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COMPUTERISED GAS CHROMATOGRAPHIC–MASS SPECTROMETRIC AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF SEDIMENTARY BENZOPORPHYRINS

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SUMMARY

Recently developed gas chromatographic–mass spectrometric (GC–MS) and high-performance liquid chromatographic (HPLC) procedures for the analysis of petroporphyrins have been employed in the search for benzoporphyrins in six sediments, a bitumen and a petroleum. In addition, structurally assigned mono-benzocycloalkanoporphyrins (benzo-CAPs) isolated from Boscan crude oil (Cretaceous, Venezuela) have been used in a co-chromatographic study of these minor components (*ca.* 1% of total porphyrins). The petroporphyrins are generally obtained by demetallation of metalloporphyrins from samples representing a variety of geographical locations and ages ranging from Permian (*ca.* 235 million years) to Palaeocene (*ca.* 60 million years). The data show that, where the benzo-CAPs were detected, up to 24 components can occur, although the dominant member in each case is the C₃₃ compound. Studies of a chromatographic fraction from Boscan crude oil, enriched in high-carbon-number benzo-CAPs, indicates that the series extends from at least C₃₀ to C₃₈ in this sample. The GC behaviour of the benzo-CAPs as the bis(*tert.*-butyldimethylsiloxy)Si(IV) [(TBDMSO)₂Si(IV)] derivatives is characterised by the high Kováts retention indices (*ca.* 3900–4300; OV-1 column). Similarly, under normal-phase HPLC conditions, the free-base benzo-CAPs elute after the major alkylporphyrins. HPLC co-chromatography of the free-bases provides a rapid means of identification of the major benzo-CAPs (C₃₂ and C₃₃), while the GC–MS procedure allows more detailed characterisation and quantitation of the benzo-CAP distributions as their (TBDMSO)₂Si(IV) derivatives.

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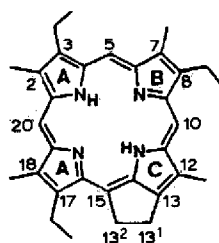
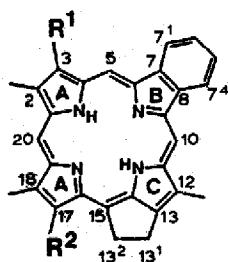
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INTRODUCTION

Computerised gas chromatography–mass spectrometry (GC–MS) of porphyrins as their bis(*tert.*-butyldimethylsiloxy)Si(IV) [(TBDMSO)₂Si(IV)] derivatives has been demonstrated as a useful means of analysis of the complex mixtures found in sedimentary organic matter^{1–5}. High-performance liquid chromatography (HPLC) of free-base porphyrins on 3- μ m silica has been reported as a complementary method of analysis^{6,7}. Both techniques provide a convenient means of identifying specific petroporphyrin components in complex mixtures via co-chromatography with compounds of known structure. Such an approach allows assignment of components without the necessity of time-consuming isolation of individual components. The structural assignment of selected members of the petroporphyrins is an essential step in the geochemical interpretation of the overall distributions⁸.

The occurrence of so-called “rhodoporphyrins” (structural class⁴ A-6, A-8) has been widely reported in petroporphyrin mixtures^{9–13}. Their presence has been inferred from the distinctive rhodo-type electronic absorption spectra⁹ and/or molecular ions of appropriate masses in MS¹⁰ and GC/MS⁵ studies. More recently, structural assignment of isolated rhodoporphyrin components from Boscan crude oil (Cretaceous, Venezuela), using ¹H NMR techniques, has identified them as monobenzo-cycloalkanoporphyrins (benzo-CAPs, 1–4, structural class⁴ A-8).



(5)

C₃₂ (2) : R¹ = methyl , R² = ethyl

C₃₃ (1) : R¹ = ethyl , R² = ethyl

C₃₄ (3) : R¹ = ethyl , R² = propyl

C₃₅ (4) : R¹ = isobutyl , R² = ethyl

We report here the occurrence of benzo-CAPs in a variety of petroporphyrin distributions via computerised GC–MS and HPLC analysis of the (TBDMSO)₂Si(IV) derivatives and free-bases, respectively, and co-chromatography with components obtained from Boscan crude oil. Earlier studies of the petroporphyrins of Boscan oil^{2,5} are extended by investigation of a fraction enriched in the high-carbon-number (> C₃₄) benzo-CAPs.

EXPERIMENTAL

Samples

The sediment samples investigated (Table I) were as follows: Gafsa chert (Palaeocene, Tunisia), Kimmeridge shale (Late Jurassic, U.K.), Serpiano shale (Middle Triassic, Switzerland), Julia Creek oil shale (Early Cretaceous, Australia), Marl slate (Permian, U.K.), El Lajjun shale (Late Cretaceous, Jordan), La Luna shale (Cretaceous, Venezuela); Gafsa chert is the least mature and La Luna the most mature sample. The other sediments are listed in a crude order of increasing maturity. The other samples were Gilsonite bitumen (Eocene, U.S.A.) and Boscan crude oil (Venezuela). The geological descriptions of the samples have been given elsewhere¹⁵⁻²². The depositional environments are all believed to be marine except for the source rock of Gilsonite bitumen, which is believed to have been deposited under saline lacustrine conditions²¹.

TABLE I

GEOLOGICAL SAMPLES INVESTIGATED FOR PRESENCE OF BENZO-CAPs BY GC-MS AND/OR HPLC CO-CHROMATOGRAPHY STUDIES

N.D. = not determined; F.B. = free base.

Sample	F.B. (%)	Ni (%)	V=O (%)	Fraction examined by	
				GC-MS	HPLC
Boscan oil	0	5	95	Total	Total
La Luna shale	0	5	95	Total	Total
Gilsonite bitumen	0	95	5	Total	Total
Marl Slate	0	67	33	Ni, V=O	Ni
El Lajjun shale	0	57	43	Ni	N.D.
Julia Creek oil shale	0	5	95	Total	Total
Serpiano shale	0	5	95	Total	Total
Kimmeridge shale	0	95	5	Total	N.D.
Gafsa chert	95	5	0	F.B.	F.B.

Isolation of porphyrins

After pulverisation (Tema mill), the samples were Soxhlet extracted [72 h, methanol-dichloromethane (1:4, v/v)] and the extracts examined by UV-VIS (dichloromethane) spectrophotometry to determine the complexing metal (V=O, λ_{\max} 530, 570 nm; Ni, λ_{\max} 510, 550 nm; free-bases, λ_{\max} 500, 530, 565, 615 nm. Where one species predominated (>90%; Kimmeridge, Gilsonite, Ni; Serpiano, Julia Creek, La Luna, V=O) the porphyrins were obtained as the free bases using methanesulphonic acid to remove the metal ions according to established procedures²³. Typically, the organic extract (1 g) was heated (V=O, 100°C, 4 h; Ni, 80°C, 1 h) with a 5-10-fold excess of methanesulphonic acid (98%, ca. 5 ml, Aldrich). The reaction mixture was diluted with methane sulphonic acid-deionised water (20:80) (ca. 30 ml), decanted through a moist filter paper and extracted with dichloromethane. The extract was neutralised (saturated sodium bicarbonate), washed with water and dried (toluene azeotrope). The

free bases obtained were purified by thin-layer chromatography (silica gel, acetone-dichloromethane, 5:95).

Where both Ni and V=O porphyrins were detected (Marl slate, 67% Ni, 33% V=O; El Lajjun shale, 57% Ni, 43% V=O) fractionation was achieved by flash chromatography²⁴ over silica, eluting with 20% and 80% dichloromethane in hexane, respectively. The demetallation procedure above was then followed on the separate fractions.

Where free-base porphyrins predominated (>90%; Gafsa chert), the extract was dissolved in diethyl ether and extracted with hydrochloric acid (5 M; 3 × 20 ml). The aqueous phase was extracted with dichloromethane (3 × 20 ml). The extract containing the porphyrin dications was neutralised (sodium bicarbonate) and dried (toluene azeotrope).

Compounds for co-chromatography

The isolation and structural elucidation of the two major benzo-CAPs (**1** and **2**) and the tentative assignment of **3** and **4** from Boscan oil used in this study, has been described elsewhere^{14,25}. In addition, a chromatographic fraction (HMW) enriched in benzo-CAPs (>90% with regard to other porphyrin types), and containing high-molecular-weight components²⁵, was obtained from Boscan oil. For the co-chromatography studies the derivatives of the C₃₃ (**1**) and C₃₂ (**2**) components were added to a derivatised aliquot of fraction HMW to give a mixture (mixture A) with the relative abundances of the benzo-CAPs approximating those in the oil.

HPLC co-injection experiments were restricted to the two major benzo-CAPs (**1** and **2**).

GC-MS analyses

Derivative formation. Established conditions³⁻⁵ for silicon insertion into the individual demetallated benzo-CAPs from Boscan oil (25–50 µg) were slightly modified due to the relatively low solubility of the pure compounds in the solvent (toluene). The reaction was conducted at increased temperature (60°C vs. ambient) and time (72 h vs. 24 h). Formation of the complex was confirmed by UV-VIS spectrophotometry. Problems of solubility were not encountered for the free-base petroporphyrin mixtures obtained after removal of metal ions and the modified conditions were not employed.

Instrumentation. Analyses were performed on a Carlo-Erba HRGC 5160 gas chromatograph with a Grob-type, cooled, on-column injector, linked to a Finnigan 4000 mass spectrometer. Data collection and processing were performed using a Finnigan Incos 2300 data system.

A flexible fused-silica capillary column (Hewlett Packard Ultra series; 25 m × 0.31 µm I.D.) coated with cross-bonded polydimethylsiloxane (0.17 µm film thickness) and helium carrier gas (0.8 kg cm⁻¹) was used. The temperature program and mass spectrometer operating conditions were as described elsewhere³⁻⁵: 50–225°C at 20°C min⁻¹, 225–300°C at 3°C min⁻¹ followed by an isothermal period at 300°C for 40 min. Data were acquired using a Finnigan program which allowed monitoring of the ion *m/z* 113 (*n*-alkanes, see below) in the selected ion monitoring mode and scanning of the range *m/z* 550–850.

Data processing and presentation. Details of the processing of porphyrin GC-MS

data have been described previously^{3-5,26}. Pseudo-Kováts retention indices (KRI) were calculated using program RRI²⁶ employing *n*-alkane (C₂₉-C₄₄) standards. The program allows conversion of GC-MS time-intensity data to a KRI-intensity format. The *n*-alkane standards were supplemented by the (TBDMSO)₂Si(IV) derivative of octaethylporphyrin (OEP) to allow greater reproducibility of KRI calculations⁵. The KRI of this standard was defined as 3800 and the alkane standards were accordingly offset by a constant value (generally <10 KRI units).

Mass chromatograms of the base peak in the porphyrin spectra were used to quantify the components (%) relative to (i) the major benzo-CAP in the distribution and (ii) the major petroporphyrin, generally C₃₂ cycloalkanoporphyrin (CAP), **5**. The presentation of data generally follows earlier formats³⁻⁵.

HPLC analyses

Analyses of the free bases were performed using a Spectra-Physics SP8700 ternary solvent delivery system and a Rheodyne 7125 injector fitted with a 20- μ l loop. Detection (UV-VIS; 405 nm) was obtained using an LCD 1202 Spectromonitor III variable-wavelength detector interfaced with a VG Minichrom data system. Analyses employed three columns (Spherisorb 3W; each 150 \times 4.6 mm I.D.; Phase Separations) in series and a flow-rate of 1.0 ml min⁻¹. A stepped solvent program⁶ involving mixtures of dichloromethane-acetone (4:1), hexane-pyridine (99:1) and hexane-acetic acid (99:1) was used.

RESULTS

Benzoporphyrins in Boscan oil

The partial mass spectrum (Fig. 1) of the (TBDMSO)₂Si(IV) derivative of **1** (the major benzo-CAP), isolated from Boscan 8-E4 oil, illustrates the characteristic

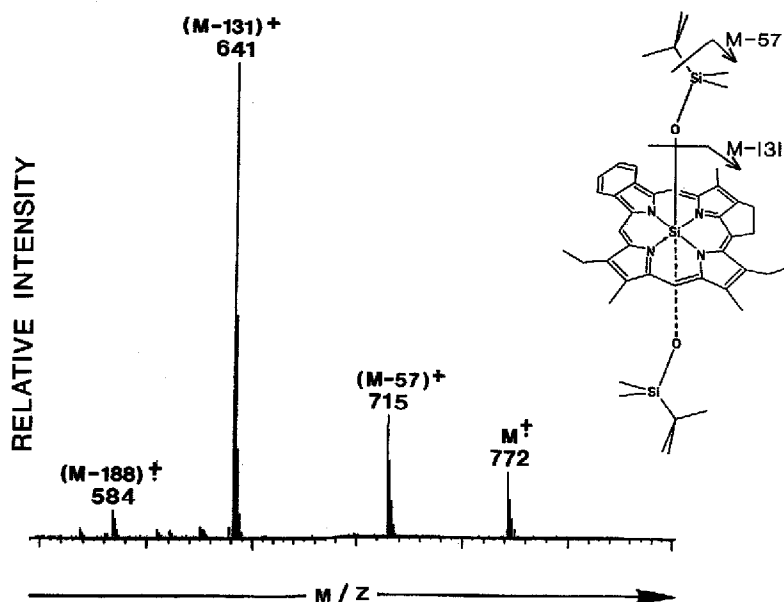


Fig. 1. Partial EI mass spectrum of the (TBDMSO)₂Si(IV) derivative of C₃₃ benzo-CAP (**1**).

fragment ions from cleavage of the TBDMSO ligands, as seen for these derivatives of other porphyrin types^{3–5}. GC–MS analysis revealed a high KRI value (4110), which agrees closely with that obtained for the derivative corresponding formally to C₃₃ benzo-CAP reported previously in the total Boscan distribution⁵. An analogous fragmentation pattern was observed for the derivative of the C₃₂ homologue (**2**; KRI 4070).

Fig. 2 shows the reconstructed ion current (RIC; m/z 550–850) obtained for the total petroporphyrin derivatives. The general resolution and peak shape are not optimal as analysis of the minor benzo-CAP components necessitated a relatively high column loading. The inset shows the partial RIC (m/z 550–850) and the mass spectrum at KRI 4380 for the fraction HMW (see Experimental) enriched in benzoporphyrins, to illustrate the extended carbon number range observed. The mass spectrum at KRI 4380 shows molecular ions at m/z 828 and 842 and major fragments $[M - 131]^+$ and $[M - 57]^+$ corresponding to C₃₇ and C₃₈ benzo-CAPs, respectively. Analysis of the total porphyrin distribution did not detect these components (presumably because of their low abundance), as observed previously⁵.

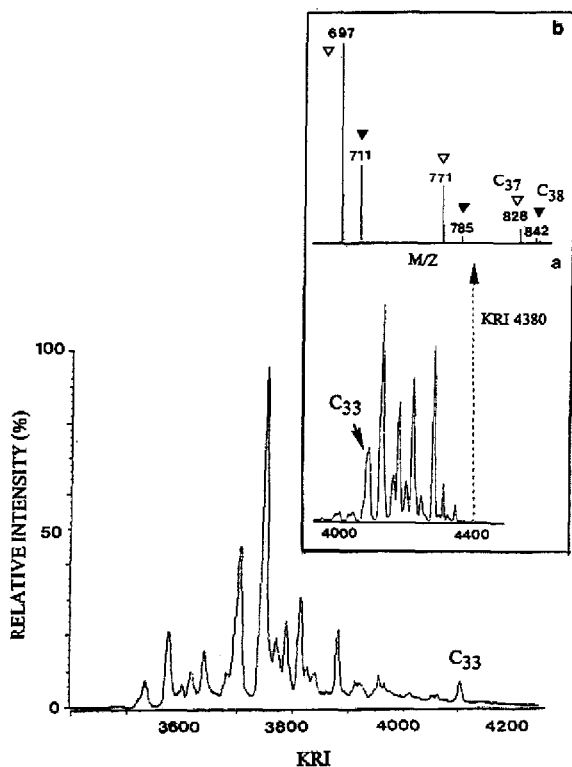


Fig. 2. Reconstructed ion current (RIC) (m/z 550–850) chromatogram of total Boscan petroporphyrins as the (TBDMSO)₂Si(IV) derivatives. The retention time scale has been converted to a KRI scale. Annotated peak indicates compound **1**. Inset shows (a) the RIC for fraction HMW (see text) with component **1** indicated, and (b) mass spectrum from (a) at KRI 4380. Although the RIC intensity at KRI 4380 is low, the presence of components corresponding to C₃₇ (▽) and C₃₈ (▼) benzo-CAP derivatives is indicated by the characteristic mass spectra (*cf.* Fig. 1). ▽: M⁺, 828; [M - 57]⁺, 771; [M - 131]⁺, 697). ▼: M⁺, 842; [M - 57]⁺, 785; [M - 131]⁺, 711.

Single ion mass chromatograms for fraction HMW (see Experimental) were examined for the intense $[M - 131]^+$ ions of all benzo-CAPs in the range C_{29} – C_{40} (Fig. 3). To ensure that the peaks detected arose from "genuine" $[M - 131]^+$ ions, and to eliminate any possible "spurious" peaks³, spectra were obtained, summed sequentially in groups of 20 scans over the entire range of benzo-CAP elution. Thus, benzo-CAPs in the range C_{30} – C_{38} were assigned. The mass chromatograms reveal the presence of structural isomers at each carbon number.

The KRI values of the benzo-CAP derivatives are high (*ca.* 3900–4400) compared to those of cycloalkano-type porphyrins (*e.g.* C_{32} CAP, **5**; KRI 3755; *cf.* Fig. 3). A combined Kovat's plot, illustrating the benzo-CAP derivatives detected in the total Boscan petroporphyrin distribution and the additional high-carbon-number (C_{36} – C_{38}) isomers detected in fraction HMW, is shown in Fig. 4a. Quantification of the major benzo-CAP (C_{33} , KRI 4110) as a percentage of the major C_{32} CAP (KRI 3755) indicated a relative abundance of 4%. Within the error limits of determination at this level and possible variations in column performance, this value agrees reasonably well with previous studies (7%)⁵. Since the abundance of other benzo-CAPs is lower, quantification of these components with respect to the total distribution of a major component such as C_{32} CAP (**5**) is perhaps not informative. Hence, in order to

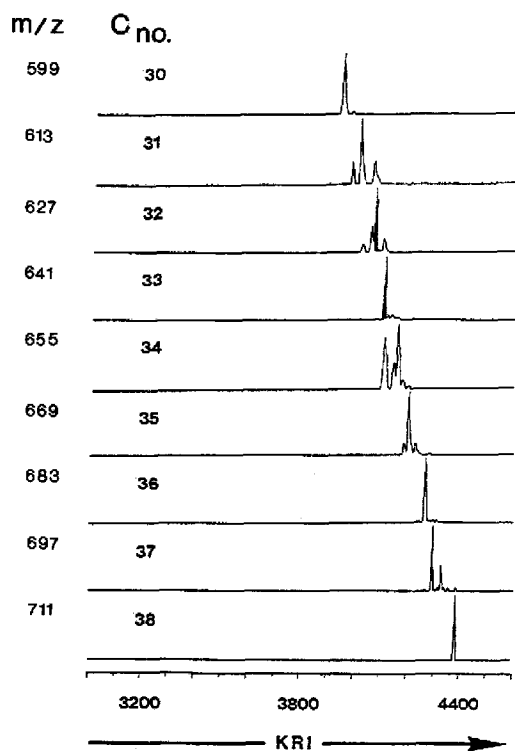


Fig. 3. Partial mass chromatograms for the $[M - 131]^+$ ions from the $(\text{TBDMSO})_2\text{Si}(\text{IV})$ derivatives of benzo-CAPs of different carbon numbers in Boscan fraction HMW. Shaded peaks indicate co-elution with derivatives of C_{32} (**2**) and C_{33} (**1**) components¹⁴.

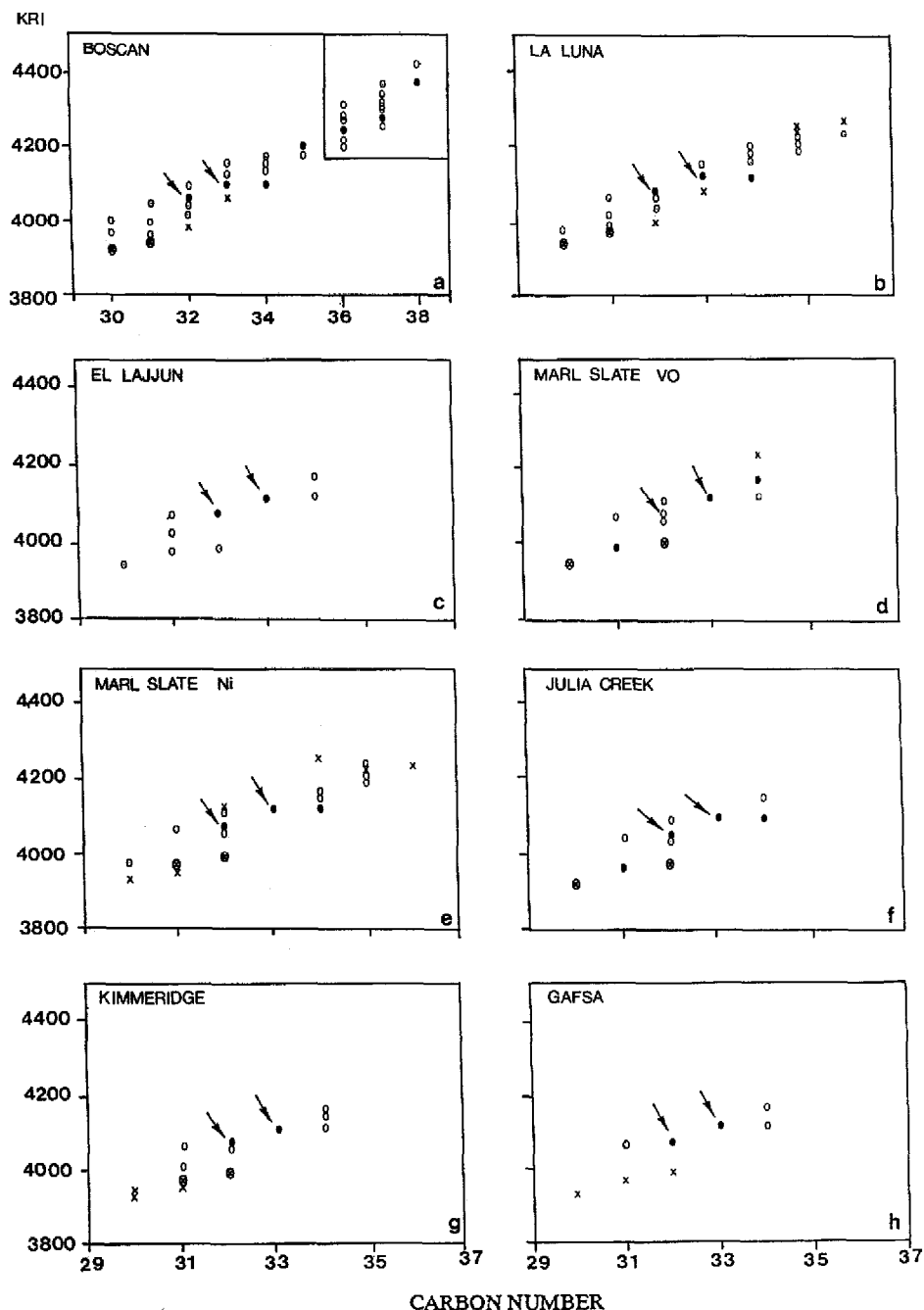


Fig. 4. Plots of KRI vs. carbon number for GC-MS analyses of total benzo-CAPs as $(\text{TBDMSO})_2\text{Si(IV)}$ derivatives in (a) Boscan oil, inset shows additional carbon number range detected in fraction HMW, (b) La Luna shale, (c) El Lajjun shale Ni porphyrins, (d) Marl slate V = O porphyrins, (e) Marl slate Ni porphyrins, (f) Julia Creek oil shale, (g) Kimmeridge shale, (h) Gafsa chert. Arrows indicate co-elution of C₃₂ (2) and C₃₃ (1) derivatives. (○) Isomer co-elutes with a component in Boscan mixture A (see text) and is clearly most abundant at carbon number (●). (×) Isomer does not co-elute with any component in Boscan mixture A but is clearly most abundant at carbon number (⊗).

compare the benzo-CAP distributions in the samples, quantification was performed relative to the major C_{33} benzo-CAP isomer (KRI 4110). This component was then quantified relative to the major C_{32} CAP (KRI 3755) for each sample. The KRI values and relative abundances of the isomers are listed in Table II and are compared with those present in the other samples studied.

Other samples

La Luna shale. Fig. 4b shows the KRI plot of the benzo-CAPs and summarises results from co-chromatography with mixture A from Boscan (see Experimental). The major benzo-CAP co-eluted with the C_{33} derivative of **1** (KRI 4110). Hence, the structures may be assumed to be identical, or at least positionally isomeric. The second most abundant co-eluted with the C_{32} derivative of **2** (KRI 4070). The major C_{34} and C_{35} benzo-CAP isomers (KRI 4109 and 4203, respectively) co-eluted with the derivatives of Boscan components tentatively proposed²⁵ to have structures **3** and **4** respectively. The major C_{36} isomer (KRI 4264) co-eluted with the major C_{36} benzoporphyrin.

HPLC co-chromatography of the C_{33} (**1**) and C_{32} (**2**) standards with the total demetallated La Luna porphyrins confirmed results from the GC-MS study (Fig. 5a).

Gilsonite bitumen. Derivatives having masses corresponding formally to benzo-CAPs were not detected and this confirms earlier GC-MS studies⁴ of this sample.

El Lajjun shale. GC-MS analysis was performed on the derivatised Ni(II) fraction. The Kovats' plot for the benzo-CAPs is shown in Fig. 4c and results from the co-chromatography experiment are illustrated. In contrast to the Boscan and La Luna distributions, the number of benzo-CAP isomers is small. The major component is, however, the derivative of the same C_{33} (**1**) component (KRI 4110). The abundance of this relative to the major C_{32} CAP component (KRI 3755) is *ca.* 2%.

Marl slate. GC-MS analysis of both the derivatised vanadyl and nickel porphyrin fractions was undertaken (Fig. 4d and e). The major C_{33} benzo-CAP derivative from both fractions again co-eluted with the derivative of the C_{33} (**1**) component (KRI 4110). Although the derivative of the C_{32} (**2**) benzo CAP (KRI 4070) in the Boscan mixture (mixture A) co-eluted with a C_{32} derivative from the vanadyl fraction, the latter is not the major C_{32} isomer (Table II). The major C_{32} isomer has a KRI of 3986. Although this component is not present in mixture A, it was detected in the Boscan total porphyrins in low abundance (Fig. 4a; Table II). Similarly, in the nickel fraction the C_{32} (**2**) standard (KRI 4070) co-eluted with a C_{32} benzo-CAP isomer, but again the major C_{32} isomer has a KRI of 3986 and is probably the same compound as that from the vanadyl fraction. In the nickel fraction, this isomer is not as predominant as in the vanadyl fraction.

Both the C_{31} components in the vanadyl fraction (KRIs 3980 and 4060) co-eluted with C_{31} components in the Boscan mixture. However, neither correspond to the major C_{31} isomer in Boscan oil (KRI 3970). In contrast, the major C_{31} isomer in the nickel fraction (KRI 3970) corresponds to the major C_{31} isomer in Boscan oil (Table II).

The major C_{34} isomer (KRI 4109) in the nickel fraction co-eluted with a compound in mixture A proposed²⁵ to correspond to the derivative of structure **3** (Table II). In contrast, the major C_{34} benzo-CAP isomer in the vanadyl fraction was at KRI 4160 (Fig. 4d; Table II).

TABLE II
RETENTION INDICES AND ABUNDANCES OF BENZO-CAPs DETECTED BY GC-MS ANALYSES OF PETROPORPHYRIN DISTRIBUTIONS AS
(TBDMSO)₂Si(IV) DERIVATIVES

Sample	Pseudo Kovals' retention index (abundance ^a)						Benzo-CAP abundance ^b
	C ₃₀	C ₃₁	C ₃₂	C ₃₃	C ₃₄	C ₃₅	
Boscan oil	3930(3)	3970(9)	3986(4)	4074(6)	4109(15)	4184(3)	4
	3968(1)	3980(4)	4022(4)	4110(100)	4143(7)	4203(6)	
	4008(2)	4010(3)	4055(7)	4120(4)	4160(6)		
La Luna shale		4060(5)	4070(30)	4134(3)	4178(4)		6
	3930(4)	3970(9)	3986(3)	4070(4)	4109(18)	4184(2)	
	3968(1)	3980(3)	4022(5)	4110(100)	4143(3)	4230(2)	
Marl slate (V=O fraction)		4010(4)	4055(7)	4135(3)	4160(5)	4226(2)	10
	3930(1)	4060(2)	4070(20)	4178(3)	4235(1)		
		3980(5)	3986(17)	4110(100)	4109(3)		
Marl slate (Ni fraction)		4060(2)	4055(3)	4070(4)	4160(6)		26
	3930(3)	3947(1)	3986(8)	4102(1)	4230(1)		
	3968(2)	3970(5)	4055(1)	4110(100)	4109(7)	4184(2)	
El Lajjun shale (Ni fraction)		4060(2)	4070(5)	4102(2)	4143(1)	4203(1)	2
	3930(1)	3970(3)	3986(6)	4111(2)	4160(2)	4217(1)	
		4010(2)	4070(25)	4110(100)	4255(1)	4226(1)	
Julia Creek oil shale		4060(1)	4102(3)	4109(2)	4160(1)		5
	3930(2)	3980(11)	3986(5)	4110(100)	4109(2)		
		4060(4)	4055(1)	4160(1)	4160(1)		
Kimmeridge shale		4060(1)	4102(3)	4109(2)	4160(1)		2
	3930(2)	3947(1)	3986(29)	4110(100)	4109(1)		
	3942(1)	3970(3)	4055(1)	4122(1)	4160(1)		
Gafsa chert		4010(1)	4070(25)	4160(1)	4160(1)		2
	3930(1)	3970(2)	3986(14)	4110(100)	4109(3)		
		4060(1)	4070(15)	4160(1)	4160(1)		

^a Relative (%) to the derivative of the major benzo-CAP in each distribution (C₃₃, I, KRI 4110).

^b Abundance (%) of the major benzo-CAP (C₃₃, I) relative to the most abundant petroporphyrin in the fraction (5 in each case).

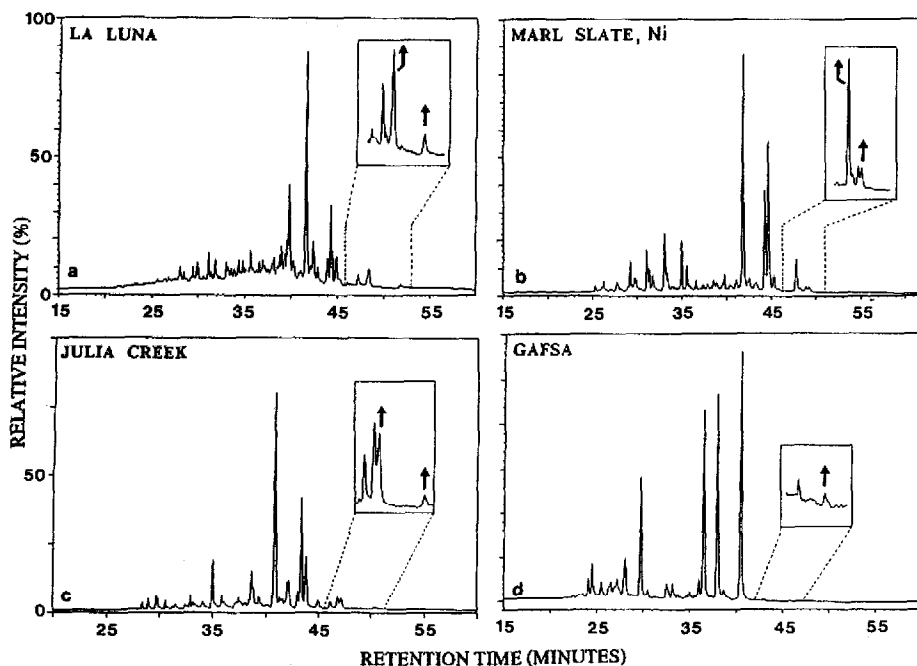


Fig. 5. HPLC chromatograms (SiO_2 ; $3 \mu\text{m}$) of total petroporphyrins as free bases from (a) La Luna shale, (b) Marl slate Ni porphyrins, (c) Julia Creek oil shale, (d) Gafsa chert. Insets in each case show enlarged regions of benzo-CAP elution (not all the peaks in this region arise from benzo-CAPs). Arrows indicate co-elution with C_{33} (1) and C_{32} (2) standards (in increasing order of retention time).

Only one C_{36} isomer (KRI 4230) was detected in the nickel fraction and this did not co-chromatograph with any of the isomers present in mixture A.

The major difference between the nickel and vanadyl benzo-CAP distributions appears to be that of a simpler overall distribution for the vanadyl fraction, with fewer isomers at most of the carbon numbers. The fact that isomers were detected in the vanadyl fraction, and no isomers corresponding to C_{35} or higher may, however, be a consequence of the somewhat lower column loading in the analysis. The total benzo-CAP concentration, as a fraction of the total porphyrins, was lower in the vanadyl fraction than in the nickel fraction. Both these factors would discriminate against the detection of very minor benzo-CAP components.

In summary, the Marl slate porphyrins contain a relatively high abundance of benzo-CAPs, with the major component (C_{33} , KRI 4110) being present in *ca.* 20% of the major C_{32} CAP in the nickel porphyrins. In addition, a relatively large number of isomers were detected at each carbon number in the nickel fraction (Fig. 4c; Table II).

The assignments were confirmed in part by HPLC co-chromatography of the C_{32} (2) and C_{33} (1) benzoporphyrin standards with the demetallated nickel fraction (Fig. 5b). The demetallated vanadyl fraction was not available for HPLC analysis.

Julia Creek oil shale. Components corresponding to benzo-CAPs in the range C_{30} – C_{34} were present and the series again maximised at C_{33} (Table II). Results from the co-chromatography experiment with mixture A are summarised on the Kovats'

plot (Fig. 4f). The major benzo-CAP again co-eluted with the derivative of the C₃₃ (1) standard, as did the C₃₂ (2) with the major C₃₂ benzo-CAP. However, a second C₃₂ isomer (KRI 3986) was present in relatively high abundance (Fig. 4f; Table II). The latter was not present in mixture A, although a C₃₂ component at this KRI was detected in the total Boscan porphyrins, albeit in low abundance. Two C₃₁ isomers were present at KRIs 3980 and 4060, the isomer at KRI 3980 being more abundant. Both co-eluted with C₃₁ isomers in mixture A and are, therefore, almost certainly the same as those present in Boscan. The C₃₄ components were present in much smaller abundance, the isomer at KRI 4109 co-eluting with the derivative of the component of proposed structure 3²⁵. The overall distribution in Julia Creek oil shale is relatively simple in terms of the number of isomers at each carbon number, with the largest number of isomers at C₃₂, where there are four (Fig. 4f; Table II).

HPLC co-chromatography confirmed the presence of the components corresponding to the C₃₂ (2) and C₃₃ (1) standards (Fig. 5c).

Serpiano shale. No porphyrin derivatives having masses corresponding to benzoporphyrins with a cycloalkano ring were detected by GC-MS. UV-VIS and/or electron impact (EI) probe MS analyses of LC chromatographic fractions obtained from the shale, however, have reported spectra²⁸ and molecular ions²⁹ corresponding formally to benzo-CAPs. It is possible that the relative abundance of these components in the total distribution is below the limits of detection of the GC-MS procedure. If so, this would indicate an abundance of less than *ca.* 2%, since this level was detected in other samples (Table II). A C₃₄ porphyrin isolated from Serpiano shale and having a molecular mass corresponding in degree of unsaturation to a benzo-aetioporphyryn, was recently reported instead to have an unusual fused-ring structure³⁰.

Similarly, HPLC analysis did not detect any peaks with the expected retention times of the benzo-CAP standards.

Kimmeridge shale. Results are summarised on the Kovats' plot (Fig. 4g). Again, the major C₃₃ isomer (Table II) and a C₃₂ isomer co-eluted with the derivatives of C₃₃ (1) and C₃₂ (2) components. However, the known C₃₂ component (2) is not the most abundant C₃₂ benzo-CAP, although it is present in a significant amount (25% of the major C₃₃ benzo-CAP). The most abundant C₃₂ isomer has a KRI of 3986 (Table II). A C₃₄ isomer (KRI 4109) again co-eluted with the derivative of the Boscan component, tentatively proposed to have structure 3. However, two other C₃₄ isomers were present in equal abundance (KRIs 4122 and 4160; Table II). The isomer at KRI 4122 was not detected in any of the other samples investigated.

The demetallated porphyrins were not available for HPLC analysis. Examination of an HPLC chromatogram from previous studies³¹ suggests, however, the presence of a peak with the appropriate relative retention time for the C₃₃ benzo-CAP (1).

Gafsa chert. The Kovats' plot (Fig. 4h) shows a relatively simple distribution with the series again maximising at C₃₃ (KRI 4110; Table II). The abundance of this component relative to the C₃₂ CAP (KRI 3755) is low, *ca.* 2%. Two C₃₂ isomers are present (KRIs 3986 and 4070), the C₃₂ (2) derivative co-eluting with the latter (Fig. 4h). Although the isomer at KRI 3986 was not present in mixture A, it was detected, in varying amounts, in all the benzo-CAP distributions studied, including the total Boscan benzoporphyrins (Table II). Similarly, there are two C₃₁ isomers at KRI 3970 and 4060 and only one of these (KRI 4060) co-eluted with a C₃₁ isomer in mixture

A (Fig. 4h). Again, the other isomer at KRI 3970 was present in a number of the samples investigated and corresponds to the major C_{31} isomer in Boscan crude oil (Table II). Both C_{34} isomers in the chert (KRIs 4109 and 4160) co-eluted with components in mixture A. The isomer at KRI 4109 is present in greater abundance (Table II) and is believed to correspond to the derivative of the C_{34} benzo-CAP (3) tentatively assigned in Boscan oil²⁵.

The HPLC chromatogram (Fig. 5d) showed the presence of a small peak at the retention time expected for the C_{33} (1) benzo-CAP standard. Co-chromatography confirmed the GC-MS results. A peak corresponding to the C_{32} (2) standard could not be detected. This is presumably a result of the very low abundance of this component in the total porphyrins.

DISCUSSION

GC-MS of the derivatised porphyrins from a range of samples has revealed the presence of benzo-CAPs in a number of them (Table II). The series were detected as free bases (Gafsa chert), nickel complexes (Kimmeridge shale), vanadyl complexes (La Luna shale, Julia Creek oil shale), or as a mixture of these metallo species (Marl slate, El Lajjun shale). The presence of components with molecular ions corresponding formally to free-base benzo-CAPs in immature sediments has been recently reported from EI-MS studies^{12,13}. The confirmation of benzo-CAPs as free bases indicates that they may be formed under very mild sedimentary conditions (*i.e.* before metal chelation). This contrasts with earlier proposed theories for their formation (the full structures being unknown at the time), such as sedimentary Diels-Alder reactions³² which might be expected to require more severe conditions.

The benzo-CAPs occur over a range of maturity, extending from the relatively immature Gafsa chert to the mature La Luna shale and Boscan oil. This fact indicates that their presence is not maturity dependent. However, they do appear to show maturity effects, as do other porphyrins, in that they are present as free bases in the most immature sample (Gafsa chert) and as metalloporphyrins in the more mature samples.

The carbon number ranges do not appear to be greatly affected over the maturity range represented by the samples (Table II; Fig. 6). The range in the more mature samples (*e.g.* La Luna shale) was *ca.* C_{30} to C_{36} , whereas in less mature samples (*e.g.* Kimmeridge shale) it was *ca.* C_{30} to C_{34} . The complexity in the more mature distributions arises more from an increase in the number of isomers at each carbon number. For example, there are five C_{32} benzo-CAP isomers in the Boscan porphyrin distribution (Fig. 4a), whereas only two are present in Gafsa chert (Fig. 4h). This can also be seen at other carbon numbers (Fig. 6). For example, there are four C_{34} isomers in La Luna shale, whereas only two are present in Julia Creek. The large number of isomers presumably results from variation in structural features, such as presence/absence of unsubstituted β -positions, the spatial relationships of the β -substituents features with the cycloalkano and benzo rings^{8,25,27}.

Carbon numbers ranging from C_{30} to at least C_{36} are present (*e.g.* La Luna shale; Table II; Fig. 6). The possibility of higher carbon numbers, below the limits of detection in the total distributions, was highlighted by examination of fraction HMW from Boscan where benzo-CAPs with carbon numbers up to C_{38} were detected.

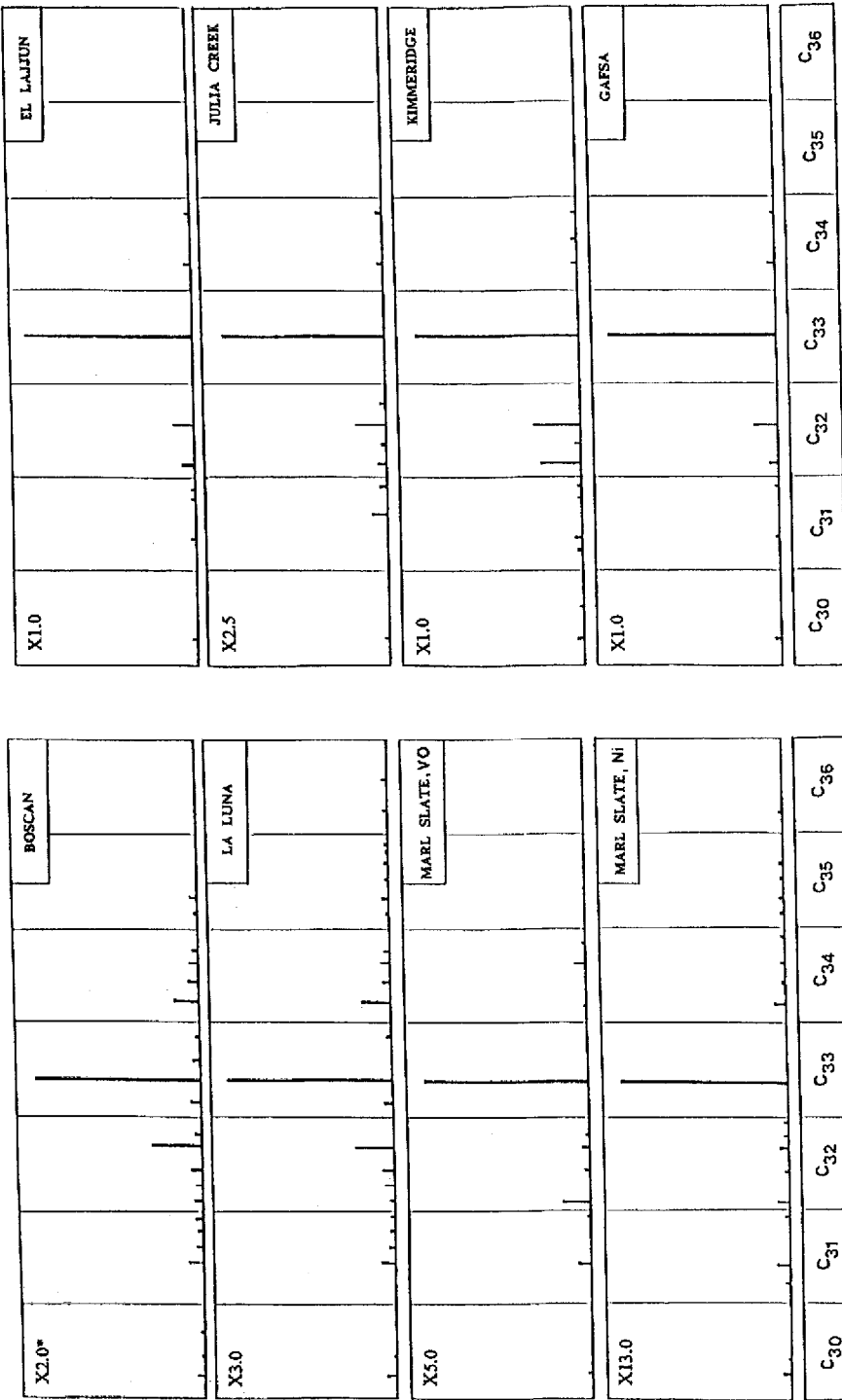


Fig. 6. Histograms illustrating relative abundances of benzo-CAP isomers at each carbon number detected by GC-MS analysis of total petroporphyrin distributions as (TBDMSO)₂Si(IV) derivatives. Abundances are relative to the major benzo CAP (C₃₃, 1) in each case. The scale on the x-axis is arbitrary, although in order of increasing KRI. Asterisk indicates relative to Gafsa chert = x 1.

The co-injection experiments (GC-MS and/or HPLC) reveal, remarkably, that in all the samples where benzo-CAPs were detected, the dominant member is the C₃₃ component (Fig. 6) corresponding to the compound previously isolated from Boscan (1)¹⁴. In addition, although other C₃₃ isomers were detected, the relative abundance of the above component (1) is generally > 90%, *e.g.* in La Luna (Fig. 6; Table II). The abundance of this major C₃₃ benzo-CAP, relative to the major porphyrin in each sample (generally C₃₂ CAP) varies from *ca.* 2% (Gafsa chert) to 26% (Marl slate, nickel fraction). The next most abundant benzo-CAP in most cases is the C₃₂ isomer (2). However, in some cases (Marl slate), an earlier-eluting C₃₂ isomer (KRI 3986) was in greater abundance. Other C₃₂ isomers, in varying amounts, were also detected (Table II). The very low relative abundance of most of the isomers, with respect to the total distributions, makes it difficult to compare these components quantitatively in the samples examined.

That the benzo-CAPs are widespread is clearly demonstrated by their occurrence in sedimentary samples from locations ranging from the United Kingdom (Kimmeridge shale) to Australia (Julia Creek oil shale) and of geological age ranging from Palaeocene (Gafsa chert) to Permian (Marl slate). However, they do not occur ubiquitously when porphyrins are present, being absent for example, from the Gilsonite bitumen. This offers some hope for the potential use of these components in geological applications. On the other hand, there is at present no apparent correlation with depositional environment among the samples examined. The two samples where benzo-CAPs were not detected were Gilsonite bitumen, whose source rock is from a lacustrine saline water depositional environment, and Serpiano shale, from a restricted marine environment.

Ideally, a study of geological samples from a wider range of depositional environments together with an examination of both the total petroporphyrin fractions and individual metalloporphyrin fractions would be desirable to search for significant geochemical correlations. In the present work, for convenience and due to the availability of samples, a combination of these approaches was employed. The results reveal, however, the striking predominance of compound 1 in all the distributions where benzo-CAPs were detected. This suggests an origin from a widely occurring specific precursor pigment rather than from sedimentary reactions of chlorophyll(s)^{32,33}. At present, an origin from a known pigment is not obvious, although the presence of the cycloethano ring clearly indicates one from chlorophylls rather than other tetrapyrroles, such as cytohaemes. In the absence of an obvious precursor, a bacterial origin has been suggested¹⁴.

CONCLUSIONS

The identification of benzo-CAPs in a variety of petroporphyrin distributions has been achieved via computerised GC-MS and HPLC analyses and co-chromatography.

(1) The GC retention times (KRI 3900-4400) of the (TBDMSO)₂Si(IV) derivatives are long compared to those of the major alkylporphyrins (*e.g.* 5, KRI 3755).

(2) The sensitivity of the GC-MS procedure has allowed detection of the less abundant benzo-CAPs proposed as 3 and 4²⁵ in a number of samples. In addition,

minor components fractionated from Boscan oil and corresponding to benzo-CAPs, were shown to be present.

(3) Up to 24 benzo-CAP components, with multiple isomers at individual carbon numbers have been detected (*e.g.* La Luna shale; Table II).

(4) Both co-chromatographic techniques have provided a means of assignment of benzo-CAPs without the use of lengthy isolation procedures for individual compounds.

(5) Benzo-CAPs occur as minor petroporphyrin components in geological samples from a range of locations and ages.

(6) The distributions studied all maximise at C₃₃ (same isomer, 1; KRI 4110).

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