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A NATURALLY OCCURRING, CHLOROPHYLL *b* RELATED PORPHYRIN

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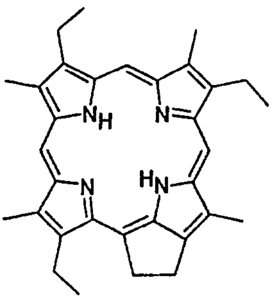
__*Abstract*__ *: A C_{31} alkyl porphyrin, occurring in an oil shale as the V=O complex, has been isolated as the free base acetylated derivative whose structure has been determined by ^1H NMR spectroscopy; the parent compound provides the first circumstantial evidence for a link between sedimentary porphyrins and chlorophyll b.*

Firm evidence for a long-assumed[1] link between sedimentary alkyl porphyrins and chlorophyll *a* has been provided recently by the structure analysis[2-4] of deoxophylloerythroetioporphyrin (DPEP, 1) which occurs widely in sediments and petroleums as the nickel(II) and/or vanadyl complexes, the structure being determined unambiguously using n.O.e. difference studies[3,4] and X-ray diffraction[5]. In addition, the Ni(II) complex of a C_{31} component with a methyl-substituted five membered ring (2) has also been isolated from an oil shale; it appears to have originated from degradation of chlorophyll *c* under sedimentary conditions *via* acid catalysed rearrangment, decarboxylation, and reduction of the vinyl group[6].

In the present study we report the identification of a novel C_{31} sedimentary porphyrin, 13,15-ethano-3,8,17-triethyl-2,12,18-trimethylporphyrin (3a) which occurs as the vanadyl complex in the Serpiano oil shale (Triassic,Monte San Giorgio, Switzerland; *ca.* 1000 ppm of metalloporphyrins[7]). The structure determination has been carried out on the acetylated derivative (3b) as the Zn(II) complex.



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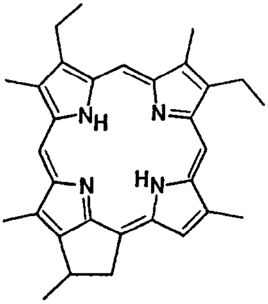

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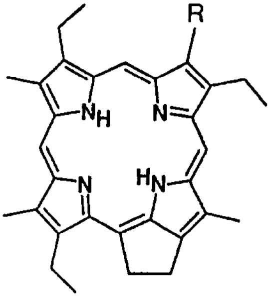

3a : R = H
3b : R = CH_3CO

The total metalloporphyrins (mainly V=O) were extracted from the finely-powdered shale (3.5 Kg) and demetallated as described previously[8]. Friedel-Crafts acetylation[9] of components with a free β-H, as the Cu(II) complexes, allowed ready chromatographic separation into three fractions (Kieselgel G, CH_2Cl_2 developer). After treatment with methanesulphonic acid (98%, N_2, 100°C, 2h) the fraction of intermediate polarity (R_f = 0.56) gave a free base, de-acetylated product[10], while the fraction of greatest polarity (R_f = 0.37) gave a free base product with the majority of the components still bearing the acetyl group. Preparative HPLC of the latter product afforded a number of porphyrins including the major component in the fraction. Analytical HPLC analysis (Spherisorb 3μ; 3 x 250 by 4.6 mm i.d. connected in series) of this component showed a single symmetrical peak, >95% pure with respect to other separated porphyrins. Low resolution mass spectrometry (40 eV) indicated, however, the presence of at least three coeluting compounds with an exocyclic alkano ring in each case: I, 504 ($M^{+\cdot}$, 100%), 489 (11.4), 252 (8.5); II, 490 ($M^{+\cdot}$,21.6), 475 (2.4), 245 (0.7) and III, 448 ($M^{+\cdot}$, 39.7), 433 (9.1), 224 (4.7). The electronic spectrum (λ_{max} = 505, 547, 576, 629; rel. int. 100:34:38:59; Soret omitted) was compatible with a free base porphyrin containing an exocyclic ring and with an extended chromophore. High resolution mass spectrometry (70 eV) indicated that the molecular ion of I corresponded to a monoacetylated component $C_{33}H_{36}N_4O$ (observed 504.2912, required 504.2919). Zn(II) insertion (zinc acetate/MeOH, reflux, 5 min.) and purification (TLC, Kieselgel G, 5% acetone/toluene developer; distillation at 10^{-4} Torr, 6h) gave a fraction, containing only I and II, which was analysed by 200 MHz ^1H NMR. Structure <u>3b</u> is proposed for the major compound (I).

The NMR spectrum (Figure, Table) of the fraction, as the Zn(II) complex, showed for <u>3b</u> the presence of 3β-methyls, 3β-ethyls, 3 *meso*-H's and one other methyl group (CH_3CO). The

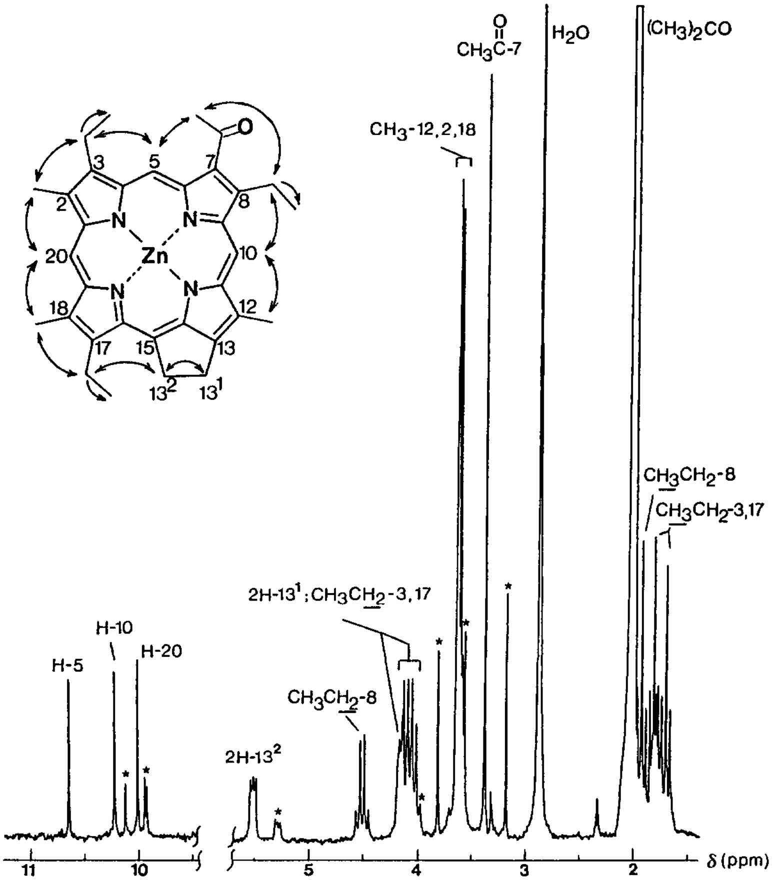

Figure . Partial 200 MHz ^1H NMR spectrum of fraction containing mainly <u>3b</u> (as zinc complex); * indicates signals from minor component with $M^{+\cdot}$ 490 (free base;see text). Arrow indicates n.O.e. observed (see Table).

presence of the five membered exocyclic ring (i.e. -CH_2CH_2- moiety) was confirmed by selective decoupling experiments. Decoupling also showed that the methylene quartet at 4.49 ppm was coupled with the methyl triplet at 1.97 ppm. The two ring protons at C-13^1 were partly obscured by the two $CH_3\underline{CH_2}$ quartets at *ca.* 4.10 ppm but were observed when the triplets at 1.86 and 1.75 ppm were irradiated to decouple the methylene, signals.

Appropriate n.O.e. experiments were carried out (summarised in Table) and the relative position of the substituents around the macrocycle could be established (Figure). Thus the *meso*-H at 10.66 ppm is flanked by a β-ethyl sustituent and the acetyl group, the latter being on the same pyrrole as the high frequency β-ethyl substituent. The *meso*-H at 10.25 ppm is flanked by the latter substituent and the high frequency β-methyl group (3.63 ppm). Since the remaining *meso*-H (10.04) is flanked by the other two β-methyls (3.61, 3.59 ppm) which are on the same pyrrole rings carrying the two low frequency β-ethyls ($CH_3\underline{CH_2}$ *ca.* 4.10 ppm), one of which flanks the methylene group (5.46) in the exocyclic ring, the structure is established. Although the methyls at 3.61 and 3.59 ppm could not be distinguished from each other because of the close chemical shift dispersion of the two methylene signals at *ca.* 4.10 ppm the assignment of the structure is not affected. Low power irradiation was used throughout n.O.e. experiments to obtain good frequency selectivity.

Table . ^1H NMR data (200 MHz) for 3b (Zn(II) complex) in $(CD_3)_2CO/C_5D_5N$.

δ (ppm)	Multiplicity	n.O.e.*	Assignments
10.66	s	4.10, 3.38	H-5
10.25	s	4.49, 3.63	H-10
10.04	s	3.61, 3.59	H-20
5.46	m	4.14, 4.10	2H-13^2
4.49	q	10.25, 3.38, 1.97	$CH_3\underline{CH_2}$-8
4.14	t	5.46	2H-13^1
*ca.*4.10†	2q	10.66, 5.46, 3.61, 3.59, 1.86, 1.75	$CH_3\underline{CH_2}$-3 $CH_3\underline{CH_2}$-17
3.63	s	10.04, 4.10, (10.25)§	CH_3-12
3.61	s		CH_3-2 or 18
3.59	s		CH_3-2 or 18
3.38	s	10.66, 4.49	CH_3CO-7
1.97	t	n.d.	$\underline{CH_3}CH_2$-8
1.86	t	n.d.	$\underline{CH_3}CH_2$-3
1.75	t		$\underline{CH_3}CH_2$-17

* Chemical shifts where enhancements seen when δ signal irradiated.

† Average shift of the two overlapping quartets.

§ When the methyls at 3.61 and 3.59 ppm were irradiated a small enhancement at 10.25 ppm was also observed due to partial saturation of CH_3 at 3.63 ppm.

n.d. Not detemined.

The position of the acetyl group at C-7 in 3b shows that the parent alkyl porphyrin (3a), occurring in the shale as the vanadyl complex, has a β-H at this position. We propose that the skeleton of 3a arises from chlorophyll *b* and is a marker for the presence of this precursor in organisms present when the sediment was deposited 250 x 10^6 years ago.

This proposal is made by strict analogy with the sequence of reactions thought to link C_{32} DPEP (1) and its presumed precursor chlorophyll *a*[1-3], except that in the case of the pathway leading to 3a removal of the formyl group at C-7 in chloropyll *b* would also be required. This could occur by acid-catalysed removal[11] occurring under sedimentary conditions or perhaps by oxidation to a carboxyl group which could be subsequently decarboxylated under sedimentary conditions. The relative abundance of 3a to 1 in the shale is *ca.* 2-5%, which might be expected since chlorophyll *a* is more abundant than chlorophyll *b* in the biosphere.

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