_8$ requires: C, 67.59; H, 6.52; N, 7.88), τ, (0.1M), 0.07, 0.08, 0.11, 0.13 (each 1H, s) *meso*-H, 5.23 (2H, t) OCH_2, 5.8 (8H, m) α-CH_2CH_2, 6.34, 6.35, 6.37 (each 3H, s) OMe, 6.43, 6.49, 6.51, 6.59 (each 3H, s) Me, 6.8 (6H, m) β-CH_2CH_2 and 7.98 (3H, s) COMe, λ_{max} 400 (ϵ 176,000), 502 (14,600), 532 (9700), 568 (6600) and 621 nm (4400).

4 - (2 - Acetoxyethyl) - 2,6,7 - tri(2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**11b**). This compound was similarly prepared from porphyrin **10b**. The product was crystallised from CH_2Cl_2–n-hexane and had m.p. 143–144°. (Found: C, 67.66; H, 6.44; N, 7.91. $C_{40}H_{46}N_4O_8$ requires: C, 67.59; H, 6.52; N, 7.88%), τ, (0.1M), 0.11 (2H, s), 0.26 (2H, s) *meso*-H, 5.24 (2H, t) OCH_2, 5.8 (8H, m) α-CH_2CH_2, 6.40, 6.42, 6.43 (each 3H, s) OMe, 6.49, 6.57, 6.59, 6.62 (each 3H, s) Me, 6.8 (6H, m) β-CH_2CH_2 and 8.00 (3H, s) COMe, λ_{max} 400 (ϵ 171,000), 501 (13,100), 532 (8500), 568 (6500) and 620 nm (2600).

2 - (2 - Hydroxyethyl) - 4,6,7 - tri - (2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**12a**). Porphyrin **11a** (183 mg) in 5% H_2SO_4 in MeOH (80 ml, v/v) was set aside overnight in the dark before being poured into NaOAc aq and extracted with CH_2Cl_2. The organic phase was washed with H_2O, dried (Na_2SO_4), evaporated to dryness, and the residue was chromatographed on alumina (Grade V, elution with CH_2Cl_2). Evaporation of the red eluates and crystallisation of the residue from CH_2Cl_2–n-hexane gave the *porphyrin* (153 mg, 91%), m.p. 216–217°. (Found: C, 68.13; H, 6.74; N, 8.44. $C_{38}H_{44}N_4O_7$ requires: C, 68.24; H, 6.63; N, 8.38%), τ, (0.1M), 0.18, 0.21, 0.22, 0.35 (each 1H, s) *meso*-H, 5.7 (10H,m) 6.85 (6H, m) CH_2CH_2, 6.39 (3H, s) 6.40 (6H, s) OMe, 6.56, 6.62, 6.68, 6.73 (each 3H, s) Me, λ_{max} 400 (ϵ 161,000), 501 (12,600), 532 (8500), 569 (5800) and 620 nm (3700).

4 - (2 - Hydroxyethyl) - 2,6,7 - tri - (2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**12b**). Compound **11b** was similarly transformed and gave a 95% yield of the *hydroxyethylporphyrin*, crystallised from CH_2Cl_2–n-hexane, m.p. 183–184°, resolidifying and then re-melting 195–196°. (Found: C, 68.13; H, 6.63; N, 8.27. $C_{38}H_{44}N_4O_7$ requires: C, 68.24; H, 6.63; N, 8.38%), τ, (0.1M), 0.09 (1H, s) 0.13 (3H, s) *meso*-H, 5.7 (10H, m) 6.8 (6H, m) CH_2CH_2, 6.37 (9H, s) OMe, 6.50 (9H, s) 6.60 (3H, s) Me, λ_{max} 400 (ϵ 162,000), 500 (12,900), 532 (8400), 568 (6200) and 620 nm (2800).

2 - (2 - Chloroethyl) - 4,6,7 - tri(2 - methoxycarbonylethyl) - 1,3,5,8 - tetramethylporphin (**13a**). Compound **12a** (135 mg) in CH_2Cl_2 (80 ml) and DMF (15 ml) was treated with solid K_2CO_3 (5.5 g) and then $SOCl_2$ (5.0 ml) and then stirred at room temp. for 3 hr. The mixture was poured carefully into H_2O, extracted with CH_2Cl_2, which was dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on alumina (Grade III, elution with CH_2Cl_2) and the red eluates where evaporated. The residue was crystallised from CH_2Cl_2–n-hexane to give the *chloroethylporphyrin* (116 mg, 90%), m.p. 174–175° lit.[6] 173–174°); a mixed

m.p. with authentic material[6] showed no depression (Found: C, 65.97; H, 6.55; N, 8.04. Calc. for $C_{38}H_{43}ClN_4O_6$: C, 66.40; H, 6.31; N, 8.15%).

4 - (2 - *Chloroethyl*) - 2,6,7 - *tri*(2 - *methoxycarbonylethyl*) - 1,3,5,8 - *tetramethylporphin* (**13b**). This compound was similarly prepared from porphyrin **12b**; it was recrystallised from CH_2Cl_2–n-hexane and had m.p. 189–190° (lit.[6] 190–191°); a mixed m.p. with authentic material showed no depression.

4,6,7 - *Tri* - (2 - *methoxycarbonylethyl*) - 1,3,5,8 - *tetramethyl* - 2 - *vinylporphin*, "*Harderoporphyrin trimethyl ester*" (**2b**). Porphyrin **13a** (85 mg) in CH_2Cl_2 (20 ml) was treated with a saturated soln of zinc(II) acetate in MeOH (2 ml); the mixture was then warmed on a water-bath for 5 min before being poured into water and extracted with CH_2Cl_2 which was dried (Na_2SO_4) and evaporated to dryness. The residue was taken up in THF (20 ml) and 1M t-butoxide in t-butyl alcohol (50 nl) was added. After standing in the dark for 48 hr the mixture was treated with HOAc (10 ml) and then pyridine (10 ml), and it was then poured into H_2O, extracted with CH_2Cl_2 which was dried (Na_2SO_4) and evaporated to dryness. The residue was set aside overnight in 5% v/v H_2SO_4 in MeOH (50 ml) before being poured into NaOAc aq. CH_2Cl_2 was added and the organic phase was washed with H_2O, dried (Na_2SO_4), and evaporated. The residue was chromatographed on alumina (Grade III, elution with CH_2Cl_2) and evaporation of the red eluates gave a residue which was crystallised from CH_2Cl_2–n-hexane to give the *vinylporphyrin* (58 mg; 70%), m.p. 197–198° (lit.[6] 203–204°); a mixed m.p. with an authentic sample[6] showed no depression. (Found: C, 70.20; H, 6.73; N, 8.81. Calc. for $C_{38}H_{42}N_4O_6$: C, 70.13; H, 6.51; N, 8.61%).

2,6,7 - *Tri* - (2 - *methoxycarbonylethyl*) - 1,3,5,8 - *tetramethyl*-4 - *vinylporphin*, "*Isoharderoporphyrin trimethyl ester*" (**2c**). This porphyrin was similarly prepared from porphyrin **13b**; it was crystallised from CH_2Cl_2–MeOH, m.p. 214–216° (lit.[6] 216–218°). A mixed m.p. with authentic material[6] showed no depression (Found: C, 70.08; H, 6.78; N, 8.81. Calc. for $C_{38}H_{42}N_4O_6$: C, 70.13; H, 6.51; N, 8.61%).

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