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PETROPORPHYRINS—II[1]

THE PRESENCE OF PORPHYRINS WITH EXTENDED ALKYL SUBSTITUENTS

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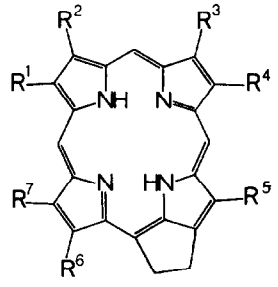
Abstract—The vanadyl porphyrins from Boscan oil (Cretaceous, W. Venezuela) were isolated as 3 fractions, and degraded to maleimides (1*H*-pyrrole-2,5-diones) by chromic acid. Analysis of the products by GC-MS, using multiple ion detection to enhance sensitivity, revealed a major homologous series of 3-Me components with n-alkyl side chains extending to C_{11}, and a minor series with branched alkyl side chains, the Me branch being at C-1. The origin of the extended alkyl groups is discussed.

Alkyl petroporphyrins occur mainly as complex distributions of vanadyl or nickel complexes of 2 major series: deoxophylloerythroetio (DPEP) porphyrins **1** and aetio porphyrins **2**. Initially they were thought to be produced solely from decarboxylation, reduction, oxidation and dealkylation of chlorophyll *a*,[2,3] but mass spectrometric studies[4] have shown the presence of high carbon number porphyrins ($\geq C_{35}$) which could not be the product of such processes. It was proposed that these high carbon number compounds were derived from chlorophylls with extended alkyl substituents, e.g. chlorobium chlorophylls[4–6] or from a random process such as transalkylation.[4,7] There is insufficient evidence to confirm either hypothesis because electron impact mass spectrometry (EIMS) provides only limited information on the number and type of substituents attached to the macrocycle. Recent analyses of the metastable peaks in the mass spectra of some vanadyl petroporphyrin mixtures indicate the presence of long (C_4–C_8) branched or straight chain alkyl substituents.[8] Degradation of porphyrin mixtures to maleimides (1*H*-pyrrole-2,5-diones) and subsequent analysis by gas chromatography-mass spectrometry (GC-MS) supplements the EIMS data on the intact compounds by providing information on the nature of the β substituents. Although this technique has been applied previously to petroporphyrin mixtures,[9,10] assignments of the products were tentative because of the lack of synthetic standards. We report the presence of a more complex mixture of maleimides than previously detected, together with the assignment of some of the products by coinjection with synthesised standards.



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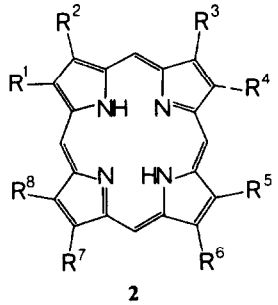

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RESULTS AND DISCUSSION

Boscan 9K3 crude oil (Cretaceous, W. Venezuela) was selected because of its high abundance of vanadyl porphyrins (*ca* 4000 ppm) and wide carbon number range (C_{27}–C_{39}).[4,11] The vanadyl porphyrins were separated chromatographically into three fractions (Table 1), each of which was demetallated,[12] and oxidised with CrO_3 to give 1*H*-pyrrole-2,5-diones.[13]

Gas chromatographic analyses of the products from each fraction showed two major components assigned as 3,4-dimethyl-1*H*-pyrrole-2,5-dione **3** and 3-methyl-4-ethyl-1*H*-pyrrole-2,5-dione **4** by coinjection with standards on GC and GC-MS (Table 2). Previous studies[9,10] misassigned the 3,4-dimethyl compound as the isomeric 3-ethyl-1*H*-pyrrole-2,5-dione **5** because standards were not available.

GC-MS analysis (Table 2, Table 3) confirmed the presence of higher carbon number 1*H*-pyrrole-2,5-diones; the spectra showed molecular ions and characteristic ions at *m/e* 125 (base peak) and *m/e* 138 observed previously in 3-methyl-1*H*-pyrrole-2,5-diones with long chain (e.g. C_{15}) reduced isoprenoid substituents.[14] The ion *m/e* 125 is thought to be the result of a McLafferty-type rearrangement (Fig. 1).[15]

Table 1. Carbon number distributions of Boscan 9K3 vanadyl porphyrin fractions by mass spectrometry

Fraction No.	DPEP Abundances % (Carbon Number)*														Aetio Abundances % (Carbon Number)*													
	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)	(39)	(40)	(27)	(28)	(29)	(30)	(31)	(32)	(33)	(34)	(35)	(36)	(37)	(38)	(39)	(40)
1	-	-	-	-	2	14	20	25	30	48	50	44	33	25	-	-	-	-	10	65	100	92	75	50	39	27	15	5
2†	-	-	-	8	39	100	72	50	26	15	9	5	3	-	-	-	20	40	47	30	16	7	4	-	-	-	-	-
3	-	-	5	54	100	74	22	5	-	-	-	-	-	-	5	15	20	9	-	-	-	-	-	-	-	-	-	-

* Corrected for ^{13}C contributions; intensities of lower C no. porphyrins are approximate (±5%) due to the difficulty in distinguishing fragment from molecular ions.

† There is at least one other minor porphyrin series present (mainly in fraction 2) whose molecular ions are 2 a.m.u. lower than those of the DPEP series, and which may contain 2 isocyclic rings (C no. range C_{32}-C_{39}).

Table 2. 1H-pyrrole-2,5-diones from oxidation of demetallated Boscan vanadyl porphyrins

Component				No. of isomers	Approx. Abundance¶			TMS derivative
R^1	R^2	Structure	Coinjection*	observed †	Fraction 1	Fraction 2	Fraction 3	coinjection*
H	H	11	-	-	n.d.	(6×10^{-3})**	n.d.	✓
CH_3	H	12	-	-	n.d.	(7×10^{-2})**	n.d.	✓
CH_3	CH_3	3	✓	-	3.9×10^{-1}	2.9×10^{-1}	3.3×10^{-1}	✓
CH_3	C_2H_5	4	✓	-	1	1	1	✓
CH_3	$CH(CH_3)_2$††	-	-	-	3×10^{-2}	2×10^{-2}	1×10^{-2}	-
C_2H_5	C_2H_5	10	✓	-	n.d.	(3×10^{-2})**	n.d.	✓
CH_3	n-C_3H_7††	-	-	-	3.8×10^{-1}	3.0×10^{-1}	2.2×10^{-1}	-
CH_3	$CH(CH_3)C_2H_5$	8	✓	-	1.2×10^{-2}	1×10^{-2}	6×10^{-2}	✓
CH_3	$CH_2CH(CH_3)_2$	9	✓	-	4×10^{-3}	3×10^{-3}	2×10^{-3}	✓
C_2H_5	n-C_3H_7 ††	-	-	-	n.d.	(1×10^{-2})**	n.d.	-
CH_3	n-C_4H_9	6	✓	-	1.6×10^{-1}	1.2×10^{-1}	8.2×10^{-2}	✓
(C_6 isomers) ¶¶		-	-	2	2×10^{-2}§	9×10^{-3}§	6×10^{-3}§	-
CH_3	n-C_5H_{11}††	-	-	-	4.0×10^{-2}	2.4×10^{-2}	1.7×10^{-2}	-
(C_7 isomers) ¶¶		-	-	4	9×10^{-3}§	5×10^{-3}§	3×10^{-3}§	-
CH_3	n-C_6H_{13}	7	✓	-	1.7×10^{-2}	1×10^{-2}	7.5×10^{-3}	✓
(C_8 isomers) ¶¶		-	-	5	6×10^{-3}§	2×10^{-3}§	1×10^{-3}§	-
CH_3	n-C_7H_{15}††	-	-	-	8×10^{-3}	4×10^{-3}	4×10^{-3}	-
(C_9 isomers) ¶¶		-	-	6	3×10^{-3}§	1×10^{-3}§	abs	-
CH_3	n-C_8H_{17}††	-	-	-	3×10^{-3}	1.2×10^{-3}	abs	-
(C_{10} isomers)¶¶		-	-	3	1.5×10^{-3}§	3×10^{-4}§	abs	-
CH_3	n-C_9H_{19}††	-	-	-	1.5×10^{-3}	4×10^{-4}	abs	-
(C_{11} isomers)¶¶		-	-	3	8×10^{-4}§	3×10^{-4}§	abs	-
CH_3	n-$C_{10}H_{21}$††	-	-	-	8×10^{-4}	4×10^{-4}	abs	-
(C_{12} isomers) ¶¶		-	-	1	abs	2×10^{-4}	abs	-
CH_3	n-$C_{11}H_{23}$††	-	-	-	4×10^{-4}	2×10^{-4}	abs	-

* On OV-1.

† In addition to components assigned.

¶ Estimated from m/e 125 and $M^{+\cdot}$ (MID data), relative to 3-methyl-4-ethyl-1H-pyrrole-2,5-dione.

** Estimated from mass fragmentograms of $(M-15)^+$ ion of TMS derivative.

†† Assigned tentatively from log plots (Fig. 2) and/or mass spectral data.

¶¶ C no. refers to total number of carbon atoms in alkyl substituents.

§ Abundances refer to major isomer.

n.d. = not determined. abs = absent.

Table 3. Mass spectra of 1H-pyrrole-2,5-diones and their TMS derivatives

Component R^1	Component R^2	Structure	Mass spectrum* m/e (%)	Mass spectrum* TMS derivative m/e (%)
H	H †	11	97(100), 69(35), 54(62), 53(19)	154(86), 127(5), 110(5), 100(8), 75(100), 53(10).
CH_3	H †	12	111(100), 93(7), 68(85), 67(8), 53(4)	183(1), 168(100), 97(12), 94(2), 75(48), 73(6), 67(28)
CH_3	CH_3 †	3	125(78), 97(3), 82(7), 70(3), 54(100), 53(28), 52(9)	197(1), 182(100), 111(9), 108(10), 100(8), 81(22), 77(16), 75(79), 54(18), 53(52)
CH_3	C_2H_5 †	4	139(100), 124(41), 121(7), 110(10), 106(8), 96(22), 68(37), 67(93), 66(16), 53(54)	211(2), 196(100), 125(4), 122(7), 100(7), 95(9), 84(6), 77(17), 75(43), 67(33), 53(12)
C_2H_5	C_2H_5 †	10	153(68), 138(100), 124(4), 120(11), 110 (11), 95(7), 92(10), 82(6), 81(11), 79(7), 67(84), 53(12)	225(4), 210(100), 194(5), 180(2), 136(6), 100(5), 81(15), 77(10), 75(22), 67(7), 53(4)
CH_3	n-C_3H_7 §	-	153(95), 138(60), 125(80), 124(50), 110 (75), 95(45), 67(100), 56(80), 54(55), 53 (80)	225(5), 210(100), 194(5), 181(5), 136(10), 109(5), 100(10), 81(10), 75(20), 53(5)
CH_3	$CH(CH_3)C_2H_5$ †	8	167(62), 152(31), 139(24), 138(51), 125 (100), 124(40), 109(47), 96(19), 95(36), 81(66), 67(91), 53(21)	239(11), 224(90), 197(18), 196(10), 195(16), 194(14), 100(32), 95(23), 75(100), 73(30), 67(38)
CH_3	$CH_2CH(CH_3)_2$ †	9	167(13), 152(3), 125(100), 124(11), 107(9), 97(16), 96(10), 81(9), 79(6), 54(19), 53 (15)	239(16), 224(100), 208(10), 197(11), 182(22), 181(40), 180(12), 100(22), 81(28), 75(91), 73(36), 53(37)
CH_3	n-C_4H_9 †	6	167(16), 152(9), 139(8), 138(33), 125(100), 124(16), 109(6), 107(7), 97(14), 96(14), 95(20), 81(16), 67(34), 54(24), 53(25)	239(6), 224(100), 197(9), 194(5), 181(10), 150(3), 125(2), 117(4), 100(5), 95(5), 87(5), 77(26), 75(35), 73(12), 67(5), 53(7)
CH_3	n-C_5H_{11} §	-	181(15), 166(10), 152(5), 139(15), 138(25), 125(100), 124(10), 95(10), 81(10), 67(15), 53(20)	253(5), 238(100), 197(10), 194(5), 181(10), 75(40)
CH_3	n-C_6H_{13} †	7	195(8), 180(5), 166(4), 153(5), 152(6), 139 (10), 138(15), 125(100), 124(10), 103(12), 102(8), 97(10), 95(9), 81(9), 67(13), 55 (12), 54(10), 53(12)	267(6), 252(100), 197(15), 144(8), 132(9), 18 (22), 100(10), 81(15), 75(45), 73(15), 67(9), 55(10), 53(14)

* Significant ions only † Spectra from GC-MS of synthesised standards § Spectra from GC-MS of oxidation products.

The degradation products were analysed further using multiple ion detection (MID) GC-MS to permit detection of the less abundant products. The following ions were selected: the molecular ions of extended alkyl-1*H*-pyrrole-2,5-diones, *m/e* 125 and *m/e* 138 characteristic of 3-methyl-1*H*-pyrrole-2,5-diones with long chain ($\geq C_3$) 4-alkyl substituents, together with *m/e* 111 and 139 expected to be diagnostic of 3-H and 3-Et analogues respectively. The analysis showed 3-methyl-1*H*-pyrrole-2,5-diones with alkyl side chains extending to C_{11} (Table 2); no additional 3-H- or 3-ethyl-1*H*-pyrrole-2,5-diones with extended alkyl side chains ($>C_2$) were detected, assuming the proposed mass spectral fragmentation pathway is valid (see below).

Isothermal MID analyses were performed to obtain plots of the log of retention time against carbon number, which showed the presence of a predominant homologous series, A, and a minor series B, *ca* 10% of A in all three fractions (Fig. 2). A number of

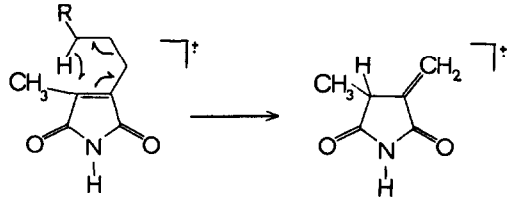


Fig. 1. Proposed rearrangement process to give *m/e* 125 in the mass spectra of 3-methyl-4-alkyl-1H-pyrrole-2,5-diones.[15]

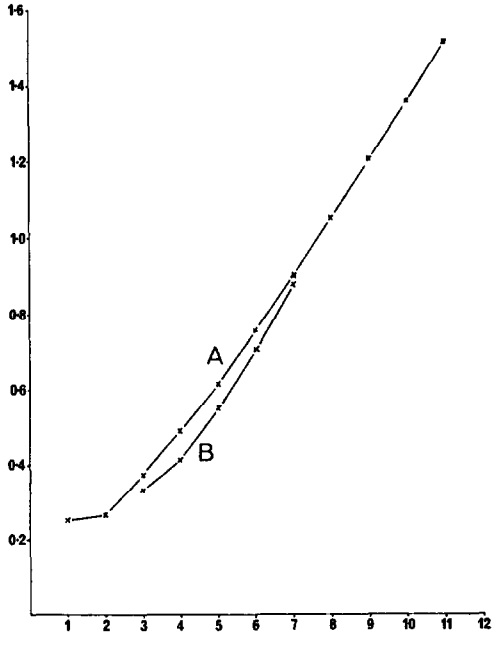
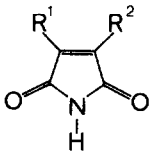


Fig. 2. Plot of log retention time (Carbowax 180°) vs carbon number of 4-alkyl substituent for 3-methyl-4-alkyl-1H-pyrrole-2,5-diones from oxidation of fraction 1. A = methyl n-alkyl series. B = methyl (1-methyl)alkyl series. Plots for the products from fractions 2 and 3 were similar but with a more restricted carbon number range.



	R^1	R^2		R^1	R^2
3	CH_3	CH_3	**9**	CH_3	$CH_2CH(CH_3)_2$
4	CH_3	C_2H_5	**10**	C_2H_5	C_2H_5
5	C_2H_5	H	**11**	H	H
6	CH_3	n-C_4H_9	**12**	CH_3	H
7	CH_3	n-C_6H_{13}	**13**	CH_3	$(CH_2)_2CO_2CH_3$
8	CH_3	$CH(CH_3)C_2H_5$			

structurally isomeric trace components were detected which may comprise other homologous series, but these components remain unidentified (Table 2). The plots are not linear for components with short ($<C_4$) alkyl substituents; this phenomenon has been observed in other homologous series.[16]

A number of 1*H*-pyrrole-2,5-diones **6–9** were synthesised by methods described previously.[5,14] GC coinjections confirmed the major homologous series, A, as 3-methyl-4-n-alkyl-1*H*-pyrrole-2,5-diones (Table 2, Fig. 2). It was necessary to carry out GC-MS coinjections with minor components using a mass spectrometric scan range reduced to 50 a.m.u., to enhance sensitivity of detection. Despite poor peak shapes on OV-1 and Carbowax it was possible to confirm **8** as a member of the homologous series B (Fig. 2, Table 2). The spectrum of **8** (Table 3) showed *m/e* 125 as base peak, which indicates the proposed mechanism (Fig. 1)[15] is not the major fragmentation pathway for members with a Me branch at C-1 in the alkyl substituent. These compounds would otherwise have been expected to show *m/e* 139 as base peak; **9** and **10** were detected in trace quantities (Tables 2 and 3) which may indicate that the chlorobium chlorophylls could be minor precursors of the Boscan petroporphyrins or that transalkylation processes occur to a limited extent.

The presence of the acidic amido proton in 1*H*-pyrrole-2,5-diones can produce poor peak shape on GC and GC-MS, making the results of coinjection studies with minor components difficult to interpret. Consequently derivatisation was employed to confirm the GC-MS coinjection data. The 1*H*-pyrrole-2,5-diones were converted to their trimethylsilylether (TMS) derivatives, which produced a marked improvement in chromatographic performance on both GC phases (OV-1 and Carbowax); these compounds are hydrolysed readily, and it is essential to store them in solution in a hydrophobic solvent, e.g. dichloromethane. The mass spectra of TMS 1*H*-pyrrole-2,5-diones (Table 3) are characterised by intense ions at $(M-15)^+$ (Fig. 3), *via* loss of a methyl radical from the TMS group, and *m/e* 75. The latter ion is usually associated with the group $SiMe_2{=}O^+H$, which might indicate that silylation occurs at oxygen rather than nitrogen, but further studies are required to confirm this hypothesis. Additional ions in the region *m/e* 180 to *m/e* 200 are probably the result of fragmentation within alkyl side chains, and may prove useful in the structural elucidation of unidentified 1*H*-pyrrole-2,5-diones. The GC-MS analysis of the derivatised fractions, and coinjection experiments showed, in addition to the components already observed, small but significant amounts of 1*H*-pyrrole-2,5-dione **11** and the 3-Me analogue **12** which were not detected in the analysis of the underivatised products. It is possible that the polar mono- and di-unsubstituted 1*H*-pyrrole-2,5-diones were preferentially absorbed on the GC-MS interface.

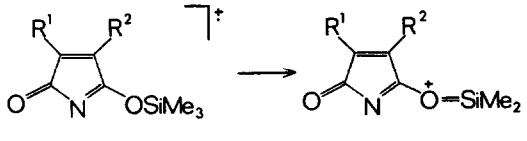


Fig. 3. Proposed fragmentation pathway to base peak, $(M-15)^+$, in the mass spectra of TMS derivatives of 3-methyl-4-alkyl-1H-pyrrole-2,5-diones.

Deuteroporphyrin-IX dimethyl ester **2** ($R^1{=}R^3{=}R^5{=}R^8{=}CH_3$; $R^2{=}R^4{=}H$; $R^6{=}R^7{=}(CH_2)_2CO_2CH_3$) was degraded in the same way to determine whether mono-unsubstituted 1*H*-pyrrole-2,5-diones were lost preferentially in the chromic acid oxidation. GC indicated the yield of **12** was indeed only *ca* 20% of that of **13**. Additionally, GC-MS analysis of the underivatised products showed no 3-methyl-1H-pyrrole-2,5-dione **12**, confirming that such compounds may be selectively absorbed on the GC-MS interface. Thus porphyrins with unsubstituted β positions may be more abundant in Boscan oil than is indicated in the present study. Mixtures of porphyrins fully substituted at the β positions show little preferential degradation of specific 1*H*-pyrrole-2,5-dione products by chromic acid.[17] From the above work it is concluded that the oxidative degradation of porphyrins to 1*H*-pyrrole-2,5-diones gives limited structural information on the nature of the β substituents. Clearly no information is obtained about meso (bridge) substituents or about the pyrrole containing the isocyclic ring present in DPEP porphyrins, as both sites are destroyed during the degradation. Similarly the data from mono- and di-unsubstituted 1*H*-pyrrole-2,5-diones are unreliable. Therefore it is difficult to draw precise conclusions on the origins of the petroporphyrins. However, it seems unlikely that naturally-occurring chlorophylls with extended alkyl substituents, e.g. chlorobium chlorophylls, are major precursors of the petroporphyrins because of the range of 1*H*-pyrrole-2,5-diones detected. Nevertheless, the presence of 3-methyl-4-(2-methyl)propyl-1H-pyrrole-2,5-dione **9** provides very

tentative evidence for the chlorobium chlorophylls as minor precursors. Similarly, random alkylation processes are unlikely to play a major role in the generation of petroporphyrins in Boscan crude because of the predominance of 3-methyl-4-alkyl-1*H*-pyrrole-2,5-diones in the products of oxidation (Table 2). This predominance suggests the operation of a specific process. One possibility is that these petroporphyrins were produced by thermal cracking of the complex organic polymer, kerogen, in sedimentary rocks in an analogous manner to the generation of the long chain n-alkanes of petroleums. The majority of organic material in sediments is eventually incorporated into kerogen which subsequently undergoes thermal cracking to yield crude oil. Supporting evidence is provided by the fact that sedimentary rocks which have a milder thermal history than crude oil, e.g. the bitumen Gilsonite, do not contain porphyrins with extended alkyl side chains in significant amounts.[1,17] Similarly, high carbon number porphyrins ($\geq C_{35}$) were detected only in sediments well within the zone of oil generation in a suite of sediment samples from the Paris Basin.[18] It appears that the most feasible method by which the petroporphyrin precursors are attached to kerogen is *via* anti-Markownikov binding of the vinyl group originally present in chlorophyll *a*. Thermal cracking would then give rise to porphyrins containing long n-alkyl substituents.

In summary, oxidative degradation of each of the three demetallated vanadyl porphyrin fractions from Boscan crude produces a wide variety of 3-methyl-1*H*-pyrrole-2,5-diones with 3-methyl-4-n-alkyl compounds as the major products. Random alkylation processes or the defunctionalisation of chlorobium chlorophylls are discounted as major routes to the petroporphyrins of Boscan crude oil.

EXPERIMENTAL

The ^{1}H NMR and decoupled ^{13}C NMR spectra were run on JEOL PS-100 and JEOL PFT-100 spectrometers respectively; $CDCl_3$ was used as solvent, with TMS as internal reference; chemical shifts are expressed in δ (ppm). The tentative ^{13}C NMR assignments are calculated from the Grant-Paul equation, and comparison with the chemical shifts of 3,4-dimethyl-1*H*-pyrrole-2,5-dione **3**. The following abbreviations are used: s = singlet, bs = broad singlet, t = triplet and m = multiplet. IR spectra were run on a Perkin–Elmer 197 spectrophotometer as solutions in $CHCl_3$. UV-visible absorption spectra were obtained on a Unicam SP-800 spectrophotometer. GC analyses were performed on a Carlo–Erba 2150 fitted with glass capillary columns (20 m × 0.25 mm i.d., OV-1 and Carbowax). Direct insertion probe mass spectra were run on a Varian MAT CH-7, and GC-MS was carried out on a Finnigan-4000 mass spectrometer interfaced directly (glass lined stainless steel, 0.3 mm i.d.) with a Finnigan 9610 gas chromatograph. Data from both mass spectrometers were acquired and processed by a DEC PDP-8e computer with a RTS/8 V2B operating system. MID analyses were carried out using a Promim Multiple Ion Detector fitted with 4 channels. The sampling time was 100 ms. High resolution mass spectra were carried out on a AE1 MS902 using the peak matching technique with perfluorokerosene as internal standard, and samples were introduced by direct insertion probe. Melting points were determined using a Kofler hot-stage apparatus. Preparative tlc was carried out on kieselgel H (Merck H60; 20 × 20 × 0.05 cm) pre-eluted with ethyl acetate, and reactivated at 100°C (2 hr). All solvents were distilled before use.

Compounds **4** and **10** were synthesised by the method of Ellsworth and Aronoff;[13] 3-methyl-(2-methyl)propyl-1*H*-pyrrole-2,5-dione and 3-methyl-4-butyl-1*H*-pyrrole-2,5-dione were prepared by the method of Holt and Morley.[5] All the alkylated acetoacetates were prepared by the procedure of Marvel and Hager.[19] The analytical data (m.p., IR, MS, ^{1}H NMR, ^{13}C NMR, HRMS) compared favourably with data given from a recent synthesis.[20]

Isolation of the petroporphyrins of Boscan 9K3. Boscan 9K3 oil (60 g) in CH_2Cl_2 (100 ml), was applied to an alumina column (800 g, BDH Grade II), and developed using CH_2Cl_2, CH_2Cl_2/MeOH and MeOH respectively. The eluates were combined and evaporated to dryness to yield a black viscous liquid (50 g) which was dissolved in toluene and chromatographed on alumina (800 g) eluting with toluene/hexane, toluene, toluene/CH_2Cl_2, CH_2Cl_2, CH_2Cl_2/MeOH and MeOH respectively. The eluates were monitored by UV-visible absorption spectrometry, and the porphyrin-containing fractions, which eluted in the range 30% toluene/CH_2Cl_2 to 60% toluene/CH_2Cl_2, were combined and evaporated to dryness. The crude vanadyl porphyrin fraction (4 g) in 10% CH_2Cl_2/hexane was applied to a silica gel column (150 g Hopkins and Williams MFC, 200 mesh) and eluted with hexane, hexane/CH_2Cl_2 and CH_2Cl_2. The eluates were monitored by UV-visible absorption spectrometry. Three porphyrin fractions were obtained; fraction 1, the least polar, and fraction 2 eluted in 30% CH_2Cl_2/hexane and fraction 3, the most polar eluted in 50% CH_2Cl_2/hexane. Each was crystallised using CH_2Cl_2/MeOH and analysed by mass spectrometry (Table 1). Yields were fraction 1 (60 mg), fraction 2 (120 mg) and fraction 3 (60 mg).

Demetallation and degradation of the petroporphyrin fractions. Fraction 1 (10 mg) in methanesulphonic acid (1 ml) was heated at 110° (90 min.). The cooled mixture was neutralised by careful addition of sat $NaHCO_3$ aq (20 ml), and extracted with CH_2Cl_2 (6 × 25 ml). The organic layer was dried (Na_2SO_4) and evaporated. The demetallated porphyrins were purified further by column chromatography (alumina, 25 g) eluting with CH_2Cl_2 and CH_2Cl_2/MeOH as eluants. The eluates were monitored by UV-visible absorption spectrometry, and the porphyrin containing fractions, which eluted in CH_2Cl_2, were combined and evaporated.

The porphyrins were dissolved in CF_3CO_2H (1 ml) and a solution of CrO_3 (3.3 g) in dilute H_2SO_4 (12.5 ml, 25% w/v) was added. The mixture was cooled in an ice bath (2 hr), and left to stand at ambient temp (2 hr). The mixture was extracted with ether (5 × 10 ml). The organic phase was washed with $NaHCO_3$ aq (10 ml) and water (10 ml), dried (Na_2SO_4) and evaporated.

The products were analysed by GC and GC-MS on OV-1 and Carbowax glass capillary columns programmed from 60° to 260° at 4°/min. Isothermal MID analyses were carried out at 150° using Carbowax glass capillary columns. The elution order of the 1*H*-pyrrole-2,5-diones on OV-1 and Carbowax is similar.

Fractions 2 and 3 were also treated as above.

Degradation of deuteroporphyrin IX dimethyl ester **2** ($R^1 = R^3 = R^5 = R^8 = CH_3$; $R^2 = R^4 = H$; $R^6 = R^7 = (CH_2)_2CO_2CH_3$). To the ester in CF_3CO_2H (0.5 ml) was added a soln of CrO_3 (1.1 g) in dilute H_2SO_4 (12.5 ml, 25% w/v). The reaction was carried out and the products isolated as above. GC analysis showed the ratio of **12** to **13** to be 1:4.2; however analysis on GC-MS showed the presence of only **13**.

Derivatisation of 1H-*pyrrole-2,5-diones.* Both synthesised 1*H*-pyrrole-2,5-diones, and the degradation products of fractions 1, 2 and 3 (*ca* 100 μg in 50 μl CH_2Cl_2) were converted to their trimethylsilyl derivatives by the addition of bistrimethylsilyltrifluoroacetamide (BSTFA, *ca* 25 μl) with warming (30 min). Excess reagent was evaporated under N_2.

At no time was the sample allowed to become dry, to prevent hydrolysis.

3-Methyl-4-hexyl-1H-pyrrole-2,5-dione **7**. Ethyl-2-acetyloctanoate (3.2 g) in ether (10 ml) was cooled in an ice bath, and an aqueous soln of NaCN (1.4 g in 3 ml) was added. The mixture was stirred, and dil H_2SO_4 (2 ml, 45% w/v) was added dropwise over 3 hr to the cold mixture. Initially reaction was vigorous, and the organic phase turned yellow. The mixture was treated with a further aliquot of NaCN (1.5 g) in water (4 ml), and dil H_2SO_4 as described above, and left to stand overnight. The mixture was separated, and the aqueous phase was extracted with EtOAc (2 × 10 ml), and the combined organic phase was dried (Na_2SO_4) and evaporated. The brown oil (2.2 g) was heated under reflux (7 hr) with concentrated HCl (10 ml). The organic phase was separated, dried (Na_2SO_4), and distilled to give the crude anhydride (600 mg) distilling at 255–265°.

The crude anhydride (300 mg) was stirred with urea (300 mg) at 160° for 6 hr, a further aliquot of urea (300 mg) being added after 3 hr. The cooled dark brown oil was neutralised to pH 7 by dilute HCl, and extracted with ether. The organic layer was dried (Na_2SO_4) and evaporated. The product (R_f *ca* 0.3) was purified on tlc using 10% acetone/hexane as developer. Yield 80 mg (5% overall). Purity (GC on OV-1 capillary) > 95%.

For **7**: m.p. 59–61°C; IR 3350 cm^{-1} (NH), 1720 cm^{-1}(CO); MS (Table 3); ^{1}H NMR 0.88 (3H; t, $C\underline{H}_3(CH_2)_5$), 1.1–1.7 (8 H; m, $CH_2(C\underline{H}_2)_4CH_3$), 1.98 (3 H; s, $C\underline{H}_3$-ring), 2.40 (2 H; t, $C\underline{H}_2(CH_2)_4CH_3$) and 7.5 (1 H; bs, N$\underline{H}$); ^{13}C NMR 8.55 ($\underline{C}H_3$-ring), 14.08 ($(CH_2)_5\underline{C}H_3$), 22.57 ($(CH_2)_4\underline{C}H_2CH_3$), 23.66 ($CH_2\underline{C}H_2(CH_2)_3$), 28.15 ($(CH_2)_2\underline{C}H_2(CH_2)_2CH_3$), 29.24 ($\underline{C}H_2(CH_2)_4CH_3$), 31.55 ($(CH_2)_3\underline{C}H_2CH_2CH_3$), 137.97 ($CH_3\underline{C}$=C), 142.28 ($CH_3C$=$\underline{C}$), 172.25 and 172.55 (2C=O); (HRMS Found: $M^{+\cdot}$ 195.125; calc. for $C_{11}H_{17}NO_2$: 195.125).

3-Methyl-4-(1-methyl)propyl-1H-pyrrole-2,5-dione **8**. Ethyl-2-acetyl-3-methylpentanoate (35 g) was converted into **8** in 0.5% overall yield using the procedures described above. Purity (GC on OV-1 capillary) > 95%. For **8**: m.p. 37–39°C; IR 3250 cm^{-1} (NH), 1720 cm^{-1} (CO) MS (Table 3); ^{1}H NMR 0.87 (3 H; t, $CH(CH_3)CH_2C\underline{H}_3$), 1.23 and 1.30 (3 H; d, $CH(C\underline{H}_3)C_2H_5$), 1.5–1.9 (2 H; m, $CH(CH_3)C\underline{H}_2CH_3$), 2.00 (3 H; s, $C\underline{H}_3$-ring), 2.71 (1 H; m, $C\underline{H}(CH_3)C_2H_5$) and 8.07 (1 H; bs, N$\underline{H}$); ^{13}C NMR 8.55 ($\underline{C}H_3$-ring), 12.44 ($CH(\underline{C}H_3)C_2H_5$) 18.38 ($CH(CH_3)CH_2\underline{C}H_3$), 27.85 ($CH(CH_3)\underline{C}H_2CH_3$), 32.76 ($\underline{C}H(CH_3)C_2H_5$), 137.54 ($CH_3\underline{C}$=C), 145.31 ($CH_3C$=$\underline{C}$) and 197.186 (2C=O); (HRMS Found: $M^{+\cdot}$ 167.095; Calc. for $C_9H_{13}NO_2$: 167.095).

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