Tetrahedron Vol. 39, No. 24, pp. 4109 to 4119, 1983
Printed in Great Britain.

0040-4020/83 $3.00 + .00
Pergamon Press Ltd.

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY OF PETROPORPHYRINS

Self-Aggregation Effects, Nuclear Overhauser Enhancements, and Spin-Lattice Relaxation used in Structural Elucidation.

Jostein Krane*, Tore Skjetne, Nils Telnæs+
Department of Chemistry, NLHT, University of Trondheim, 7055 Dragvoll, Norway
Malvin Bjorøy and Hauk Solli
Continental Shelf Institute, Haakon Magnussons gt. 1b, 7000 TRONDHEIM, Norway

(*Received in the UK* 29 *June* 1983)

Abstract: The substitution pattern of the two major petroporphyrins of Marl Slate, the ETIO III and the C_{32} DPEP, is determined by NMR spectroscopy alone, using self-aggregation effects, nuclear Overhauser enhancements and spin-lattice relaxation times. Protoporphyrin IX dimethyl ester was used as a model compound.

INTRODUCTION

Petroporphyrins in geological samples occur as complex mixtures[1]. It has been suggested that they are derived from naturally occurring chlorophylls[2]. Through various stepwise defunctionalization reactions chlorophyll a is thought to give rise to two major series of petroporphyrins: the deoxophylloerythroetio (DPEP) and etio types. Members of these series with more than 32 carbon atoms are thought to arise from either chlorophyll variants such as those found in photosynthetic bacteria, or through diagenetic transalkylation[3].

Although the DPEP- and etio-porphyrins constitute the major porphyrin-series found in the geosphere, a third porphyrin series, the rhodo-type, is present in some samples[4]. The rhodoporphyrins are thought to be alkylbenzoporphyrins, and may arise from geological ring closure and an aromatization step, or from a Diels-Alder type reaction[2].

---

* To whom correspondence should be addressed.

\+ Taken in part from N. Telnæs thesis submitted in partial fulfilment for a dr.scient. degree.

The large number of different individual porphyrins found in geological matrixes makes it essential to isolate single carbon-number species, and elucidate their structure in order to provide evidence for the various hypotheses put forward for the origin of petroporphyrins. The maximum utilization of porphyrins as biological markers, in fact, requires structural determination of individual fossil species.

No physicochemical technique alone, except for x-ray diffraction analysis, would very likely render the structures, but a combination of mass spectroscopy (MS)[1,5,6], visible absorption spectroscopy[7] and nuclear magnetic resonance spectroscopy (NMR)[8-12] might provide information sufficient to elucidate porphyrin structures. The advancement of high-field NMR spectrometers equipped with powerful software for spectral manipulation makes this relatively insensitive method a very viable tool for structural elucidation in solution, even on submilligram quantities.

In this paper we propose a general strategy for reliable assignment of NMR spectra of petroporphyrins. As examples we are utilizing protoporphyrin IX dimethylester (**1**) and two petropor-

phyrins: a C_{32}DPEP (**2**) and a C_{32}ETIO (**3**), and we do not make use of the assumption that chlorophyll a is the parent precursor. The aim is to establish the relative and absolute positioning of the substituents on the aromatic tetrapyrrole nucleus. It is of particular importance to distinguish between meso protons, methyl and ethyl groups which are magnetically different, but are in very similar molecular environments and have similar chemical shifts. Conventional chemical shift arguments using series of related compounds are not accurate enough because of shift differences of less than 0.1 ppm, and because of the porphyrins ability for self-aggregation[13]. We report here that self-aggregation shifts of both the free bases and their zinc(II) complexes, nuclear Overhauser enhancements and spin-lattice relaxation times (T_1) provide the necessary information to elucidate structures.

EXPERIMENTAL SECTION

The protoporphyrin IX (Sigma) was treated with 5% sulphuric acid in methanol (distilled in glass) to obtain the protoporphyrin IX dimethylester (**1**). The two petroporphyrins **2** and **3**, were isolated from Marl Slate (Yorkshire, England) and the purification of these will be dealt with in full detail in a forthcoming paper. Mass spectroscopy was done by the direct insertion probe (DIP) technique on a Hewlett-Packard 5985 mass spectrometer. Ionization voltage was 70 eV, source temperature 250°C, and DIP-temperature was programmed from 80 to 300°C. All the NMR spectra were obtained in $CDCl_3$ solution with TMS as the internal reference. Concentrations employed were 1-3 mg/ml.

The 400 MHz ^1H NMR spectra were obtained at room temperature on a Bruker WM-400 instrument (Bruker Spectrospin). The instrument is equipped with an ASPECT 2000 computer. All the spectra were run with 32K data points over 6024 Hz, giving an acquisition time of 2.72 sec. and a digital resolution of 0.37 Hz. Quadrature detection and phase cycling were employed. Spin-lattice relaxation rates were obtained by the inversion recovery sequence, using two parameter non-linear least squares fitting of the data (Eq. $Y=A3-A2\ \exp(-t/T_1)$). The nuclear Overhauser enhancement (nOe) measurements were obtained by pre-irradiation of the different proton chemical shift frequencies for 5 sec. After collecting 8 scans, preceeded by 2-4 dummy scans, at one selected irradiation frequency, the FID was stored, and the irradiation frequency was changed. For every fourth frequency a reference FID with irradiation at an off-resonance frequency was acquired. The entire cycle was repeated 10-100 times under computer control with addition of the new data to those already stored. The accumulated FID's were processed identically with the same line broadening (1 Hz) and phase corrections. Low power irradiation were sometimes necessary in order to achieve high frequency selectivity, which leads to loss of enhancement intensity. The 2D ^1H correlated spectrum was obtained with a $(90^0\text{-}t_1\text{-}90^0\text{-}t_2)$. sequence (COSY-90, Bruker Automated Program). The spectral width in both frequency domains was 4425 Hz with a digital resolution of 8 Hz (1024 data points with zero filling in the F1 dimension). 64 scans were stored for each increment of t. After the 2D Fourier transformation with absolute value data and sine bell multiplication in both F1 and F2, the matrix was symmetrized.

RESULTS AND DISCUSSION

The protoporphyrin IX dimethylester (**1**) together with a C_{32} DPEP (**2**) and a C_{32} ETIO (**3**) porphyrin were used in this work.

Protoporphyrin IX dimethylester (**1**): The structure of **1** is given in Figure 1. The NMR spectrum of the free base is given in Figure 2a and shows the following resonances: singlets at 3.73, 3.72, 3.65 and 3.63 ppm due to the four methyl groups in 1-, 3-, 5- and 8 positions, the two vinyl groups in 2- and 4 positions give rise to the resonances at 6.2-6.5 and 7.7-7.8 ppm.

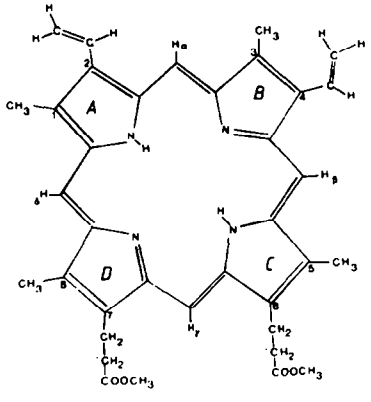

Fig. 1: The structure of **1**, protoporphyrin IX dimethylester.

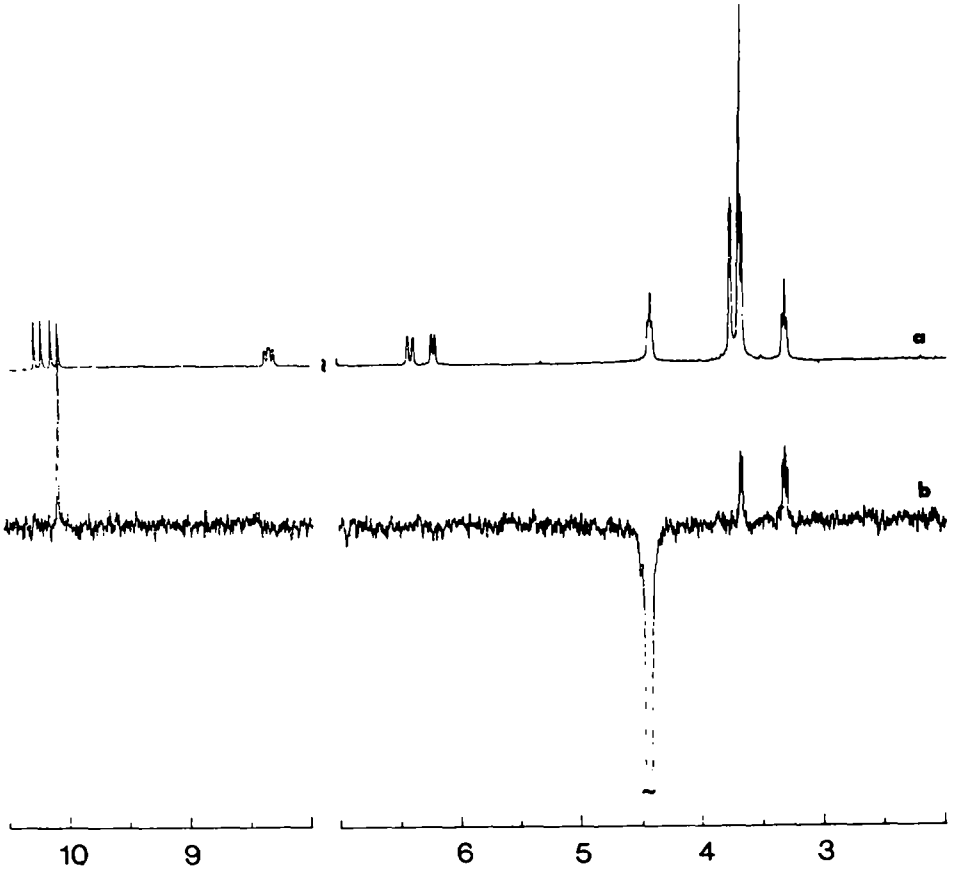

Fig. 2: a) NMR spectrum of 1
b) NOe difference spectrum of 1 after irradiation of the resonance at 4.41 ppm.

The meso protons give nicely resolved singlets between 10.0 and 10.5 ppm. The singlet at 3.66 ppm and the two triplets at 3.29 and 4.41 ppm. are due to the propionate methylenes and the methoxy groups in the 6- and 7 positions. Even at 400 MHz the resonances from the substituents in 6- and 7 positions are not resolved at the particular concentration used.

The total assignment is therefore not straight forward. Resolution enhancement by Gaussian multiplication reveals a small difference in chemical shift for the methylene protons in 6'- and 7' positions and also for the methylene protons in 6''- and 7'' positions. The methoxy resonances remain unresolved.

Further spectral assignments were made by nuclear Overhauser enhancement difference spectroscopy. Figure 2b shows the nOe difference spectrum after irradiation of the resonance at 4.41 ppm belonging to the methylene protons in 6'- and 7' positions, which gives rise to a nOe effect at the high-field meso proton resonance. There are also increases in the intensities of the two high-field methyl resonances and of the methylene protons in the 6''- and 7'' positions. The meso proton receiving the nOe effect must be the γ-proton situated between the two methylene groups in 6'- and 7' positions. The methyl groups receiving nOe enhancement must be in the 5- and 8 positions, because they are attached to the same pyrrolic rings as the methylene protons in the 6'- and 7' positions. The effects at the methylene protons in 6''- and 7'' positions are as expected since they are attached to neighbouring carbon atoms.

We have irradiated all of the remaining resonances in a similar way and thus have been able to assign the other resonances in the spectrum. The α-, β-, γ-, and δ-meso protons have chemical shifts of 10.25, 10.19, 10.05 and 10.11 p.p.m, respectively. The methyl groups in the 1-, 3-, 5- and 8 positions give rise to singlets at 3.72, 3.73, 3.63 and 3.65

ppm, respectively. These results are in excellent agreement with those published by Sanders *et al.*[13]. It should be pointed out that the chemical shift values given above are not limiting shifts. A change in concentration can lead to significant variations in individual shift values[9].

Zn(II) complex: Addition of Zn(II) acetate has previously been shown to give dramatic effects on the relative position of the chemical shifts of porphyrins[9]. The NMR spectrum of the Zn(II) complex of **1** is presented in Figure 3. The spectral assignments were made by the use of nOe difference spectroscopy in the same manner as for the free base. The results are presented in Table 1.

These results are in good agreement with the results obtained by Abraham *et al.*[9]. In order to prevent aggregation they added two mol. equiv. of pyrrolidine. The NMR spectrum after addition of two mol. equiv. of pyrrolidine to the Zn(II) complex is given in Figure 4. Our assignment, after such addition, was made using nOe difference spectroscopy and the results are also given in Table 1. Our assignment differs

Table 1: Chemical shifts (ppm) for the Zn(II) complex and pyrrolidine Zn(II) complex of **1**.

	Zn(II)-complex of **1**	Zn(II)-complex of **1** with pyrolidine
α	9.11	10.23
β	9.29	10.14
γ	8.98	9.94
δ	8.99	10.07
Methyl-1	3.36	3.77
Methyl-3	3.34	3.76
Methyl-5	3.37	3.63
Methyl-8	3.29	3.65
Methoxy	3.65	3.68
	3.63	
Methylene-6'	4.11	4.42
Methylene-7'	4.08	
Methylene-6''	3.02	3.28
Methylene-7''	2.99	

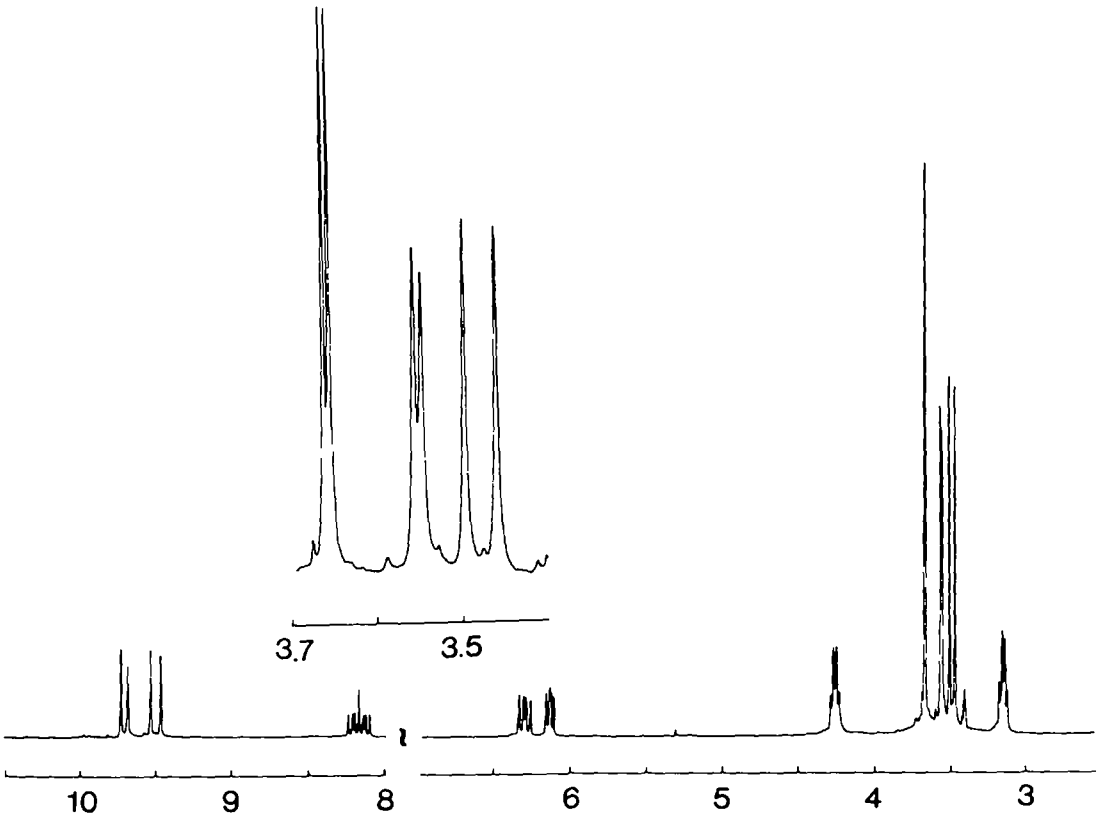

Fig. 3: NMR spectrum of the Zn(II) complex of **1**.

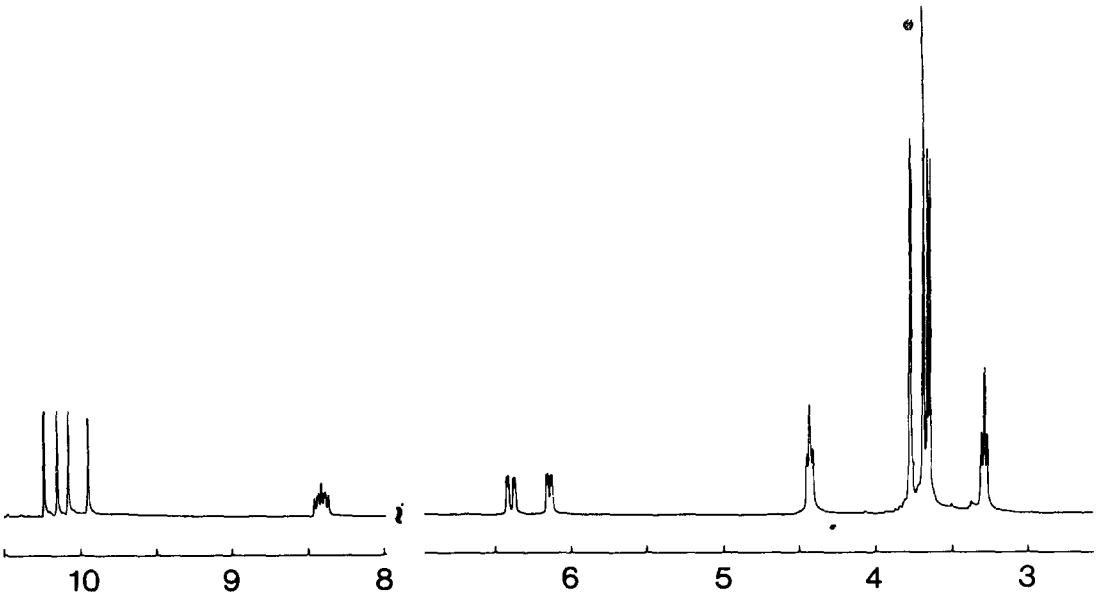

Fig. 4: NMR spectrum of the pyrrolidine Zn(II) complex of 1.

from that of Abraham et al.[9] in that the methyl groups in the 5- and 8 positions should be interchanged, i.e. the chemical shifts are 3.63 and 3.65, respectively, for the methyl groups in the 5- and 8 positions.

T_1 measurements were performed on the Zn(II) complex and the pyrrolidine Zn(II) complex.
The T_1 values for the meso protons and the methyl groups are listed in Table 2.

The T_1 values show without exception an increase in going from the Zn(II) complex to the pyrrolidine Zn(II) complex. This reflects disaggregation of the Zn(II) complex upon the addition of pyrrolidine.

Another striking feature of these T_1 values is the close agreement with the substitution pattern. The γ-meso protons of the Zn(II) complex and the pyrrolidine Zn(II) complex which are relaxed by the propionate methylenes through dipole-dipole interactions, have significantly shorter T_1 values than the remaining meso-protons in both the Zn(II) complex and the pyrrolidine Zn(II) complex.

Table 2: T_1 (s) values for porphyrins and porphyrin complexes used in this paper.

	Meso proton				Methyl group			
	α	β	γ	δ	1-	3-	5-	8-
Zn(II) complex of 1	0.64	0.70	0.45	0.67	0.53	0.53	0.54	0.54
Pyrrolidine - Zn(II) complex of 1	0.81	0.85	0.53	0.79	0.64	0.60	0.61	0.64
2	0.97	1.06	-	1.09				
Zn(II) complex of 2	0.71	0.73	-	0.77				
Zn(II) complex of 3	0.73	0.73	0.64	0.86				

C_{32} DPEP (**2**): The mass spectrum of this compound gives a molecular ion at M/e 476. This is consistent with a porphyrin of the deoxyphylloerythroetio type with 32 carbon atoms. The NMR spectrum of the free base is given in Figure 5. A rough assignment shows four methyl groups, three ethyl groups, three meso protons and an isocyclic five-membered ring. The structural elucidation was carried out as outlined for **1**. Difference nOe spectroscopy makes the total assignment fairly easy. Irradiation of the low-field meso proton (10.01 ppm) gives enhancements of two methyl groups at 3.63 ppm and at 3.55 ppm. When irradiating the meso proton at 9.90 ppm, enhancements of the methyl resonance at 3.68 ppm, the ethyl group resonances at 3.96 (quartet) and 1.77 (triplet) ppm is observed. Irradiation of the last meso proton, at 9.96 ppm, gives a positive nOe to the methyl resonance at 3.56 ppm and the ethyl groups resonances at 4.13 (quartet) and 1.88 (triplet) ppm. When the protons in the isocyclic five-membered ring at 5.32 ppm are irradiated, positive nOe effects are observed for the ethyl group resonances at 4.01 and 1.72 ppm, and the protons in the isocyclic ring at 3.99 ppm.

We label the protons in the isocyclic five-membered ring at 3.99 and 5.32 ppm as the α- and β-protons, respectively, and this labelling will be used throughout the paper. The CH_2 group attached to the pyrrole ring is, according to this scheme, the α-position in the isocyclic ring and the CH_2 group attached to the γ-methine carbon in the porphyrin nucleus is labelled the β-group.

Irradiation of the ethyl and methyl group resonances provides further information about the connectivity between substituents in the compound and serves as a crosscheck for the information provided by the irradiation of the meso protons. Based on the information above, the substitution pattern in **2** is as shown in Figure 6. Included in the Figure are the chemical shifts for the partjcular concentration used.

The structural elucidation is not based on the assumption that **2** is derived from chlorophyll a. The substitution pattern determined is, however, identical to the one postulated by Treibs as the main degradation product from chlorophyll[14,15], and strongly supports Treibs' hypothesis that chlorophylls are the major precursors of the petroporhyrins found in crude oil and bitumen. The substituent pattern in the C_{32} DPEP isolated from Marl Slate is identical to that of the C_{32} DPEP species that Quirke et al.[10] isolated from Gilsonite.

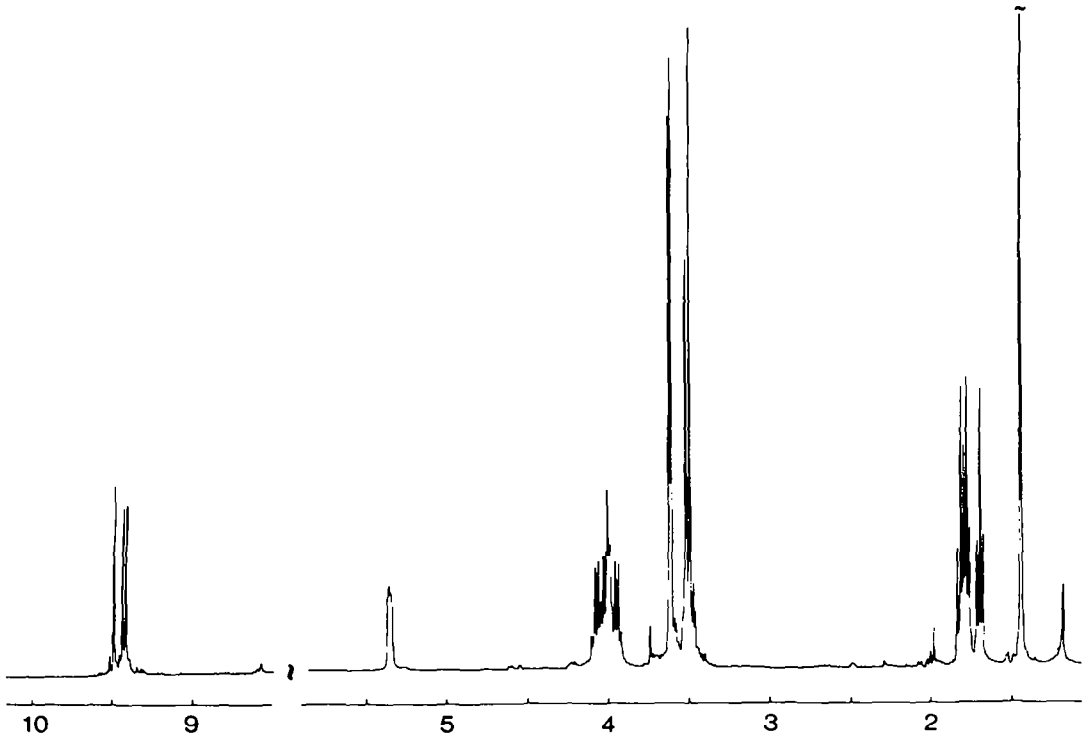

Fig. 5: NMR spectrum of C_{32}DPEP (**2**).

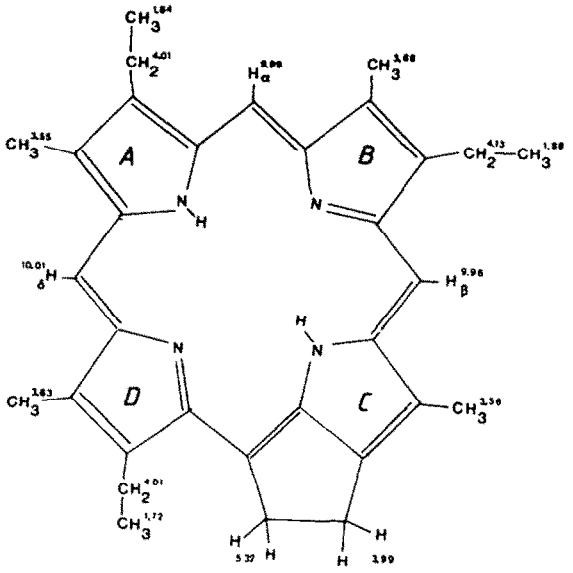

Fig. 6: The structure of C_{32}DPEP (**2**), with the chemical shifts in ppm.

T_1 measurements on **2** indicate some differences in the relaxation of the meso protons similar to those shown for **1**, however, the differences are much smaller, as given in Table 2.

Zn(II) complex of **2** : Figure 7 shows that the resonances in the NMR spectrum of the Zn(II) complex of **2** are better resolved than was the case for the free base. In addition, the relative positions of the resonances have changed (Table 3). The methyl group in 3 position resonates at lowest field in both forms, but the position of the methyl groups in 1- and 8 positions have interchanged in going from the free base to the Zn(II) complex of **2** at this particular concentration.

Table 3: Chemical shifts (ppm) for the Zn(II)-complex of **2** .

Group	Chemical shift
α	9.60
β	9.41
δ	9.27
Methyl-1	3.44
Methyl-3	3.48
Methyl-5	3.35
Methyl-8	3.18
α-isocyclic	3.70
β-isocyclic	4.71

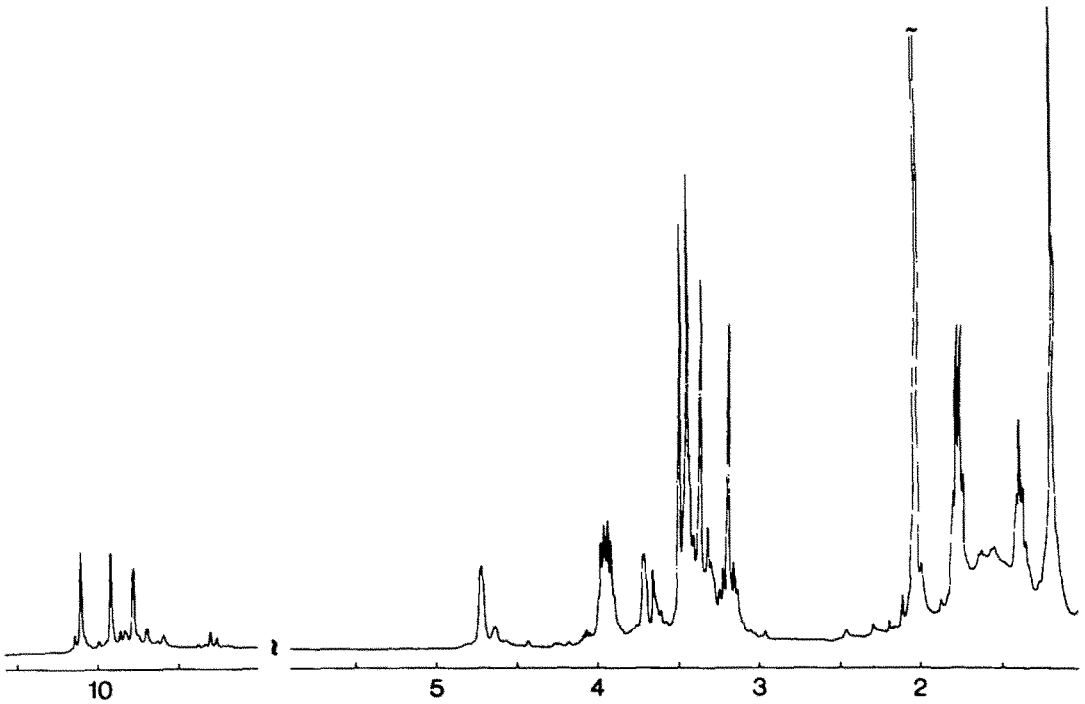

Fig. 7: NMR spectrum of the Zn(II) complex of **2**. The sample is contaminated by approx. 20% of other porphyrins. The resonance at 2.1 ppm is due to acetate, and the resonance at 1.25 ppm is due to water.

The improved shift dispersion, particularly for the methyl groups and the shift to higher field for the multiplet in the isocyclic ring, facilitates the interpretation of the results. The resonance from the methylene protons of the ethyl group in the 7 position has shifted upfield as well, and appears now in the methyl region as shown in the 2D COSY-90 spectrum in Figure 8, where there is a crosspeak at 1.38 ppm showing the scalar coupling between the methylene and the methyl in the ethyl substituent. We also observe a crosspeak (1.77-1.78 ppm) representing the coupling between the methylenes and the methyl groups in 2- and 4 positions. At 3.70 ppm another crosspeak appears due to the scalar coupling between the α- and β -protons of the five membered isocyclic ring. The COSY spectrum even reveals a coupling between the α-protons of the isocyclic ring and the methyl group in the 5 position. The rest of the crosspeaks are due to impurities in the sample. This experiment was not set up for observation of small, long range couplings, still it is possible to observe some small crosspeaks for coupling between meso protons and methyl groups. These long range couplings explain the variation in intensities of the methyl resonances, even though their T_2 values are approximately equal, which should give almost the same half-height line width. We assume that the T_1's are approximately equal to the T_2's in the porphyrins we have investigated.

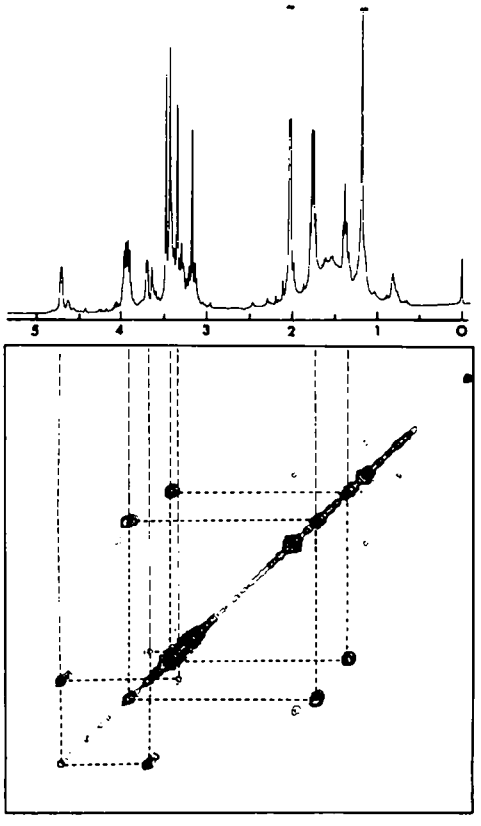

Fig. 8: COSY-90 spectrum of the Zn(II) complex of **2**, in the range of 5.0-0.0 ppm.

The nOe results are summarized in Table 4 for the meso protons of the Zn(II) complex of **2**.

The results do not represent maximum enhancements because of incomplete presaturation. The nOe from a methyl group to a neighbouring meso proton range from 7.7-8.8%. Because of the small difference in chemical shift for some of the methyl groups, selective irradiation of one of the methyl resonances is difficult to achieve. Irradiation of one resonance therefore results in a easily interpretable effect to the neighbouring meso proton and smaller effects to other meso protons due to the low selectivity of the irradiation.

Table 4: nOe for meso protons of the Zn(II) complex of **2**.

Resonance irradiated	Chemical shift (ppm)	% nOe		
		α-meso	β-meso	δ-meso
Methyl-1	3.44	0.8	1.2	7.8
Methyl-3	3.48	7.9	0.0	0.9
Methyl-5	3.35	0.9	7.7	1.8
Methyl-8	3.18	0.0	0.3	8.8

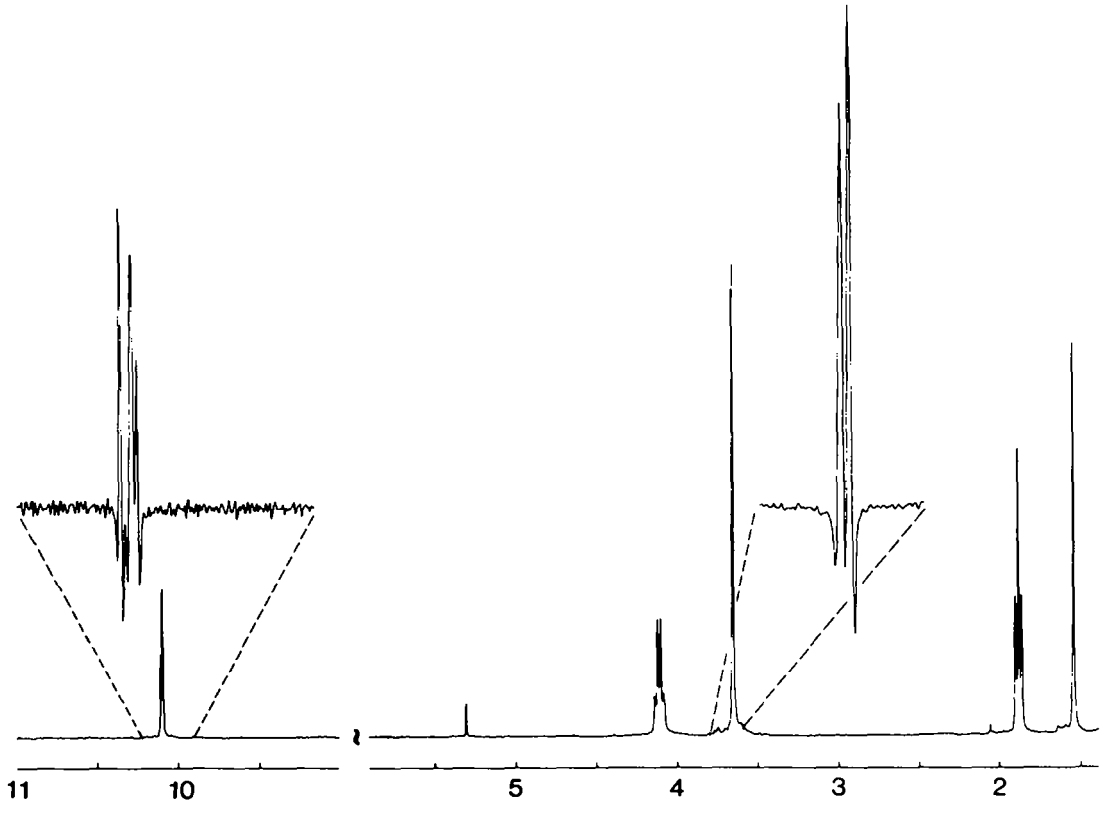

Fig. 9: NMR spectrum of C_{32} ETIO porphyrin, the insert show the meso protons and the methyl groups after resolution enhancement using gaussian multiplication.

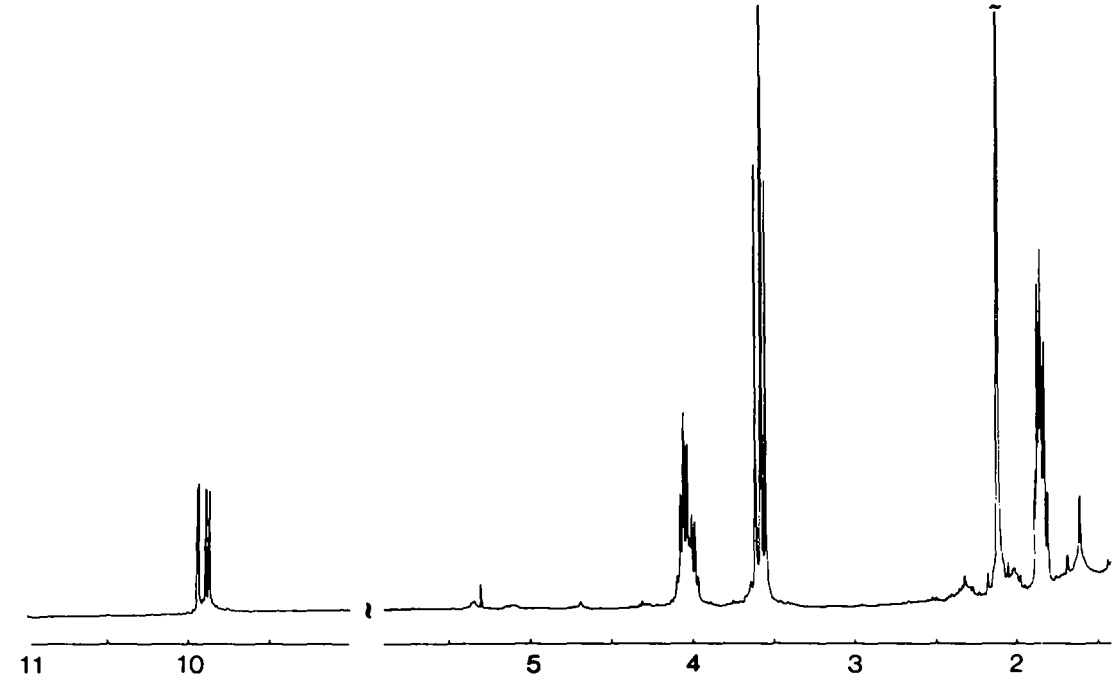

Fig. 10: NMR spectrum of the Zn(II) complex of **3**. The resonance at 2.1 ppm is due to acetate.

C_{32} ETIO Porphyrin (**3**): The mass spectrum of this compound gives a molecular ion at M/e 478, consistent with a ETIO type porphyrin with 32 carbon atoms. The NMR spectrum of **3** is shown in Figure 9. The results for the free base are easily interpreted, but give little structural information. Integration shows four methyl groups, four ethyl groups and four meso protons. Both the ethyl and the methyl group resonances are poorly resolved. Gaussian multiplication gives some resolution enhancement and reveals three meso and two methyl resonances. Structural elucidation based on this spectrum would be extremely difficult, if not impossible.

Zn(II) complex of **3**: The NMR spectrum becomes almost completely resolved after addition of one mol. equiv. of Zn(II) acetate, as shown in Figure 10, and the structural assignment using nOe difference spectroscopy is now relatively straightforward. The substitution pattern of **3** is given in Figure 11, together with the chemical shifts for the particular concentration used. The nOe results are summarized in Table 5. The structure of **3** is identical with that of the ETIO III porphyrin[11].

The conclusion is based on nOe, but care must be exercised in interpreting the results. Due to the small shift dispersion of the methyl groups, the selectivity is reduced. Irradiation of the methyl group in the 3 position, at 3.55 ppm, affects the methyls resonating at 3.53 and 3.56 ppm. Ideally, when irradiating the methyl group at 3.55 ppm, one should observe a nOe to the α-meso proton only, but due to the closeness in shift to the methyl groups at 3.53 and 3.56 ppm, which are positioned on either side of the δ-meso proton in the structure, an even larger nOe is observed to this proton. With this irradiating power there is even a small disturbance of the ethyl groups which causes a small nOe to the γ-meso proton.

Table 5: nOe results for the Zn(II) complex of **3**.

Resonance irradiated	Chemical shifts (ppm)	% nOe			
		α-meso	β-meso	γ-meso	δ-meso
Methyl-1	3.53	3.2	1.1	0.0	13.0
Methyl-3	3.55	9.4	1.6	0.6	14.5
Methyl-5	3.60	2.4	9.6	1.3	4.1
Methyl-8	3.56	9.1	2.5	0.7	14.3
Methylene-2	3.97	5.2	0.0	0.0	0.0
Methylene-6	4.05	0.0	4.5	9.0	0.0
Methylene-4/7	4.03	0.0	7.4	9.8	0.0

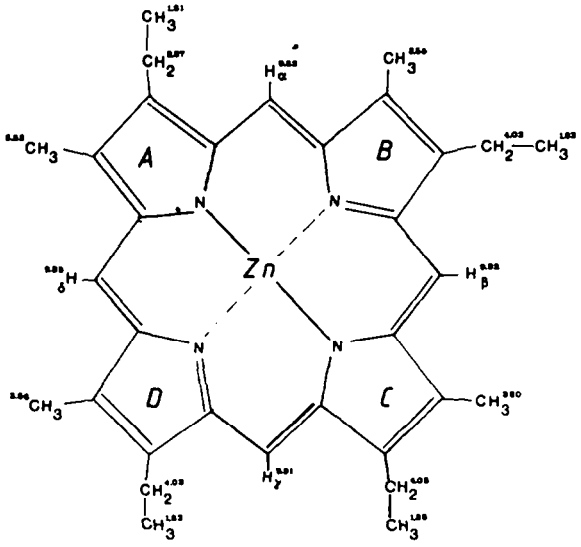

Fig. 11: Structure of the Zn(II) complex of **3** with chemical shifts in ppm.

The T_1 values for the Zn(II) complex of **3** show an excellent agreement between expected and observed values. The δ-meso proton positioned between the two methyl groups in the 1- and 8 positions has the longer T_1 value, while the γ-meso proton positioned between the two ethyl groups in the 6- and 7 positions has the shorter T_1 value. The α- and β-meso protons, which have identical neighbouring substituents have intermediate T_1 values. The T_1 values are listed in Table 2.

Addition of 2 mol. equiv. of pyrrolidine gives a disaggregated complex and the NMR spectrum shown in Figure 12 is very similar to that of the free base (Figure 9) and shows the limiting chemical shifts.

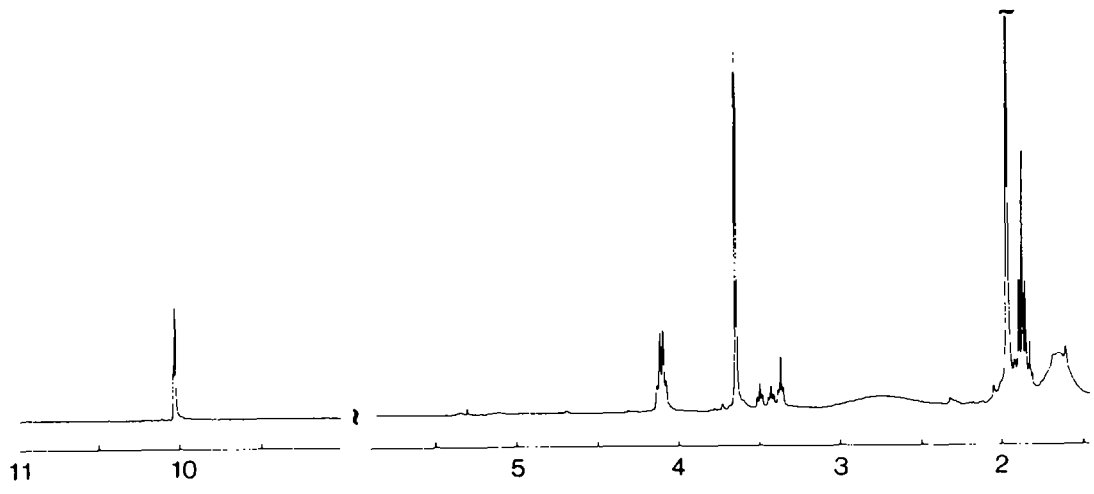

Fig. 12: NMR spectrum of the pyrrolidine Zn(II) complex of **3**. The resonance at 1.95 ppm is due to water.

CONCLUSION

These results taken together provide an almost overdetermined set of assignments for **1**, **2** and **3**. There is obviously no need to resort to chemical modification or biosynthetic labelling.

We have utilized the described procedure to determine a whole series of petroporphyrin structures, which will be published in the near future.

Acknowledgements- We wish to thank Dr. A.G. Douglas at the Organic Geochemistry Unit, University of Newcastle upon Tyne for supplying extracts from Marl Slate, and the Norwegian Research Council for Science and the Humanities (NAVF) for a grant towards the purchase of the NMR spectrometer.

REFERENCES

[1] E.W. Baker, J. Am. Chem. Soc., 88, 2311 (1966).

[2] E.W. Baker and S.E. Palmer, "Geochemistry of Porphyrins" in "The Porphyrins", D. Dolphin (Ed.), Academic Press, New York, N.Y., Vol. I, pp. 485, (1978).

[3] J.M.E. Quirke, G.J. Shaw, P.D. Soper and J.R. Maxwell, Tetrahedron, 36, 3261 (1980).

[4] T.F. Yen, L.J. Boucher, J.P. Dickie, E.C. Tynan and G.B. Vaughan, J. Inst. Pet. (London), 55, 87 (1969).

[5] A. Ekstrom, H. Loeh and L. Dale, "Symposium on Geochemistry and Chemistry of Oil Shales", Amer. Chem. Soc., Seattle 1983, pp. 166.

[6] G.J. Shaw, J.M.E. Quirke and G. Eglinton, J. Chem. Soc. Perkin I, 1655 (1978).

[7] G.W. Hodgson and B.L. Baker, Chem. Geol., 2, 187 (1967).

[8] J.J. Katz, L.L. Shipman, T.M. Cotton and T.R. Janson, "Chlorophyll Aggregation. Coordination Interactions in Chlorophyll Monomers, Dimers and Oligomers" in "The Porphyrins", D. Dolphin (Ed.), Acedemic Press, New York, N.Y., Vol. V, pp. 401 (1978).

[9] R.J. Abraham, S.C.M. Fell and H. Pearson, Tetrahedron, 35, 1759 (1979).

[10] J.M.E. Quirke, J.R. Maxwell, G. Eglinton and J.K.M. Sanders, Tetrahedron Letters, 21, 2987 (1980).

[11] J.M.E. Quirke and J.R. Maxwell, Tetrahedron, 36, 3453 (1980).

[12] J.M.E. Quirke, G. Eglinton and J.R. Maxwell, J. Am. Chem. Soc., 101 7693 (1979).

[13] J.K.M. Sanders, J.C. Waterton and I.S. Denniss, J. Chem. Soc. Perkin I, 1150 (1978).

[14] A. Treibs, Ann., 509, 103 (1934).

[15] A. Treibs, Angew. Chemie, 49, 682 (1936).