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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF FREE-BASE PORPHYRINS

II. STRUCTURE EFFECTS AND RETENTION BEHAVIOUR

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SUMMARY

The high-performance liquid chromatographic separations, on 3-μm silica, of a variety of free-base alkyl porphyrins of the type isolated from sediments and petroleums, and of a number of their acetylated counterparts, have been compared. Members of pseudohomologous series and structural isomers of both aetioporphyrins and cycloalkano porphyrins are readily separable, although the method appears to be less effective for the separation of positional isomers. Substitution of a β-methyl substituent for a β-hydrogen atom results in an increase in t_R, presumably partly as a result of increased basicity. The effect of the introduction of an acetyl substituent is to shorten t_R in the cycloalkano components, despite the polarity increase, but to increase t_R in the aetio components. The differences in t_R are explicable, therefore, in terms of an interplay between basicity and polarity, brought about by changes in the structural features of the substituents. The availability of isolated components for coinjection provides a method for routine studies of the distributions of the components of sediments and petroleums.

INTRODUCTION

The preceding communication[1] describes a high-resolution high-performance liquid chromatographic (HPLC) method, using 3-μm silica, for the analysis of free-base alkyl porphyrins of the type obtained after demetallation of the metalloporphyrins typically found in sedimentary organic matter. Rapid and efficient separations are obtained by addition of varying amounts of both acetic acid and pyridine to the mobile phase and it is apparent that, in general, the retention times of components with an exocyclic alkano ring (*e.g.* 1a) are longer than those without such a structural

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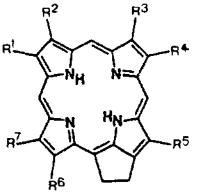
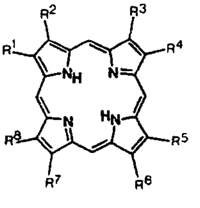
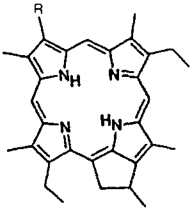
feature (*e.g.* 2a). This behaviour was attributed mainly to an increase in basicity for the cycloalkano species.



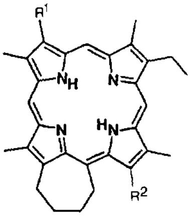
1a: $R^{1,3,5,7} = CH_3$, $R^{2,4,6} = CH_2CH_3$
1b: $R^{1-3,5,7} = CH_3$, $R^{4,6} = CH_2CH_3$
1c: $R^{1,5,7} = CH_3$, $R^{2,4,6} = CH_2CH_3$, $R^3 = H$
1d: $R^{1,5,7} = CH_3$, $R^{2,4,6} = CH_2CH_3$, $R^3 = COCH_3$



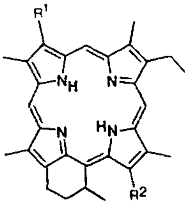
2a: $R^{1,3,5,8} = CH_3$, $R^{2,4,6,7} = CH_2CH_3$
2b: $R^{2,4,5,7,8} = CH_3$, $R^{1,3,6} = CH_2CH_3$ or
$R^{2,4,6-8} = CH_3$, $R^{1,3,5} = CH_2CH_3$ or
$R^{2-5,8} = CH_3$, $R^{1,6,7} = CH_2CH_3$ or
$R^{1,3-5,8} = CH_3$, $R^{2,6,7} = CH_2CH_3$
2c: $R^{1-3,5-7} = CH_3$, $R^{4,8} = CH_2CH_3$ or
$R^{1-3,5,6,8} = CH_3$, $R^{4,7} = CH_2CH_3$
2d: $R^{1-6,8} = CH_3$, $R^7 = CH_2CH_3$
2e: $R^{1-8} = CH_2CH_3$
2f: $R^{1,3,5,8} = CH_3$, $R^{2,4,7} = CH_2CH_3$, $R^6 = H$
2g: $R^{1,3,5,7} = CH_3$, $R^{2,4,6,8} = CH_2CH_3$
2h: $R^{1,3,5,8} = CH_3$, $R^{2,4,6} = CH_2CH_3$, $R^7 = H$
2i: $R^{1,3,5,8} = CH_3$, $R^{2,6,7} = CH_2CH_3$, $R^4 = H$
2j: $R^{1,3,5,8} = CH_3$, $R^{4,6,7} = CH_2CH_3$, $R^2 = H$
2k: $R^{1,3,5,8} = CH_3$, $R^{2,4,7} = CH_2CH_3$, $R^6 = COCH_3$



3a: $R = CH_2CH_3$
3b: $R = CH_3$



4a: $R^1 = CH_2CH_3$, $R^2 = H$
4b: $R^1 = CH_3$, $R^2 = H$
4c: $R^1 = CH_2CH_3$, $R^2 = COCH_3$
4d: $R^1 = CH_3$, $R^2 = COCH_3$



5a: $R^1 = CH_2CH_3$, $R^2 = H$
5b: $R^1 = CH_3$, $R^2 = H$
5c: $R^1 = CH_2CH_3$, $R^2 = COCH_3$
5d: $R^1 = CH_3$, $R^2 = COCH_3$

In the present study we have examined the separation of a wide variety of structural types of alkyl porphyrins and acetyl derivatives, and an attempt has been made to rationalise the retention time behaviour in terms of differences in the structural features.

EXPERIMENTAL

Isolation of sedimentary porphyrins

The total metalloporphyrins were extracted from Gilsonite bitumen and Serpiano oil shale as described previously[2–4]. The Friedel–Crafts acetylation procedure used for the separation of β-unsubstituted components has also been described previously[4–6]. Preparative-scale HPLC afforded a number of compounds which were further purified by zinc(II) insertion, thin-layer chromatography and removal of volatile impurities under high vacuum; the zinc complexes were then analysed by ^{1}H NMR[2–8].

HPLC analyses

HPLC analyses were performed using a Spectra-Physics SP8700 ternary solvent delivery system and a Rheodyne 7125 injector valve, with 10-μl (analytical scale) or 200-μl (preparative scale) injection loops. Detection (400 nm) was obtained using an LDC 1202 spectromonitor II variable-wavelength detector. The analytical-scale conditions were as described in the preceding communication[1]. The preparative-scale conditions were similar, except that there were minor changes in the solvent programme (Table I) and the analyses were performed on Spherisorb S 5W (250 × 10 mm I.D.); back-pressure (*ca.* 800 p.s.i.) and flow-rate (5.0 ml min^{-1}) were kept constant throughout.

TABLE I

PROGRAMME FOR TERNARY SOLVENT SYSTEM USED IN PREPARATIVE-SCALE ANALYSES OF SEDIMENTARY PORPHYRINS

Solvents (doubly distilled): A = methylene chloride–acetone (4:1); B = hexane–pyridine (99:1); C = hexane–glacial acetic acid (99:1).

Time (min)	*Solvent*		
	A (%)	*B (%)*	*C (%)*
0	15	15	70
14	15	15	70
20	25	25	50
25	25	25	50
30	15	15	70
35	15	15	70

RESULTS AND DISCUSSION

The structures of a variety of demetallated alkyl porphyrins isolated from Gilsonite bitumen (Eocene) and Serpiano oil shale (Triassic) have been determined[2–7] using NMR methods, after isolation of the individual components by HPLC under preparative conditions. Most of the separations are illustrated by Fig. 1. Fig. 1a shows the distribution of fully alkylated demetallated porphyrins from Serpiano shale, along with the retention positions marked for selected Gilsonite components

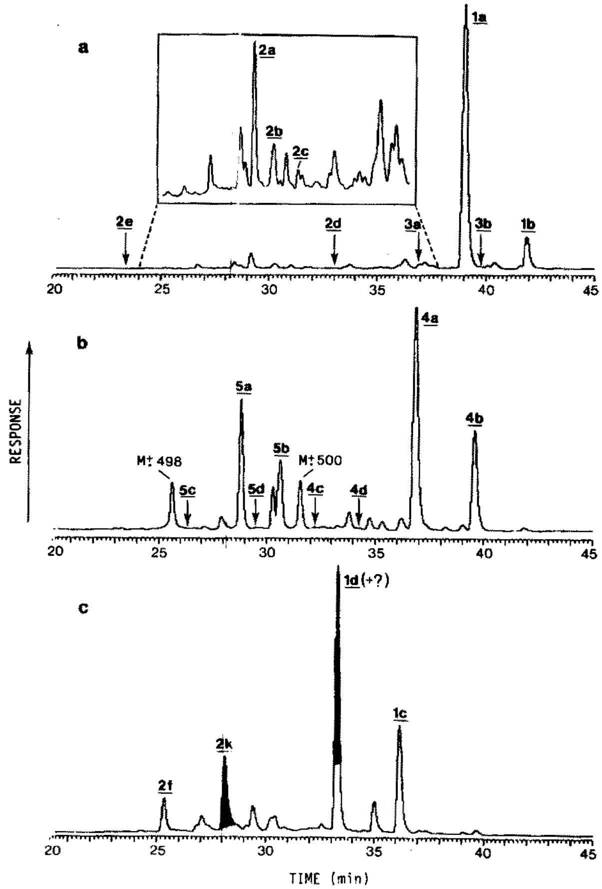


Fig. 1. Analytical-scale HPLC chromatograms (3-μm silica) of Serpiano oil shale demetallated vanadyl porphyrins. (a) Components fully alkylated at β positions, with retention positions of octaethylporphyrin (2e) and selected components from Gilsonite bitumen marked. (b) Components with a β-hydrogen substituent, with retention positions of selected acetylated counterparts marked. (c) Components with a β-hydrogen substituent, and acetylated counterparts (shaded). For conditions see Experimental.

which do not occur in Serpiano shale. Fig. 1b shows components present in a fraction obtained by acetylation of the total demetallated porphyrins of Serpiano shale, followed by deacetylation with methanesulphonic acid; the retention positions of a number of components prior to removal of the acetyl group are shown for comparison. Fig. 1c shows a similar fraction which contains both components with an acetyl substituent (*i.e.* from incomplete deacetylation) and their deacetylated counterparts. A number of points can be discussed in the light of these distributions and the separations achieved.

Separation of carbon numbers within series

Within a pseudohomologous series it is apparent that the effect of increasing the molecular weight by addition of a methyl group to a β-alkyl substituent is to decrease the retention time (Fig. 1a), as suggested previously[9]. Hence, within the fully alkylated aetioporphyrins t_R C_{32} (2a) < t_R C_{31} (2b) < t_R C_{30} (2c) < t_R C_{29} (2d). Furthermore, the t_R of octaethylporphyrin (C_{36}; 2e), which is not known to occur in sedimentary organic matter, is far shorter than those of these four sedimentary components (Fig. 1a). The same effect is observed for the various pairs of fully alkylated cycloalkano species [compare t_R values of 1a (C_{32}) and 3a (C_{33}) with those of 1b (C_{31}) and 3b (C_{32}) in Fig. 1a]. It is also apparent for components with a β-hydrogen substituent, as seen in Fig. 1b for the pairs 4a (C_{32}) and 4b (C_{31}) and 5a (C_{32}) and 5b (C_{31}), and for their acetyl derivatives (see below).

This decrease in t_R, from extending the alkyl substituents, appears to result from a decrease in polarity, as implied previously[9].

Separation of structural isomers

The relatively short t_R values on silica of alkylporphyrins with a β-hydrogen substituent have been noticed previously[5,9–11]. For a given carbon number, a cycloalkano porphyrin with a β-hydrogen substituent has a shorter t_R than its fully alkylated counterpart. Thus, the two C_{32} mono β-hydrogen components (4a and 5a) with a seven-membered exocyclic ring and a methyl-substituted, six-membered exocyclic ring elute well before the C_{32} fully alkylated component with a five-membered exocyclic ring (1a). A similar situation exists for the C_{31} homologues (4b, 5b and 1b). That the t_R difference cannot be solely the result of the changes in the exocyclic ring size comes from comparison of the isomeric C_{31} components 1b (Fig. 1a) and 1c (Fig. 1c), each with a five-membered ring. The presence of the β-hydrogen in 1c shortens t_R, in addition to the expected reduction for the presence of an ethyl substituent in 1c in place of a methyl substituent in 1b. The same behaviour can be observed for the C_{30} fully alkylated aetioporphyrin (2c in Fig. 1a) and the C_{30} mono β-hydrogen species (2f in Fig. 1c).

Calculation of equilibrium constants for porphyrins with different substituents have shown that electron-donating groups increase the basicity towards proton acceptance[12]. It was also shown in our previous paper[1] that addition of small amounts of pyridine to the HPLC solvent system improves chromatographic behaviour by way of reducing peak broadening and shortening of retention times, indicating the importance of basicity. Thus, the equilibrium between the porphyrin mono-cation (PH_3^+) and the free base (PH_2) appears to be important under the HPLC conditions used. It seems reasonable to suggest, therefore, that the substitution of an electron-donating methyl group for a β-hydrogen substituent increases the t_R value by increasing the basicity. In this context, it is interesting to note that, under these HPLC conditions, the di-cations (PH_4^{2+}) are barely retained on the column, eluting at a point corresponding to the dead volume[13].

It is difficult to rationalise completely the effect of changes in the exocyclic ring structure in the cycloalkano porphyrins. Components with a five-membered ring would be expected to have, in the absence of other structural differences, longer retention times (as appears to be the case) than those with a six- or seven-membered ring, since the strained five-membered ring is responsible for reducing the planarity of the porphyrin ring system[14] and, thus, for increasing the basicity[15]. It is interesting also to consider the relative t_R values (Fig. 1a) of the C_{32} 13,15-ethanoporphyrin (1a) and the C_{32} 13^1-methyl-13,15-ethanoporphyrin (3b). In the absence of any other structural difference, the presence of a β-methyl at C-3 instead of an ethyl substituent would be expected to increase markedly the t_R value (*cf.* t_R of 1a and 1b). The t_R value of 3b is, however, only slightly greater than that of 1a, presumably as a result of the presence of the 13^1-methyl substituent countering this effect in the same manner to a similar extent. On the other hand, the two components with a methyl-substituted, six-membered exocyclic ring (5a, 5b) have short t_R values in comparison with their seven-membered ring counterparts (4a, 4b). It appears that the components with the methyl-substituted, six-membered ring are less strained; hence, it would be expected that the porphyrin nucleus is more planar and the components less basic. In addition, the methyl substituent is likely to be in an axial position, causing a decrease in the interaction between the nitrogens and the stationary phase.

It is difficult to separate structural isomers having the same alkyl substituents (*i.e.* positional isomers) since the differences in basicity and polarity are minimal. This is illustrated in Fig. 2, with the partial separation of aetioporphyrins III (2a) and I (2g) on 5-μm silica. Under conditions identical with those in Fig. 1, the C_{30} aetioporphyrin (mainly 2f) isolated from Serpiano oil shale showed essentially one peak (Fig. 3a); under reversed-phase conditions[16] (courtesy of Professor A. H. Jackson) it was partially separated into four peaks (Fig. 3c), indicating that minor amounts of the other three positional isomers (2h, 2i, 2j) could be present (*cf.* Fig. 3b). The suggestion that reversed-phase HPLC might be more efficient for separation of positional isomers of alkyl porphyrins has been made previously[11].

Effect of acetylation

The acetylated cycloalkano porphyrin derivatives have shorter t_R values than their parent compounds, irrespective of the structural type. The observed t_R values of several acetyl derivatives (5c, 5d, 4c, 4d) are indicated by arrows in Fig. 1b for ready comparison with those of their parent compounds (5a, 5b, 4a, 4b). In Fig. 1c the t_R value of the acetyl derivative (1d) is clearly shorter than the parent compound

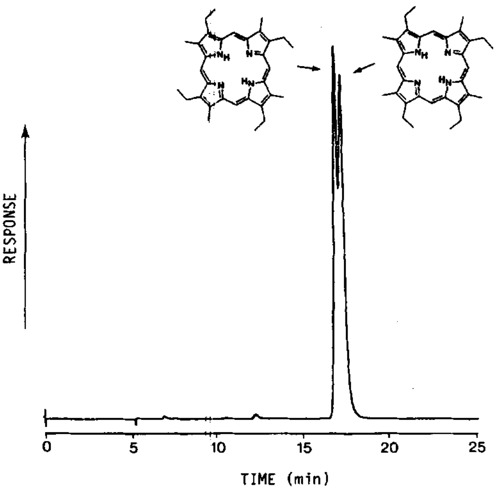


Fig. 2. Analytical-scale HPLC chromatograms (5-μm silica; three 250 × 4.6 mm I.D. columns) of a mixture of aetioporphyrins III and I (2a and 2g, respectively). Otherwise, conditions as for Fig. 1.

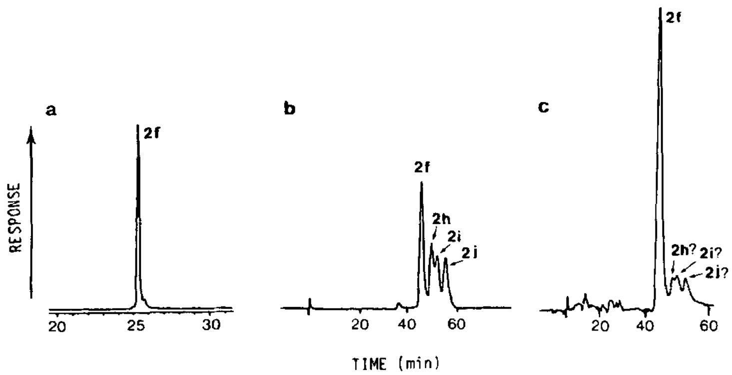


Fig. 3. Analytical-scale HPLC chromatograms. (a) Component 2f from Serpiano shale under normal phase (3-μm silica) conditions; other conditions as for Fig. 1. (b) A mixture of synthetic isomers under reversed-phase conditions (PE ODS HC-Sil x-1 10W, acetonitrile, flow-rate 0.2 ml min^{-1}). (c) Component 2f under reversed-phase conditions (as for b).

(1c). The separations are explicable in terms of differences in basicity in bringing about the separations. The electron-withdrawing effect of the acetyl substituent causes a reduction in basicity[12] resulting in a shortening of the t_R value, despite the increase in polarity (as shown by the lower R_F value of the acetyl derivatives on silica gel with methylene chloride as developer[5,8]). The opposite behaviour is observed for the C_{30} acetylated aetioporphyrin (2k), *i.e.* the parent compound (2f) has the lower t_R value (Fig. 1c). In this case the polarity increase resulting from the introduction of the acetyl substituent into the more planar aetioporphyrin (which presumably has less interaction with the stationary phase) appears to be more important than a reduction in basicity. These results emphasise the importance of both polarity and basicity changes in bringing about the separations.

Isolation of individual components using semi-preparative conditions

With the exception of octaethylporphyrin (2e) and aetioporphyrin I (2g), all of the compounds discussed were either obtained from the porphyrin fractions of Serpiano oil shale[3–6,8] shown in Fig. 1, or from Gilsonite bitumen[2,7,10], using semi-preparative HPLC on 5-μm silica. To illustrate the approach, Fig. 4a shows a typical chromatogram of the fraction shown under analytical conditions in Fig. 1b, from which six subfractions were collected at the points shown. Analytical HPLC chromatograms of these subfractions are shown in Fig. 4b; each of the fractions was obtained in sufficient purity (more than 95%) with respect to other alkyl porphyrins to allow structure analysis by probe mass spectrometry and ^{1}H NMR after removal of non-porphyrin impurities by distillation (see Experimental). This resulted in assignment of 5a, 5b, 4a and 4b and isolation of two other components with molecular ions at *m/z* 498 and 500; structural studies of these components are in progress.

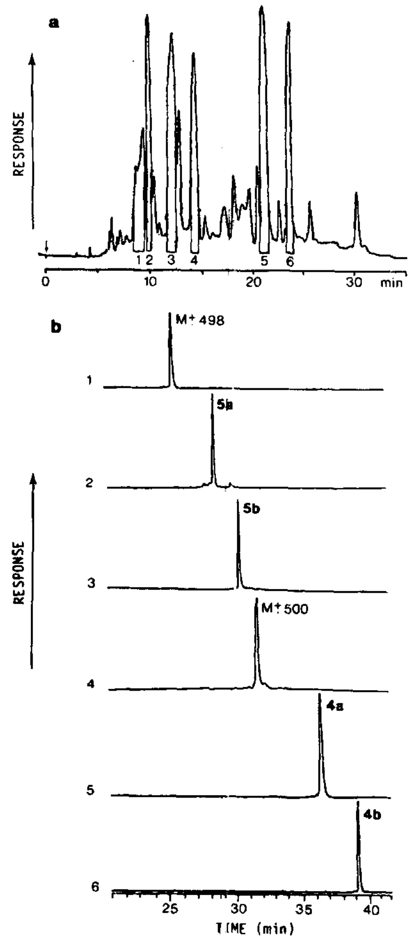


Fig. 4. (a) HPLC chromatograms of fraction in Fig. 1b under semipreparative conditions (5-μm silica) with points of collection of six fractions indicated (1–6); for conditions, see Experimental. (b) Analytical-scale HPLC chromatograms (3-μm silica) of the individual fractions; conditions as for Fig. 1.

CONCLUSIONS

(1) Normal-phase HPLC on 3-μm silica is confirmed as a valuable analytical tool in the separation of the free-base alkyl porphyrins obtained by demetallation of the complex mixtures of metalloporphyrins present in sediments and petroleums.

(2) Of the compounds examined, both members of pseudohomologous series and structural isomers are readily separable, although the method appears to be less effective than reversed-phase HPLC for separation of positional isomers.

(3) Comparison of the structural features of the various components, and of the components with a β-hydrogen substituent with their acetyl derivatives indicates that the separations under the solvent conditions used result from differences in basicity and polarity.

(4) Use of the method under preparative conditions has already allowed the isolation of more than fourteen components from the Triassic Serpiano oil shale and the Eocene Gilsonite bitumen, the majority of them in more than 95% purity.

(5) Using the isolated components for coinjection, the method allows the distributions ("fingerprints") of the demetallated porphyrins from sediments and petroleum to be compared on a routine basis.

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