Tetrahedron Vol 40, No. 20, pp 4033 to 4039, 1984
Printed in the U S A

0040-4020/84 $3.00 + .00
Pergamon Press Ltd

PORPHYRINS WITH A NOVEL EXOCYCLIC RING SYSTEM IN AN OIL SHALE

M.I. CHICARELLI, G.A. WOLFF, M. MURRAY and J.R. MAXWELL*

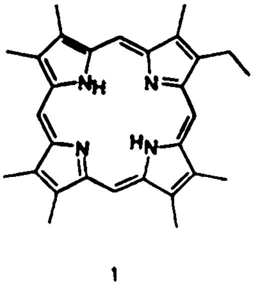
Organic Geochemistry Unit, University of Bristol, School of Chemistry,
Cantock's Close, Bristol BS8 1TS, U.K.

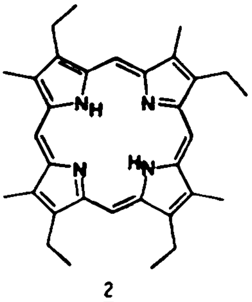
(*Received in UK* 19 *June* 1984)

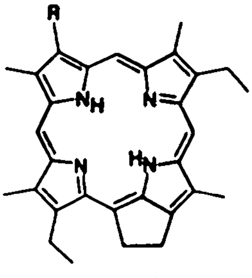
Abstract - A C_{32} and a C_{31} alkyl porphyrin, each present in Serpiano oil shale as the vanadyl complex, have been isolated as the demetallated compounds and their structures determined by ^1H NMR spectroscopic analysis of the zinc complexes, using selective decoupling and nuclear Overhauser effects. The occurrence of these chiral optically inactive compounds with a methyl-substituted, six membered exocyclic ring extends the known series of isomeric structural types of sedimentary alkyl porphyrins.

INTRODUCTION

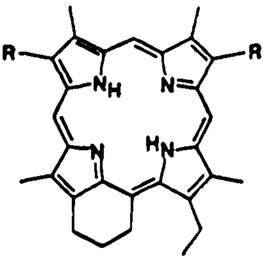
Alkyl metalloporphyrins (mainly Ni^{II} or V=O) occur widely in sedimentary rocks and petroleums and comprise complex mixtures containing mainly aetio components and components with an exocyclic ring (DPEP's)[1-5]. Structural analysis of individual components isolated from these mixtures is essential to provide information both about the origins of the compounds in terms of their biological precursors and of the sedimentary degradative pathways giving rise to them. Since the first unambiguous structure determination of two aetio porphyrins (1, 2) from a bitumen[6,7], most studies have been concerned with DPEP components⁺. At least four structural types with an exocyclic alkano ring have been found, including members with a five[8-11] (e.g. 3a,3b), six[12] (either 4a or 4b) and seven membered ring[13,14] (5a to 5c). A C_{31} component with a methyl-substituted five membered ring (6) has also been isolated recently from an oil shale[15]. The structures of some of these compounds clearly suggest an origin from known chlorophylls. For example, 3a and 3b may have originated from degradation of chlorophyll a[8,10], and 6 from chlorophyll c via acid catalysed rearrangement followed by decarboxylation and reduction of the vinyl group under sedimentary conditions[15].


1

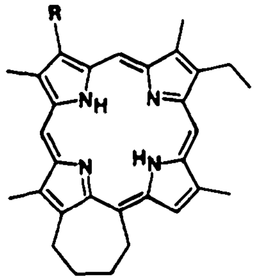

2


3a: $R=C_2H_5$
3b: $R=CH_3$

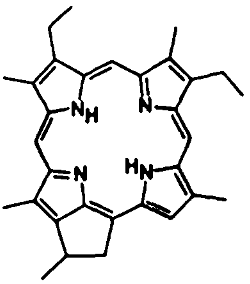
⁺ For convenience, all components with an exocyclic ring are referred to as DPEP types although the term applies strictly to components of the type represented by 3.



4a: R=CH_3, R'=C_2H_5
4b: R=C_2H_5, R'=CH_3



5a: R=C_2H_5
5b: R=CH_3
5c: R=H



6

In the present study, two members of a novel alkyl porphyrin type with a methyl-substituted six membered ring have been isolated from the total metalloporphyrins (mainly V=O) of Serpiano oil shale (Triassic,Monte S.Georgio,Switzerland), which is known to contain a high concentration (*ca.* 1000 ppm) of metalloporphyrins[2]. Friedel-Crafts acylation[16] of components with a free β position, as the Cu^{II} complexes, allowed ready chromatographic separation from fully alkylated components. The two compounds, with a free β position, have been characterised as the deacetylated free bases, using mass spectrometry and electron absorption spectrophotometry, and their structures elucidated using ^{1}H NMR and n.O.e. difference studies. The latter approach has been applied previously to porphyrins[8,9,13-15,17] and related macrocyclic compounds[18,19].

EXPERIMENTAL

Methods

Low resolution mass spectra were obtained, using the direct insertion probe technique, on a Finnigan 4000 spectrometer coupled to an INCOS 2300 data system: ionisation voltage 40 eV, source temperature 250°C and probe temperature programmed from 90° to 300°C. High resolution spectra were recorded on a VG MS9 spectrometer coupled to a VG data system: ionisation voltage 70 eV, source temperature 200°C and probe temperature programmed from 50° to 250°C; resolution *ca.* 10,000; perfluorokerosene as internal reference.

A Perkin-Elmer 551 spectrophotometer was used to obtain electronic spectra from solutions in CH_2Cl_2 (1 cm cell). ^{1}H NMR spectral data were obtained, in $CDCl_3$ with TMS as internal reference, on a JEOL FX 200 FT instrument. Concentrations used were typically 2-5 mg ml^{-1}. Optical rotation measurements were recorded on a 241 MC Perkin-Elmer polarimeter using solutions in CH_2Cl_2 (2 cm cell). High pressure liquid chromatography (HPLC) analyses were performed using a Spectra Physics SP 8700 tertiary solvent delivery system and Rheodyne 7125 injector. Detection (400 nm) was carried out using an LDC 1202 Spectromonitor II variable wavelength detector. Analytical analyses were obtained with three columns (Spherisorb 3W; each 250 x 4.6 mm) connected in series, using a combination of solvents similar to that described previously[20]. Preparative-scale HPLC was also carried out on Spherisorb 5W (250 x 10 mm).

Isolation

The total metalloporphyrins were extracted from the shale (3.5 kg) and demetallated as described previously[6]. The product of Friedel-Crafts acylation of the Cu^{II} complexes[16] was separated into three fractions by TLC (Kieselgel G, CH_2Cl_2 developer). Treatment with methanesulphonic acid (98%, N_2, 100°C, 2h) of the fraction of intermediate polarity (R_f = 0.56) gave a free base, deacetylated product (Fig.1). Preparative HPLC afforded a number of components including 7a and 7b.
3,8-Diethyl-2,7,12,18-tetramethyl-15,17-(15^1-methyl)propanoporphyrin (7a): LRMS (40 eV) significant ions 476 (100%, $M^{+\cdot}$), 461 (36), 238 (22, M^{2+}); HRMS found 476.2948, $C_{32}H_{36}N_4$ requires 476.2940; $\lambda_{max}(\varepsilon)$ soret 400 (*ca.* 135,000), 498 (*ca.* 10,500), 533 (*ca.* 3,200), 568 (*ca.* 4,200), 622 (ca. 980); ^{1}H NMR, see text.
8-Ethyl-2,3,7,12,18-pentamethyl-15,17-(15^1-methyl)propanoporphyrin (7b): LRMS (40 eV) significant ions 462 (100%, $M^{+\cdot}$), 447 (26), 231 (18, M^{2+}); HRMS found 462.2790, $C_{31}H_{34}N_4$ requires 462.2790; $\lambda_{max}(\varepsilon)$ soret 400 (*ca.* 131,000), 498 (*ca.* 7,500), 532 (*ca.* 3,500), 568 (*ca.* 4,000), 623 (*ca.* 700); ^{1}H NMR, see text.

RESULTS AND DISCUSSION

Analytical HPLC analysis (Fig.1) of the monoacetylated Cu^{II} fraction, after removal of Cu and deacetylation, showed the presence of a mixture of components with a free β position. Preparative-scale HPLC afforded four components (> 95% pure by analytical HPLC). Two were 5a and 5b (Fig.1), the major one coeluting with 5a[13] on coinjection. Component 5b, which has also been isolated previously[14], was assigned by comparison of NMR spectral data with 5a and by using n.O.e. difference studies (*cf.* ref. 13).

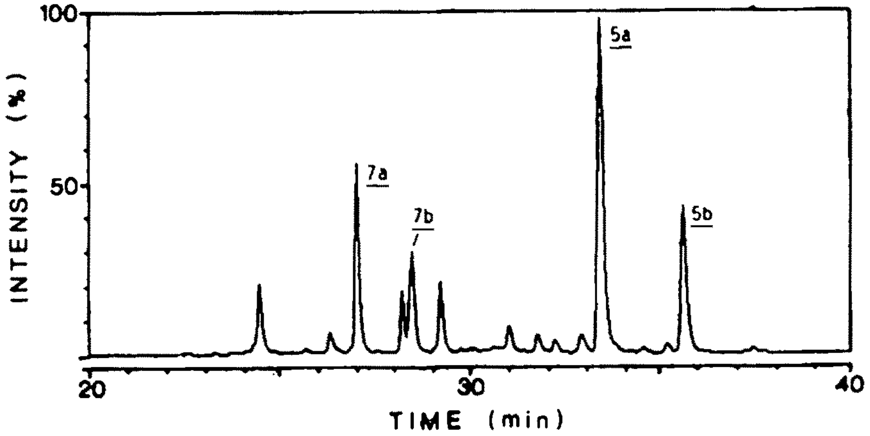
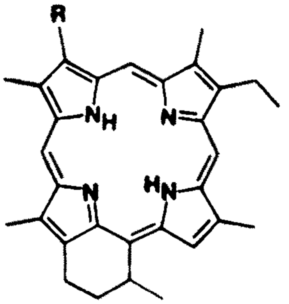


Figure 1. HPLC trace of a free base porphyrin fraction containing components with a free βH position, from Serpiano oil shale. Numbers refer to structures in text.

Two other components also had electronic absorption spectra and low resolution mass spectra suggesting the presence of an exocyclic alkano ring in each case; high resolution mass spectrometry indicated that the molecular ions corresponded to $C_{32}H_{36}N_4$ and $C_{31}H_{34}N_4$ respectively.



7a : R = C_2H_5
7b : R = CH_3

The 200 MHz ^1H NMR spectrum (Fig.2; Table 1) of 7a (as Zn^{II} complex) showed the presence of 4β-methyls, 2β-ethyls, 3 meso-H's and 1 β-H. The presence of the $-CH_2CH_2CH(CH_3)$-moiety in the exocyclic ring was confirmed by decoupling and n.O.e. difference experiments (Table 1). Furthermore, spatial connections between the $-CH_2CH_2CH(CH_3)$-moiety and H-13 and CH_3-12 (3.77 ppm) could also be established (Fig.2 and Table 1).

Some difficulty was experienced with these experiments. The small enhancements, in part due to slow molecular tumbling[17], result from the low power irradiation necessary to achieve good selectivity at 200 MHz. The principal source of difficulty was, however, poor instrument stability resulting from thermal variations, and the absence of suitable software for interleaving the control and irradiated measurements. The resulting variations in signal positions, phases, and line-shapes produce signals in the difference spectra which either have a dispersion form (from position or phase changes) or a double dispersion form (from line width changes), as shown in Fig.3. Detection of n.O.e. enhancements from these difference spectra was greatly facilitated by integration of the difference spectra. Both the dispersion and double dispersion line shapes have a net integral of zero, whereas any enhancement caused by a n.O.e. will give a net positive integral. Application of this technique is shown in Fig.3(a), where artifacts resulting from instrument instability are clearly distinguished by their net integrals of zero, and the enhancement of H-13 shows a net positive integral when the signal at 5.58 ppm (H-15^1) was irradiated. Figure 3(b)

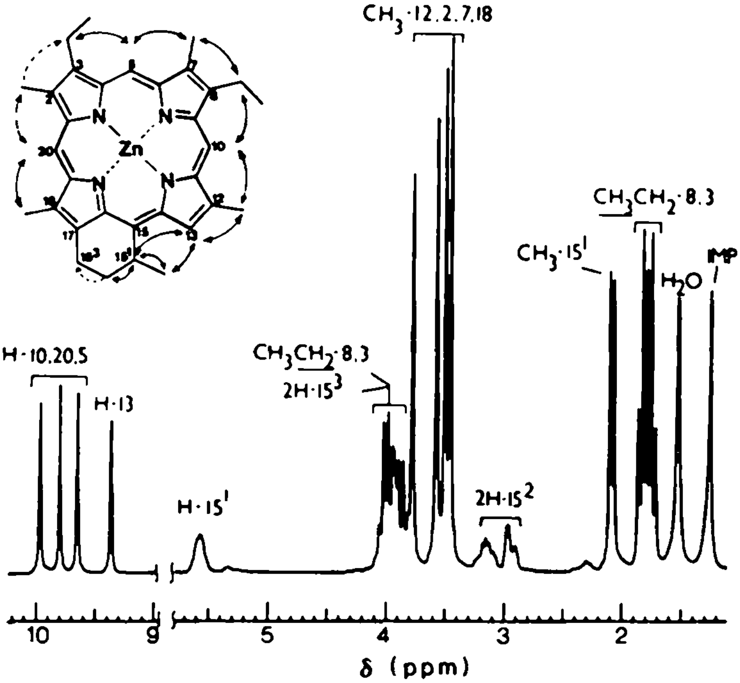


Figure 2. Partial ^1H NMR spectrum of 3,8-diethyl-2,7,12,18-tetramethyl-15,17(15¹-methyl)propanoporphyrin (7a), as zinc complex. Numbers indicate positions carrying the appropriate protons. Arrows indicate nuclear Overhauser enhancements observed; dotted arrows indicate weak enhancement observed.

Table 1. ^1H NMR chemical shifts (200 MHz), coupling constants and n.O.e. results for 7a, as Zn^{II} complex in $CDCl_3$.

δ (ppm)	Multiplicity	Coupling Constants*	n.O.e.†	Assignments
9.92	s	-	3.96, 3.77	H-10
9.72	s	-	3.56, 3.40	H-20
9.55	s	-	3.83, 3.46	H-5
9.36	d	0.9 (3.77)	5.58, 3.77, 2.10	H-13
5.58	m	? (3.17, 2.98, 2.10)	9.36, 3.17, 2.98, 2.10	H-15¹
3.96	q	7 (1.81, 1.71)	9.92, 3.46	$CH_3\underline{CH_2}$-8
3.83	q		9.55	$CH_3\underline{CH_2}$-3
3.90	t§	n.d.	n.d.	2H-15³
3.77	d	0.9 (9.36)	9.92, 9.36	CH_3-12
3.56	s	-	9.72, 3.83**	CH_3-2††
3.46	s	-	9.55, 3.96	CH_3-7
3.40	s	-	9.72	CH_3-18††
3.17, 2.98	2m	n.d.	5.58, 3.90**	2H-15²
2.10	d	7 (5.58)	9.36, 5.58	CH_3-15¹
1.81	t	7 (3.96, 3.83)	n.d.	$\underline{CH_3}CH_2$-8
1.71	t			$\underline{CH_3}CH_2$-3

* J = H (δ coupled nuclei)
† Chemical shifts where enhancements seen when δ signal irradiated.
§ Obscured by $CH_3\underline{CH_2}$ quartets, but observed when CH_3 at 1.81 and 1.71 ppm decoupled.
** Weak enhancement observed.
†† Assignments may be reversed, see text.
n.d. Not determined.

shows how connection between the signals at 3.40 and 3.56 ppm with the flanking H-20 was established. From the n.O.e. difference measurements, the assignments given in Table 1 could be unequivocally established, with the exception of CH_3-2 and CH_3-18. The possibility of these assignments being reversed must be considered, since the close chemical shift dispersion in the methylene region (Table 1) made observation of n.O.e.'s on these signals (2H-15^3 and $CH_3\underline{CH_2}$-3) difficult when the appropriate methyl neighbours were irradiated. This does not affect, however, the structure assignment.

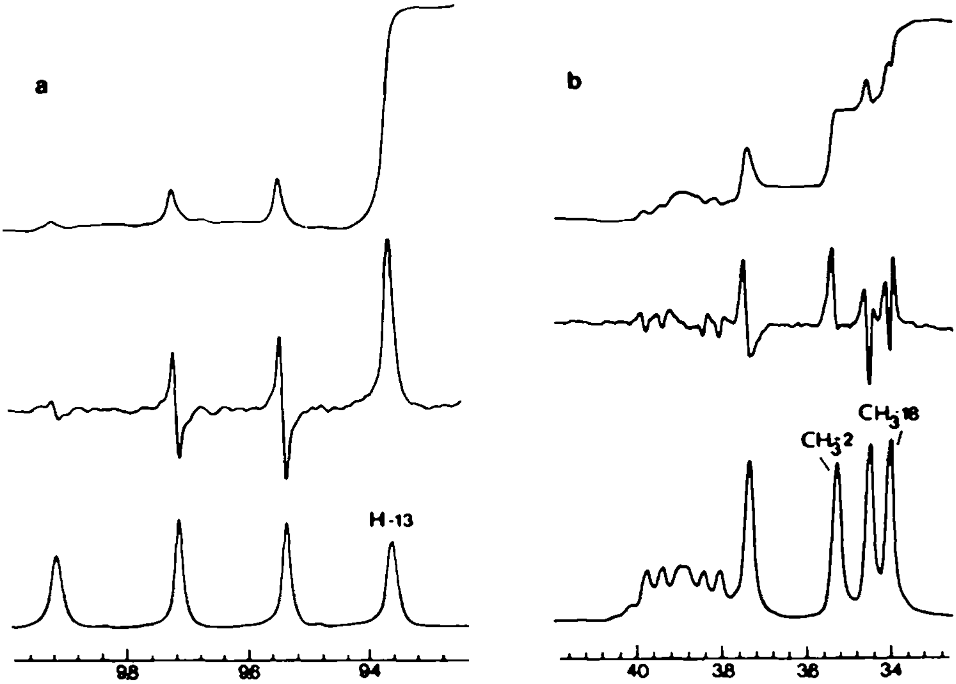
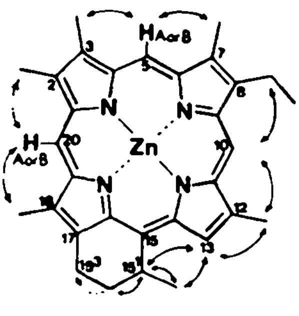


Figure 3. Bottom: Partial ^1H NMR spectrum of meso-region (a) and methylene, methyl regions (b) of 7a as zinc complex.
Middle: N.O.e. difference spectra arising from irradiation of H-15^1 (a) and H-20 (b)
Top: Integrated n.O.e. difference spectra.

The ^1H NMR spectrum of the Zn^{II} complex of 7b was essentially similar to that of the Zn^{II} complex of 7a and showed 5β-methyls, 1β-ethyl, 3 meso-H's and 1 β-H. The appropriate n.O.e. and decoupling experiments were carried out and the presence of the six membered, methyl-substituted exocyclic ring was established as before. The resulting connections between substituents for 7b (Zn^{II} complex) are summarised as follows (see also Table 2):



N.O.e. difference results revealed that meso H_A and H_B are each flanked by a pair of methyls at 3.56 and 3.38 ppm and 3.45 and 3.35 ppm, respectively. As the link between CH_3-7 and $CH_3\underline{CH_2}$-8 and between CH_3-18 and the methylene 15^3 substituent at C-17 could not be established, H_A may be at C-5 or C-20, with H_B at the alternative position. The assignments for the two pairs of methyls in question can, therefore, be interchanged; for example, if H_A is at C-20 then the methyl with a chemical shift of 3.56 ppm may be at C-2 or C-18. This does not affect the assignment of the structure. Considering the close similarity in chemical shifts of the substituents in the Zn^{II} complex of its C_{32} homologue (7a), tentative assignments of meso H_A and meso H_B and the flanking methyls could be made and are summarised in Table 2.

Table 2. ^1H NMR chemical shifts (200 MHz), coupling constants and n.O.e. results for 7b, as Zn^{II} complex in $CDCl_3$.

δ (ppm)	Multiplicity	Coupling Constants*	n.O.e.†	Assignments
9.93	s	-	3.96, 3.78	H-10
9.69	s	-	3.56, 3.38	H-20§
9.49	s	-	3.45, 3.35	H-5§
9.36	d	0.9 (3.78)	5.59, 3.78, 2.10	H-13
5.59	m	? (3.23, 2.99, 2.10)	9.36, 3.23, 2.99, 2.10	H-15^1
3.96	q	7 (1.81)	9.93	$CH_3C\underline{H_2}$-8
3.93	t**	n.d.	n.d.	2H-15^3
3.78	d	0.9 (9.36)	9.93, 9.36	CH_3-12
3.56	s	-	9.69	CH_3-2§
3.45	s	-	9.49	CH_3-7§
3.38	s	-	9.69	CH_3-18§
3.35	s	-	9.49	CH_3-3§
3.23, 2.99	2m	n.d.	5.59, 3.93	2H-15^2
2.10	d	7 (5.59)	9.36, 5.59	CH_3-15^1
1.81	t	7 (3.96)	n.d.	$\underline{CH_3}$ CH_2-8

* J = Hz (δ coupled nuclei)
† Chemical shifts where enhancements seen when δ signal irradiated.
§ Tentative assignment, see text.
** Obscured by CH_3CH_2 quartet but observed when CH_3 signal at 1.81 ppm decoupled.
n.d. Not determined.

Both 7a and 7b have a chiral centre at C-15^1 but optical rotation measurements revealed that each homologue is optically inactive, i.e. comprises a racemic mixture. The occurrence of 7a and 7b, as the vanadyl complexes, in Serpiano oil shale emphasises the complexity of the mixtures of sedimentary porphyrins containing an exocyclic alkano ring substituent. The major components in sediments with this structural feature lie in the carbon number range C_{32} - C_{30}. Thus, four C_{32} structural isomers are now known to occur having a five (3a), six (4a or 4b) or seven (5a) membered exocyclic ring or a methyl-substituted six membered ring (7a). Similarly, four C_{31} isomers can occur with a five (3b) or seven (5b) membered ring or with a methyl-substituted five (6) or six (7b) membered ring. It has been proposed that the C_{32} compound 3a retains the carbon skeleton of its presumed precursor, chlorophyll a, by vinyl reduction, ester hydrolysis, carbonyl group reduction and decarboxylation[10]. In the case of the C_{32} compound with the methyl-substituted exocyclic ring (7a), there is no known chlorophyll with the appropriate carbon skeleton which could give rise to this compound by an analogous pathway of defunctionalisation. To our knowledge, the only biological tetrapyrrole with a six membered carbocyclic ring is a nickel complex found in methanogenic bacteria[21].

The C_{31} compound with a methyl-substituted five membered ring (6) has been proposed to arise from chlorophyll c in the sedimentary column by way of acid catalysed rearrangement, vinyl reduction and decarboxylation[15]. A pathway from chlorophyll a, involving a rearrangement process, has also been suggested to account for the occurrence of sedimentary porphyrins with a seven membered exocyclic ring[14]. It is difficult at present to envisage, however, a rearrangement process of a known chlorophyll or chlorophyll product which could give rise to 7a or 7b, although it is possible that they may be related in origin to their counterparts with the seven membered exocyclic ring, by way of a ring contraction step during the sedimentary degradative pathway.

Acknowledgements

Two of us thank the Brazilian National Research Council (CNPq) (M.I.C.) and the Science and Engineering Research Council (G.A.W.) respectively for Studentships. We are also grateful to Professor R.H. Rieber (University of Zurich) for a sample of Serpiano oil shale. We thank the British Petroleum plc for HPLC facilities and the Natural Environment Research Council (GR3/2951 and GR3/3758) for low resolution mass spectrometric facilities, and Dr. K.A.G. MacNeil, University of Bristol and Mr. A.J. Bennet for high resolution mass spectrometric and optical rotation measurements respectively. We also thank Mr. N. Robinson and Mr. T. Peakman for valuable discussions.

References

1. A. Treibs, Angew.Chemie 49, 682 (1936).
2. D.W. Thomas and M. Blumer, Geochim.Cosmochim.Acta 28, 1147 (1964).
3. E.W. Baker and J.W. Louda, In: Advances in Organic Geochemistry 1981 (eds. M. Bjorøy et al.), pp.401-421, J. Wiley and Sons, Chichester (1983).
4. J.R. Maxwell, J.M.E. Quirke and G. Eglinton, In: Internationales Alfred-Treibs-Symposium, Munich 1979 (ed. A.A.Prashnowsky), pp.37-55. Universität Wurzburg (1980).
5. J.M.E. Quirke, G.J. Shaw, P.D. Soper and J.R. Maxwell, Tetrahedron 36, 3261 (1980).
6. J.M.E. Quirke, G. Eglinton and J.R. Maxwell, J.Am.Chem.Soc. 101, 7693 (1979).
7. J.M.E. Quirke and J.R. Maxwell, Tetrahedron 36, 3453 (1980).
8. J.M.E. Quirke, J.R. Maxwell, G. Eglinton and J.K.M. Sanders. Tet.Letts. 21, 2987 (1980).
9. J. Krane, T. Skjetne, N. Telnaes, M. Bjorøy and H. Solli, Tetrahedron 39, 4109 (1983).
10. C.J.R. Fookes, J.Chem.Soc., Chem.Commun., 1472 (1983).
11. A. Ekstrom, C.J.R. Fookes, T. Hambley, H.J. Loch, S.A. Miller and J.C. Taylor, Nature 306, 173 (1983).
12. G.A. Wolff, M.I. Chicarelli, G.J. Shaw, R.P. Evershed, J.M.E. Quirke and J.R. Maxwell, Tetrahedron, submitted.
13. G.A. Wolff, M. Murray, J.R. Maxwell, B.K. Hunter and J.K.M. Sanders, J.Chem.Soc., Chem. Commun., 922 (1983).
14. C.J.R. Fookes, J.Chem.Soc., Chem.Commun., 1474 (1983).
15. R. Ocampo, H.J. Callot, P. Albrecht and J.P. Kintzinger, Tet.Lett. 25, 2589 (1984).
16. J.M.E. Quirke, In: Advances in Organic Geochemistry 1981 (eds. M. Bjorøy et al.), pp. 733-745, J. Wiley and Sons, Chichester (1983).
17. J.K.M. Sanders and J.D. Mersh, Prog.Nucl.Magn.Reson.Spectrosc. 15, 353 (1983).
18. J.K.M. Sanders, J.C. Waterton and I.S. Denniss, J.Chem.Soc., Perkin Trans. 1, 1150 (1978).
19. A.R. Battersby, C. Edington, C.J.R. Fookes and J.M. Hook, J.Chem.Soc., Perkin Trans.1, 2265 (1982).
20. S.K. HajIbrahim, P.J.C. Tibbetts, C.D. Watts, J.R. Maxwell and G. Eglinton, Anal.Chem. 50, 549 (1978).
21. A. Pfaltz, B. Jaun, A. Fässler, A. Eschenmoser , R. Jaenchen, H.H. Gilles, G. Diekert, R.K. Thauer, Helv.Chim.Acta 65, 829 (1982).