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CAPILLARY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF METAL(III) AND METAL(IV) PORPHYRIN COMPLEXES

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SUMMARY

The capillary gas chromatographic (GC) analysis of silicon(IV), aluminium(III), gallium(III) and rhodium(III) alkyl substituted porphyrins, mainly as their trimethylsiloxy derivatives, is reported. Retention indices on apolar stationary phases are presented and are discussed in terms of retention index increments associated with molecular differences between various porphyrins. The dependence of retention volumes (indices) upon the oxidation state of (and hence axial ligation to) the central metal is illustrated. GC–mass spectrometric (MS) data indicate that the silicon derivatives in particular are favourably suited to GC–MS analysis and the strong diagnostic ions produced under electron impact ionisation should prove invaluable for the analysis of complex mixtures.

GC traces for the M(III) and for some of the silicon(IV) porphyrins are the first examples to be reported in the literature.

INTRODUCTION

Gas chromatography (GC) of porphyrin compounds has had a relatively long, but at the same time not particularly successful, infancy. The few reported literature works on this subject characterise the problems encountered in developing routine analytical approaches to application of GC methods to porphyrins. Almost invariably, the preceding work was limited to packed column GC using either glass bead or diatomaceous earth supports with low liquid phase loadings. It was common practice to condition (termed "porphyrinise") a column with a number of injections of a concentrated silicon porphyrin standard prior to use of the column[1] indicative of undesirable activity (and hence adsorption) within the support matrix.

Boylan and Calvin[2] first proposed, and illustrated the adequate volatility of, silicon porphyrins for GC. Their data indicated that the bistrimethylsiloxy derivative

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possessed the most favourable characteristics. The bisethoxy derivative was irreversibly adsorbed. Alturki[1] and Boylan *et al.*[3] applied the technique to some samples of naturally occurring petroporphyrins. The poorly resolved distributions which resulted made analysis complicated and offered only marginal advantages over other available techniques. Games *et al.*[4] illustrated that good porphyrin peak shapes could be attained on an OV-1 coated Gas-Chrom Q solid support. They intimated that a "biological" porphyrin (mesoporphyrin-IX dimethyl ester) should be amenable to GC analysis after appropriate derivatisation. Baker[5] attempted to extend the GC method to aluminium porphyrins, though with limited success.

Corwin and co-workers[6–8] employed hyper pressure methodologies to chromatograph a wide range of porphyrins. The peak shapes were not favourable (though the difficulties associated with the technique were considerable). This procedure decreased the partition coefficient such that enhanced porphyrin solubility in the mobile phase occurred, and thus permitted chromatographic migration of porphyrins at reduced temperatures.

Alexander *et al.*[9] reinvestigated the porphyrin GC problem, employing the recently developed technology of fused-silica capillary columns, and using on-column injection. They presented promising results for a small number of silicon porphyrins, which included aetioporphyrin-I and octaethylporphyrin, and also reported the successful GC of the trimethylsiloxy Al(III) derivative of aetioporphyrin-I. Later work, using short capillaries at high temperatures[10] indicated that a range of transition metal(II) porphyrins and oxometal(IV) porphyrins could be chromatographed under normal conditions. The inability of the conventional packed column GC method used by earlier workers to fractionate successfully complex mixtures has undoubtedly contributed to the lack of persistence of various groups in developing and extending the technique to other metals and porphyrins. Therefore, it is not surprising that high-performance liquid chromatography now plays the major role in such porphyrin analyses[11,12].

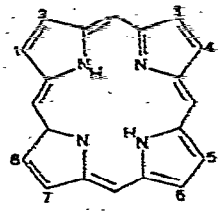
In an extensive search for new metalloporphyrin derivatives which might permit facile GC analysis for porphyrins, we have had success in chromatographing certain such compounds using capillary columns. We wish to present our preliminary results on some M(III) and M(IV) alkylporphyrin standards and discuss these results in terms of general chromatographic properties.

EXPERIMENTAL

Reagents

Free-base porphyrin compounds used for preparations were obtained from various sources. Octaethylporphyrin (OEP, 1) was bought from Porphyrin Products (Logan, UT, U.S.A.). Dr. J. G. Erdman kindly donated the aetioporphyrin type isomers -I and -III (aetio-I and -III, 2 and 3 respectively). Octamethylporphyrin (OMP, 4) was provided by Professor P. S. Clezy, and the C_{31} aetioporphyrin was isolated from a natural mixture by Mr. G. A. Wolff. Professor R. Grigg supplied the aetioporphinato-I, rhodium(III), $COCH_3$ and its rhodium(III) CH_3 analogue.

Proprietary chemicals used included hexachlorodisilane (Aldrich), triethylaluminium (Cambrian Chemicals), tris-(2,4-pentanedionato)gallium(III) (Ventron), bis(trimethylsilyl)-trifluoroacetamide (BSTFA, Applied Science Labs.), and *tert.*-



Structures of some free base porphyrins used in this study

Porphyrins	*Alkyl substituents*
1 octaethylporphyrin, OEP	1–8 = ethyl
2 aetioporphyrin-I; aetio-I	1 = 3 = 5 = 7 = methyl; 2 = 4 = 6 = 8 = ethyl
3 aetioporphyrin-III; aetio-III	1 = 3 = 5 = 8 = methyl; 2 = 4 = 6 = 7 = ethyl
4 octamethylporphyrin; OMP	1–8 = methyl

butyldimethylchlorosilane (TBDMSCl, Fluka).

A sample of oil pipe sludge obtained from British Petroleum, Exploration Division, Sunbury-on-Thames, Great Britain, was extracted with hexane and cleaned up on alumina to obtain a normal alkane standard of relatively high molecular weight containing both even and odd carbon number hydrocarbons. This was used for coinjection studies.

Preparations of metalloporphyrins

Silicon. The method of Alturki[1] and Boylan *et al.*[3] was modified to a simple reflux method, with dry toluene solvent and dry nitrogen used to prevent moisture reacting with hexachlorodisilane. This enabled insertion of silicon into a number of porphyrins in yields approaching quantitative, indicated by the lack of electronic absorption bands of the free base starting material in the final product. After completion of the reaction, volatile components were removed under vacuum then the remaining solids were refluxed in either ethanol–1 *M* hydrochloric acid or methanol–aqueous sodium hydroxide to hydrolyse the porphyrin to the dihydroxy species. This was then extracted into organic solvent and purified on alumina thin-layer chromatography (TLC). Formation of silicon dioxide in the hydrolysis step may be a drawback since some porphyrin could be irreversibly trapped in the solid silicon dioxide matrix.

Gallium. Porphinatogallium(III) compounds are easily prepared by reaction of the porphyrin with trisacetylacetonatogallium(III) in a medium of phenol as solvent[13]. Metal insertion occurs rapidly (within minutes) with the solution taking on the characteristic rosy metalloporphyrin colour when the mixture was heated to 220°C. Metal insertion, hydrolysis and initial solvent partitioning of products was carried out *in situ*. Prior to hydrolysis, a phenoxy ligand is coordinated to the gallium, in the axial position (indicated by mass spectral and X-ray crystal structure[14] analysis). Hydrolysis to the hydroxy species was carried out by refluxing the mixture with 15 ml ethanol and 1 ml aqueous sodium hydroxide (1 *M*). The product was extracted into methylene chloride, the concentrated extract passed through an alumina column, and then TLC on alumina G with methylene chloride–methanol (10:1, v/v) gave the required pure product.

Free-base porphyrin was not present in the reaction mixture on the basis of the visible spectrum recorded prior to hydrolysis, and only metalloporphyrin was obtained on the TLC plate, thereby indicating virtually complete metallation.

TABLE I

RETENTION DATA FOR M(III) AND M(IV) PORPHYRINS AS THEIR TRIALKYLSILOXY DERIVATIVES

GC peak	*Metal**	*Porphyrin*	*Substitution pattern*	*Structure type***	*Axial ligand****	*Total carbon number (TC)*	*Molecular weight†*	*Retention index (Kovats, I)*	*ΔI*	*Column‡‡*
A	Si(IV)	OMP	4	c	2 × OTMS	34	623.31	3200		1
B	(28.086)	C_{31} actio	–	c	2 × OTMS	37	678.35	3300	130	1
C		Actio-I	2	c	2 × OTMS	38	682.37	3330	30	1
D		Actio-III	3	c	2 × OTMS	38	682.37	3330	130	1
E		OEP	1	c	2 × OTMS	42	736.43	3460		1
F		OEP	1	c	2 × OTBDMS	48	822.53	3835	375	1
G		Actio-I	2	c	OTMS, F	35	612.32	≈4300		1
H	Al(III)	Actio-I	2	b	OTMS	35	592.33	4190		1
I	(26.983)	OEP	1	b	OTMS	39	648.39	4310	120	1
J	Ga(III)	Actio-I	2	b	OTMS	35	634.27	4295		1
K	(69.72)	OEP	1	b	OTMS	39	690.33	4410	115	1
L	Rh(III)	Actio-I	3	b	$COCH_3$	34	622.22	≈4950		2
M	(102.905)	Actio-I	3	b	CH_3	33	594.23	≈5250		2
N	Ni(II)†††	Actio-I	2	a	–	32	534.24	5285		2
O	(58.71)	OEP	1	a	–	36	590.30	5410	125	2

* Values in parentheses are average atomic weights.

** Structure type as given in schematic line diagram.

*** OTMS = trimethylsiloxy group [$(CH_3)_3SiO$–]; OTBDMS = *tert.*-butyldimethylsiloxy group [$((CH_3)_3C)(CH_3)_2SiO$–].

† Calculated for most abundant atomic isotopes.

‡‡ 1 = Chrompack CPSil 5 glass column; 2 = Hewlett-Packard OV-1 flexible silica column.

††† Data from ref. 10.

Aluminium. Aluminium trialkyls have been used to prepare porphinatoaluminium(III) compounds[15,16].

Porphyrin free base (1 mg) was dissolved in 10 ml freshly distilled dry toluene in a two-necked round-bottom flask previously flushed with dry nitrogen. An excess of aluminium triethyl (*ca.* 4–5 drops) was introduced into the reaction vessel using a syringe whilst continuously flushing the flask and transfer lines with dry argon, ensuring dry and oxygen-free conditions. Metallation was almost immediate, and the electronic spectrum indicated only metalloporphyrin in the mixture.

Hydrolysis of the product with ethanol–water was accompanied by formation of alumina from excess aluminium triethyl. Solvent extraction, concentration, then TLC on alumina H (eluent: methylene chloride–methanol, 5:1, v/v) afforded the pure metalloporphyrin as evidenced by its mass and visible spectra.

Trialkylsilylation of axial hydroxy groups

Trimethylsilylation may be effected by the conventional method of taking up the dihydroxysilicon(IV) porphyrin in BSTFA–pyridine and leaving at 70°C overnight, with evaporation to dryness and dissolution of the derivatised porphyrin in hexane prior to injection. Alternatively, satisfactory silylation could be obtained by adding neat BSTFA reagent to the dihydroxysilicon(IV) porphyrin directly.

tert.-Butyldimethylsilylation was carried out by reaction of *tert.*-butyldimethylchlorosilane and imidazole with the dihydroxysilicon(IV) porphyrin. The resulting product porphyrin was isolated by TLC.

The porphyrin compounds used in this work are listed in Table I, along with their assigned letter designations employed throughout the text and the figures.

Gas chromatography

The gas chromatograph employed was a Carlo Erba FTV 4160 instrument incorporating split/splitless and on-column injection. Flame ionisation detection and hydrogen carrier gas were used. Capillary columns used included a flexible silica column (0.30 mm I.D.) coated with OV-1 (Phase Separations), a flexible silica column (0.32 mm I.D.) coated with OV-1 (Hewlett-Packard) and a glass column (0.34 mm I.D.) coated with CPSil 5 (Chrompack).

Temperature programming was used throughout, with an initial oven temperature of 60°C. On-column injection was the usual injection method, although splitless procedures were also used. Average hydrogen carrier gas flow velocities were in the region of 50–120 cm sec^{-1}, and are indicated in figure legends as appropriate.

Gas chromatography–mass spectrometry system

A Carlo Erba FTV 4160 gas chromatograph was interfaced with an AEI MS 30 mass spectrometer. The gas chromatograph, with an access hole drilled through its base, was supported above the source of the MS 30. An interface heater unit was constructed and placed on top of the normal AEI glass reentrant, protruding 2.5 cm into the oven of the gas chromatograph. The glass reentrant was held by a support bracket in order to bear the interface heater without breakage of the fragile reentrant. A length of glass-lined stainless steel tubing (GLT 0.5 mm I.D., Scientific Glass Engineering, Milton Keynes) was mounted (vacuum tight) on top of the reentrant, passing through the interface heater and into the oven of the gas chromatograph. The

flexible silica capillary column was fed down the GLT directly into the source, terminating *ca.* 2–4 mm from the ion volume. The vacuum seal was made between the flexible silica and GLT using a Swagelock coupling and graphite ferrules (both from Scientific Glass Engineering). Column changing entailed undoing the top seal between the column and GLT. Interface temperatures were monitored with chromel/alumel thermocouples placed in five regions along the interface. Adequately uniform temperatures up to 350°C were found to be easily maintained.

The mass spectrometer (a magnetic sector instrument) has limiting scan rates for capillary column analyses. Normally, total scan times of 3–3.5 sec were employed, scanning from 750 to 50 a.m.u. For higher mass detection, further reduced accelerator voltages, allowing scanning from 900 a.m.u. were necessary. For direct insertion probe analyses, slower scan times were used. Usual ionisation voltages were 35 eV and 24 eV.

The mass spectrometer was operated under computer control using a Finnigan INCOS computing data system.

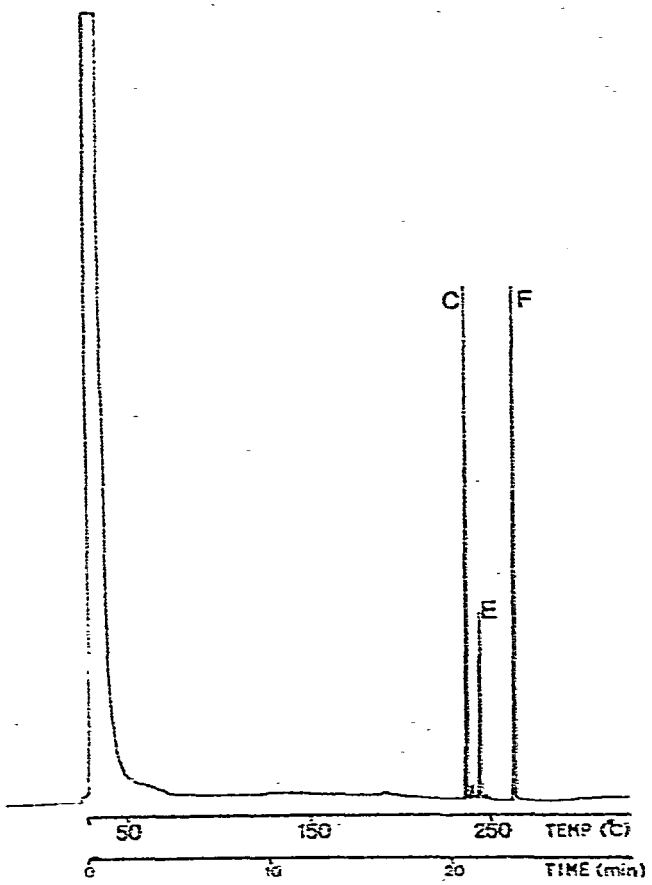


Fig. 1. Capillary gas chromatogram of silicon(IV) porphyrins using splitless injection. Injector temperature 200°C. Oven temperature: ambient at injection then programmed from 50°C to 300°C at 10°C min^{-1}. Column: 3.5 m × 0.3 mm I.D. Hewlett-Packard OV-1 coated flexible silica capillary; $\bar{u}$ *ca.* 100 cm sec^{-1}. Peaks: C = $(OTMS)_2Si$(aetio-I); E = $(OTMS)_2Si$(OEP); F = $(OTBDMS)_2Si$(OEP).

RESULTS

GC traces are illustrated in Figs. 1–6. Alkane mixtures were routinely coinjected with the porphyrins to provide a comparison of peak shapes and also to obtain Kovats indices (or, more correctly, "pseudo" Kovats indices).

Fig. 1 illustrates chromatography of three standard silicon porphyrin derivatives using splitless injection. A reduced temperature flash vapourisation injection zone of 200°C was used, since higher temperatures resulted in apparent thermal decomposition of the chelate (possible via loss of axial trimethylsilyl group(s)). This was with a short (3.5 m) OV-1 flexible silica column.

Fig. 2 represents a chromatogram of some silicon porphyrins under the more usual methodology; on-column injection at 60°C onto a longer (20 m in this example) capillary column. Peak B (a C_{31} aetioporphyrin) coeluted with normal alkane *n*-C_{33}, and is baseline-resolved from aetioporphyrin-I (C). The increased retention of *tert.*-butyldimethylsiloxy (OTBDMS) derivatives can be seen in Fig. 3. $(OTBDMS)_2$-Si(OEP) (F) eluted with apparently excellent peak shape, comparable with those of the alkane standards. The elution volume of $(OTMS)_2$Si-(aetio-III) (peak D in Fig. 3) is practically coincident with that of the aetio-I isomer (peak C in Fig. 2).

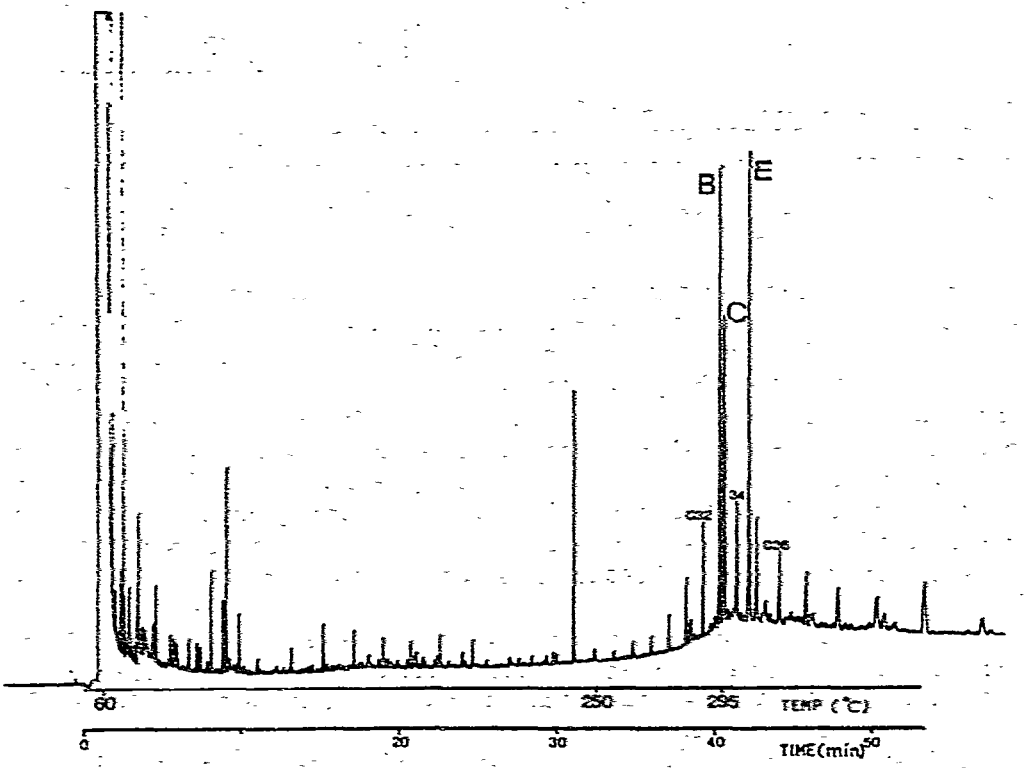


Fig. 2. Capillary gas chromatogram of silicon(IV) porphyrins coinjected with an alkane mixture. Temperature programme: 60°C to 295°C at 6°C min^{-1}, with a 20 m × 0.34 mm I.D. Chrompack CPSil 5 capillary; $\bar{u}$ *ca.* 43 cm sec^{-1}. Some alkane peaks are indicated by numbers corresponding to their total numbers of carbons (*i.e.*, C32 = *n*-C_{32}, etc.). Peaks: B = $(OTMS)_2Si(C_{31}$ aetio); C = $(OTMS)_2$Si(aetio-I); E = $(OTMS)_2$Si(OEP).

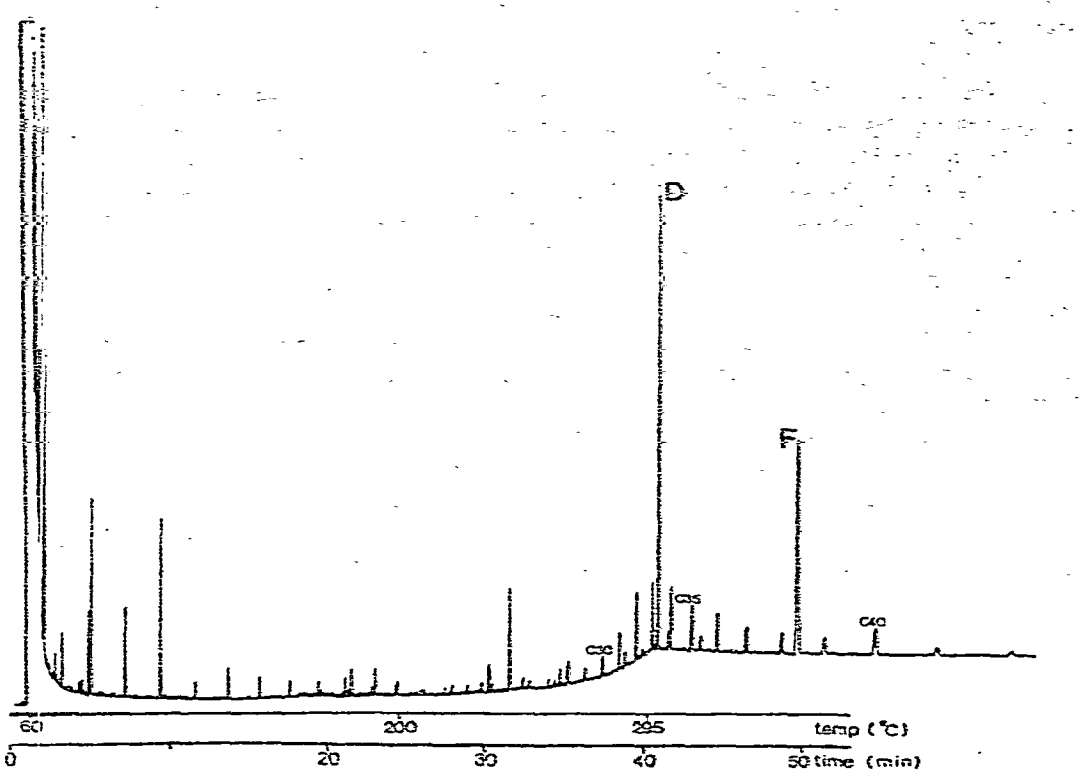


Fig. 3. Capillary gas chromatogram of silicon(IV) porphyrins coinjected with an alkane mixture. Column and conditions as for Fig. 2. Peaks: D = $(OTMS)_2Si$(aetio-III); F = $(OTBDMS)_2Si$(OEP).

Figs. 4 and 5 are the first examples to be illustrated in the literature of the GC of M(III) porphyrins. The two traces were obtained on different columns (a Hewlett-Packard OV-1 flexible silica and a Chrompack CPSil 5 glass column respectively), and on the latter column (OTMS)Al(aetio-I) (H) almost coeluted with n-C_{42}. As with the silicon derivatives, the aluminium(III) and gallium(III) porphyrins exhibited excellent peak shapes, indistinguishable from alkanes of similar elution volumes. Another M(III) porphyrin, $(COCH_3)$Rh(III) (aetio-I) (L), produced the result illustrated in Fig. 6 on a short capillary. Its elution volume was considerably larger than those of Ga(III) and Al(III) and this will be discussed later. The chromatographic peak for Rh(III) shows the overloading phenomenon associated with compounds of low solubility in the stationary phase.

GC–MS results for some selected porphyrin derivatives are presented in Figs. 7–9. The data are presented in terms of reconstructed ion currents (RIC) (or ion chromatograms) for the total ion current and as mass chromatograms where specific masses or mass ranges were selected.

Fig. 7 illustrates that the excellent chromatography obtained for the OTBDMS derivative in Fig. 3 was reproduced in the GC–MS experiment, and plots of diagnostic ions 822 (M), 765 (for M − 57) and 691 (for M − 131) corresponding to molecular ion, loss of *tert.*-butyl group and OTBDMS ligand respectively all show the same profiles as expected. The mixture from Fig. 2 was injected onto the GC–MS

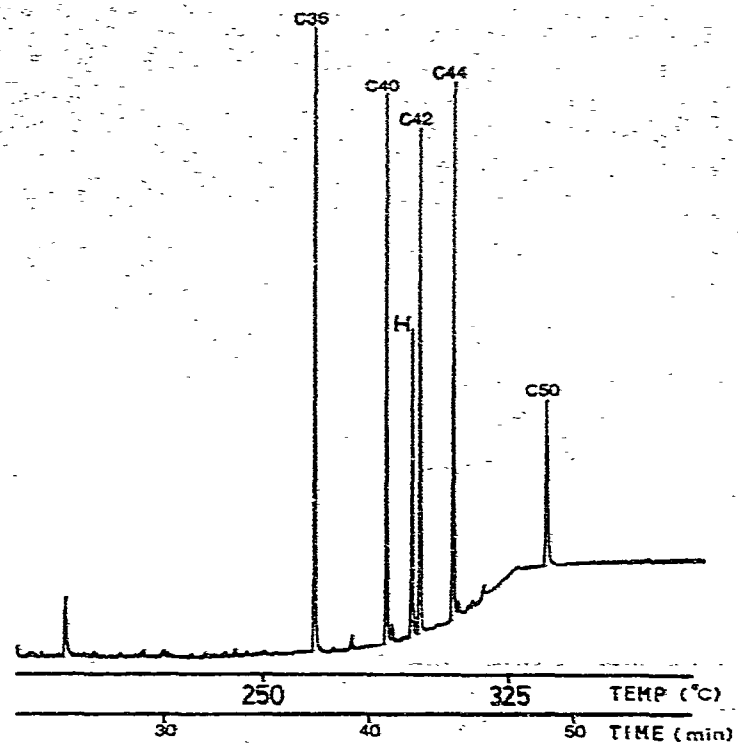


Fig. 4. Capillary gas chromatogram of (OTMS)Al(III) (aetio-I) (H) and normal alkane standards as indicated. Column: 5 m × 0.3 mm I.D. Hewlett-Packard OV-1 coated flexible silica, $\bar{u}$ *ca.* 50 cm sec^{-1}. Temperature programme: 50°C to 325°C at 6°C min^{-1}.

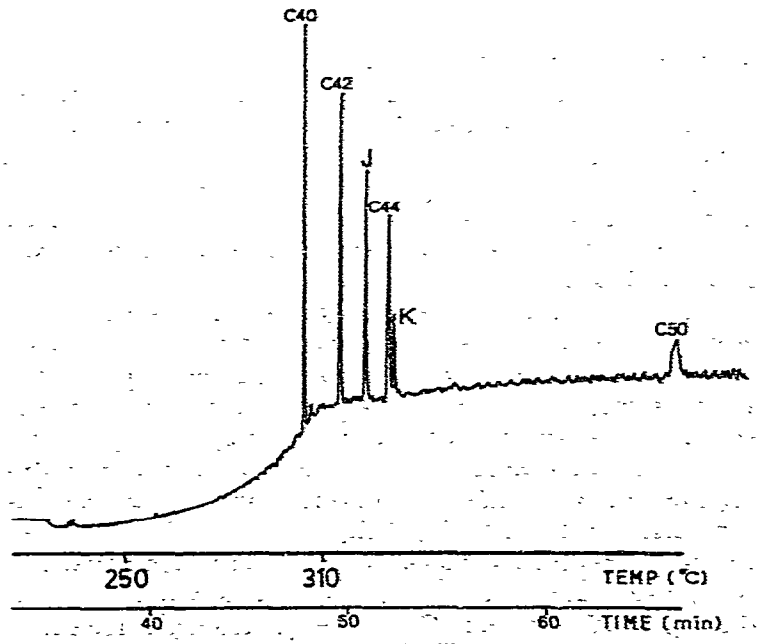


Fig. 5. Capillary gas chromatogram of gallium(III) porphyrins and normal alkane standards. Column as in Fig. 2. Temperature programme: 50°C to 310°C at 6°C min^{-1}; $\bar{u}$ *ca.* 100 cm sec^{-1}. Peaks: J = (OTMS)Ga(aetio-I); K = (OTMS)Ga(OEP).

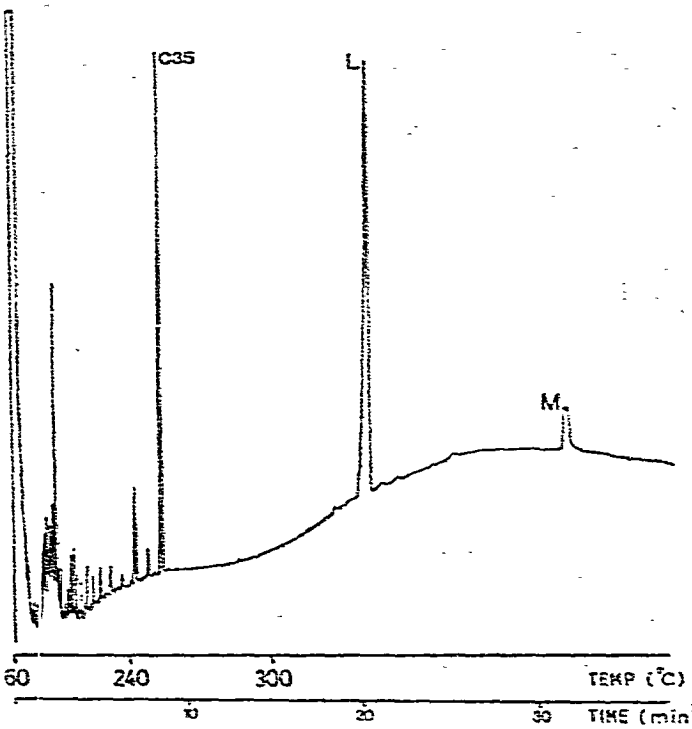


Fig. 6. Gas chromatogram of some rhodium(III) porphyrins. Column as in Fig. 1, with on-column injection. Temperature programme: 60°C at injection with ballistic heating to 240°C (in *ca.* 5 min.) followed by programming to 300°C at 6°C min^{-1}. Peaks: L = $(COCH_3)Rh(III)$(aetio-I); M = $(CH_3)Rh(III)$(aetio-I) (not confirmed).

column, with added $(OTMS)_2Si$(aetio-III), to produce Fig. 8. The C_{31} and C_{32} porphyrins were still baseline resolved. The lower (RIC) trace contains *n*-alkanes as marked, and averaging all the ions in the mass range *m/z* 550–750, which includes the major silicon porphyrin ions, produces the upper trace, thus "pulling out" the porphyrins from the hydrocarbons.

Trimethylsiloxyaluminium aetio-I was also chromatographed with the alkane standard, and the partial mass chromatogram, from scans 500 to 800, averaged over masses 500 to 600, is indicated in Fig. 9.

A summary of retention index (*I*) data calculated for all the compounds referred to are reported in Table I. These were calculated by linear interpolation between two homologues of the coinjected alkane mixture whose elutions span that of the porphyrin of interest. Linear interpolation is an approximate method of index determination under temperature programming conditions, where the logarithmic method no longer applies[17]. Values were rounded to the nearest 5 index units. Included in Table I are some retention index differences (ΔI) for various pairs of compounds, and also molecular weights of the metalloporphyrins.

Table II presents separation numbers for some selected porphyrin pairs. The accurate determination of such values may be complicated by the narrowness of peak width when using normal chart speeds, however the values quoted are sufficient to

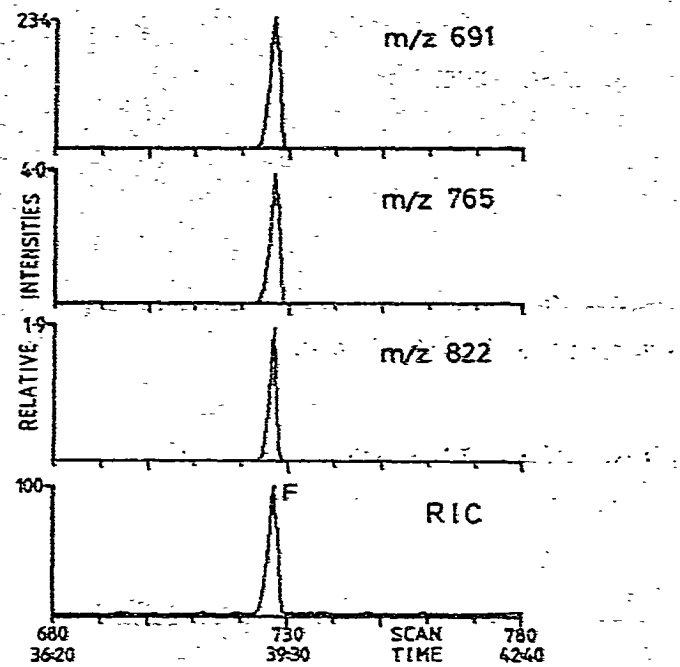


Fig. 7. Reconstructed ion chromatogram (RIC) of $(OTBDMS)_2Si$ (OEP) (F). Column and temperature programme as in Fig. 2. Data acquisition commenced at 65°C, and the isothermal temperature hold of 295°C is reached at scan number 665. Total scan time (800 to 50 a.m.u.) was 3.25 sec.

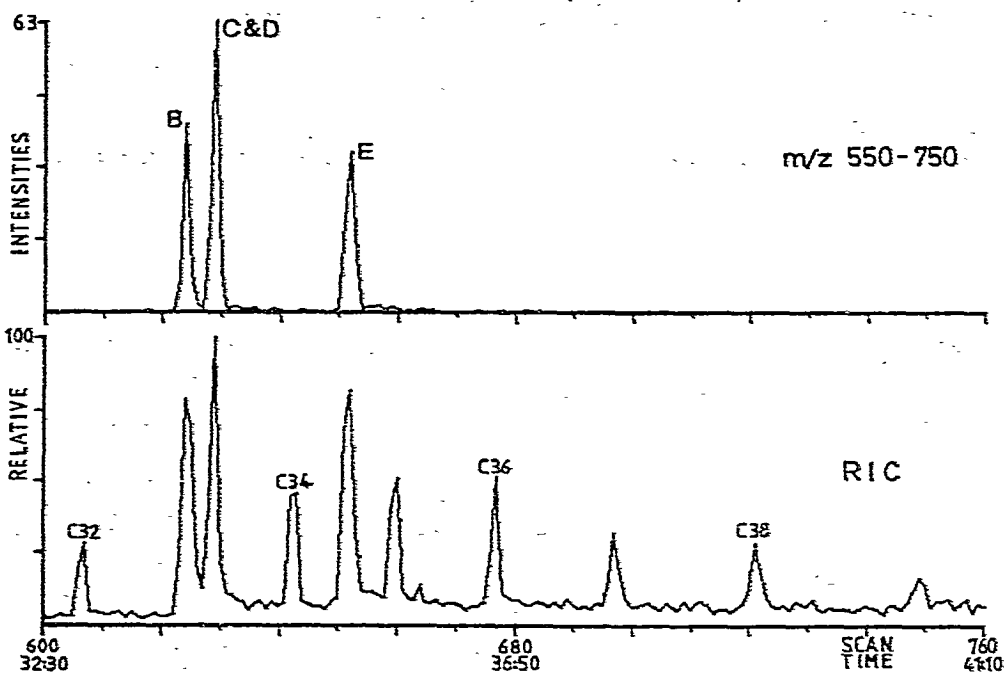


Fig. 8. RIC and summed mass chromatogram (from *m/z* 550 to 750) for GC–MS of some silicon porphyrins coinjected with the alkane mixture, displayed over scan range 600 to 760. Column and conditions as in Fig. 7. Peaks: B = $(OTMS)_2Si(C_{31}$ aetio); C + D = $(OTMS)_2Si$(aetio-I/-III); E = $(OTMS)_2Si$(OEP). Porphyrin mixture as in Fig. 2, with added aetio-III compound which is not resolved from aetio-I under these conditions.

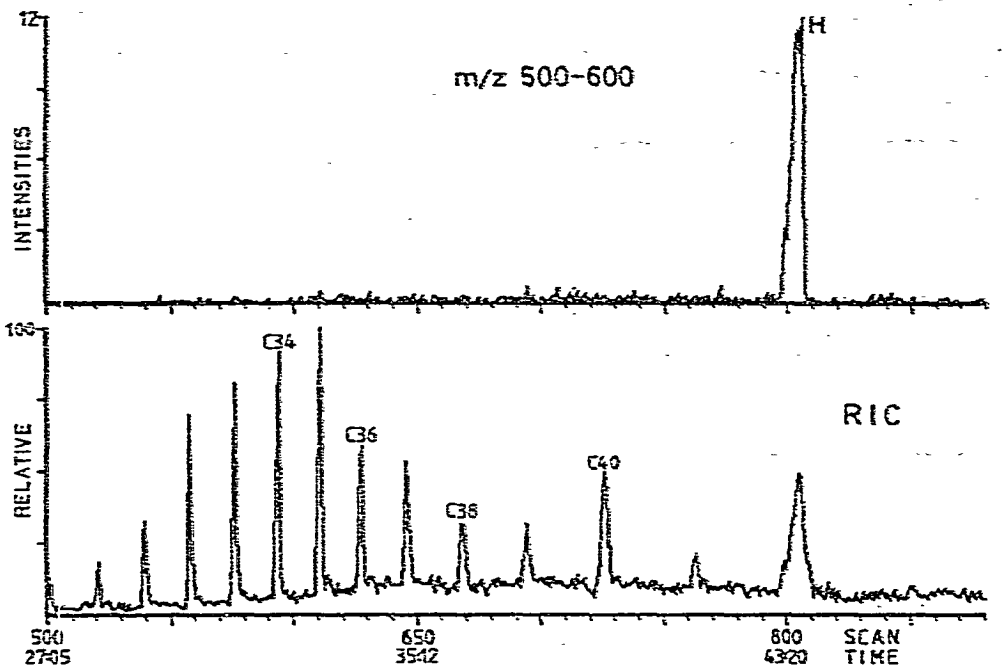


Fig. 9. RIC and summed mass chromatogram (from *m/z* 500 to 600), displayed over scans 500 to 880, for GC–MS of (OTMS)A1 (aetio-I) (H) and coinjected alkane mixture. Column and conditions as in Fig. 7. Isothermal 295°C reached at scan 625 with data acquisition commenced at 80°C.

provide an insight into various characteristics of the porphyrin peaks.

Mass spectrometric data for some of the various porphyrins reported here are tabulated in Table III.

TABLE II

REPRESENTATIVE CALCULATED RESOLUTIONS (SEPARATION NUMBERS) FOR SOME PAIRS (AETIO-I/OEP) OF METALLOPORPHYRINS AS THEIR TMS DERIVATIVES

Metal species	*Separation number*	*Column*	*Notes*
Si(IV)	12	20 m CPSil 5 glass; $\bar{u}$ *ca.* 120 cm sec^{-1}	Fig. 1, C and E.
Ga(III)	7	20 m CPSil 5 glass; $\bar{u}$ *ca.* 120 cm sec^{-1}	Fig. 5, J and K.
Al(III)	5.6	5 m OV-1 flexible silica; $\bar{u}$ *ca.* 100 cm sec^{-1}	
Ni(II)	3.5	6 m OV-1 flexible silica; $\bar{u}$ *ca.* 80 cm sec^{-1}	

DISCUSSION

Retention indices of M(III) and M(IV) porphyrins

Table I tabulates retention indices (I values) calculated for the compounds discussed in this paper. They are reported for various capillary columns as indicated, and thus relative retentions cannot be directly compared unless the same column was used to collect the data (even on these reportedly non-polar columns, retention indices may vary markedly from one column to another). Some interesting observations may be drawn from the data presented.

The index difference (ΔI) between aetio-I and OEP as their $(OTMS)_2Si$ derivatives is 130 (*i.e.*, equivalent to 1.3 carbons in a homologous *n*-alkane series). This is for a carbon number difference of 4, with OEP having extra methylene groups at positions 2, 4, 6 and 8.

This implies that for one single carbon added to the macrocycle in this manner, an increment in retention index of about 32 units can be expected. The retention difference between the C_{31} aetio and aetio-I compounds, which differ by one carbon, is almost precisely this value, at 30 units. This does not, of course, differentiate between the manner in which the homologues are related (*i.e.*, a long chain substituent may tend to increase the retention increment per added carbon), however the empirical result for the standards employed seems consistent.

A comparison of I values for the two SiOEP compounds (OTMS and OTBDMS derivatives) reveals a ΔI of 375, which is for an additional six carbons in the latter case (two derivatised hydroxy groups on the silicon). This is closer to the ΔI value expected from comparison of OTMS and OTBDMS derivatives of hydroxy groups, although it is still somewhat less than might be expected; the ΔI increments for OTBDMS over OTMS are reported to average about 240 units per derivatised functionality[18] (therefore 480 for two hydroxy groups).

Therefore, the axial ligand is affecting retention in a manner which can be related to usually observed behaviour, however the substituents on the pyrrole rings influence retention much less than expected. Successive members of a homologous series usually differ in retention by about 100 index units (*i.e.*, equivalent to one carbon of an *n*-alkane series), however the series which have received most widespread attention in the literature, *i.e.*, esters, isomeric alkylbenzenes and aromatics, simple hydrocarbons, etc. are not as complex as the porphyrins and so further data on many more porphyrins must be collected before trends in these parameters can be elucidated.

Retention of aetio-I/OEP pairs of M(III) and M(IV) porphyrins

From Table I, ΔI values for aetio-I and OEP pairs for Al(III), Ga(III) and Si(IV) can be compared. The values are 120, 115 and 130 respectively, and the similarities of these are well within experimental error. Values quoted for these pairs of porphyrins as their M(II) derivatives were in the region of 125–140[10], with data for Ni(II) given in Table I. It is apparent, therefore, that the ΔI of this pair of porphyrins is relatively independent of the metal species used in the derivatisation process. This serves to illustrate that the central metal, whether M(II), M(III) or M(IV), exerts minimal influences upon the porphyrin macrocyle.

TABLE III

DATA FOR MASS SPECTRAL ION INTENSITIES (% OF BASE PEAK) OF SOME METALLOPORPHYRIN DERIVATIVES

GC peak	*Compound*	*Substitution pattern*	*Mass spectral ion*										
			M	*M−15*	*M−L*	*M−L−15*	*M−L−30*	*M−L−45*	M^{2+}	$M-15^{2+}$	$M-L^{2+}$	$M-L-15^{2+}$	$M-L-30^{2+}$
B	$(OTMS)_2Si$-(C_{31} actio)	–	100		88				1.5		24	31	
C	$(OTMS)_2Si$-(actio-I)	2	97		100				2		22	16	
D	$(OTMS)_2Si$-(actio-III)	3	100	2.5	90	12	7	2.5	2.2	0.8	25	17.5	1
E	$(OTMS)_2Si$-(OEP)	1	100		42				1		27	10	
H	(OTMS)Al-(actio-I)*	2	100	48	96	9	6		2.3	35	6.3	22	
I	(OTMS)Al-(OEP)	1	100	24	40	9	6	3	1	28	2.5	3	
J	(OTMS)Ga-(actio-I)*	2	36	4	100	3.5	3.5		–	4	7.5	10	6.5
L	$(COCH_3)Rh$-(actio-I)	3	45	1	100	20	12	8	–	–	8	2	3.5

GC peak	*Compound*	*Substitution pattern*	*M*	*M−57*	*M−L*	*M−L−15*	*M−L−57*	M^{2+}	$M-57^{2+}$	$M-2\times57^{2+}$	$M-L^{2+}$	$M-L-57^{2+}$	$M-2L^{2+}$
F	$(OTBDMS)_2Si$-(OEP)	1	6	16	100	4	5	0.3	0.5	10	1.0	16	7

* Data for probe analysis; all others for GC-MS.

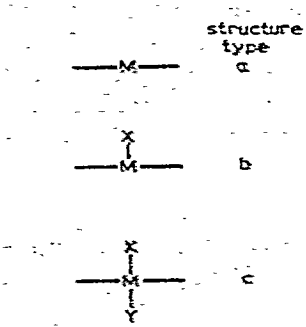
Metal oxidation state and metalloporphyrin volatility

Relative retentions, given in Table I, vary dramatically according to the degree of axial ligation about the metal and also the types of ligand (for most of this discussion the ligand will be the OTMS group, unless otherwise indicated). Structures a, b and c are schematic line drawings of M(II), M(III) and M(IV)-type porphyrins, with the porphyrin plane in bold, which represents gross structural variations in the metalloporphyrins and possible shielding properties of the axial ligand(s).

The addition of each OTMS produces a concomitant increase in volatility (decrease in retention volume) of the order of 10 carbon numbers. Thus although $(OTMS)_2Si$(aetio-I) has a molecular weight of 682, it elutes a full 2000 retention index units earlier than Ni(II)(aetio-I) (molecular weight 534). Evidently, mass is rather unimportant in determining relative GC properties (volatility) of porphyrins, and axial ligation therefore plays the major role. Porphyrins and their metal complexes can exhibit intermolecular interactions (self-association)[19,20] and some have been represented as dimeric structures[21,22]. Such a phenomenon would affect the volatility of the compounds, and associative behaviour in the liquid phase would be expected to decrease the rate of porphyrin desorption into the mobile carrier, thereby increasing the partition coefficient. Since the chromatographic experiment is assumed to occur at infinite dilution, contributions from intermolecular effects should be negligible. Of more significance will be adsorptive interaction between individual solute molecules and the column surfaces to which they are exposed, *i.e.*, column walls and/or stationary liquid phase. It is possible also to invoke absorption differences of the various types of porphyrins to account for the varying retention volumes, *i.e.*, under this model the M(II) porphyrins would have to exhibit a much larger absorption affinity to (*i.e.*, solubility in) the liquid phase, with M(III) not so soluble and M(IV) the least soluble. This is not consistent with the usually observed solubilities of these compounds in common organic solvents, where the OTMS derivatives show increased solubility over the M(II) porphyrins.

The fully shielded M(IV) porphyrins should not be subject to the adsorptive interaction described above since large axial groups prevent close approach of the molecule to adsorptive sites. More bulky groups than OTMS, such as OTBDMS, have no added advantage in preventing interactions because the retention time of the OTBDMS derivative is increased in parallel with its higher molecular weight.

For small axial ligands the shielding effect may not be so efficient as that for the trialkylsiloxy group. During some preparations of Si(IV) porphyrins, incorporation



Schematic line diagrams (structure type) of the M(II), M(III) and M(IV) porphyrins (a, b and c, respectively) used in this study. For c, X and Y are usually the same. The porphyrin plane is represented by the bold line.

of a fluorine axial ligand occurred, such that after hydrolysis a mixture of $(OH)_2SiP$, (F)(OH)SiP and $(F)_2SiP$ (P = porphyrin) was obtained (confirmed by high-resolution MS). Low resolution direct probe analysis of the mixture after conversion of OH to OTMS indicated that the bistrimethylsiloxy species sublimed before the monofluoromonotrimethylsiloxy species. GC analysis of the product obtained with aetio-I indicated that (F)(OTMS)Si(aetio-I) (G) eluted considerably later than the $(OTMS)_2Si$(aetio-I) analogue (C), with a retention index for the former of about 4300. Thus, with regard to their chromatography, compounds of the type (F)(OTMS)SiP may be considered to be in a similar category to the (OTMS)M(III) porphyrins, suggesting that the monofluoro ligand permits some interaction, as described above, to occur. This would presumably be through the face of the molecule bearing the fluorine ligand. Undoubtedly there is some potential for a localised dipole charge to exist across the Si–F bond, and this might account for the differences observed.

This is not the only example of such behaviour of small axial substituents: $(COCH_3)$Rh(III) (aetio-I) (L) had a retention index resembling that of M(II) porphyrins, although the Rh(III) compound eluted with slightly smaller retention volume (thus the $-COCH_3$ ligand might provide some measure of shielding, but not sufficient to fully shield one side of the porphyrin plane). Interestingly, (CH_3)Rh(III) (aetio-I) (M) is of lower volatility than the $-COCH_3$ ligated compound; the latter being volatilised from a mass spectrometer insertion probe before the former. This agreed with GC results where the former compound eluted about 3 carbon numbers (300 Kovats units) later than $(COCH_3)$Rh(III) (aetio-I) (Fig. 6).

The observed volatility (sublimation) for $[((CH_3)_3SiO)_2CH_3SiO]_2$Ge(IV) P (where P = porphin) prompted Kane *et al.*[23] to speculate that this compound might be amenable to GC analysis, explaining that the volatility resulted from the globular shape of the molecule. GC trials in our laboratory of $(OTMS)_2$Ge(IV) (aetio-I) and $(OTBDMS)_2$Ge(IV) (aetio-I) have not yet proved successful.

For partially shielded M(III) porphyrins [Ga(III) and Al(III)], *i.e.*, those represented by structure type b, there is still potential for adsorptive interaction through the unshielded face of the molecule. Since these compounds eluted with retention volumes midway between the Si(IV) and M(II) porphyrins, the axial OTMS ligand must enhance their volatility over that for M(II) porphyrins, and the free-base porphyrins [which elute just prior to the M(II) compounds].

Adsorptive interactions, as invoked to explain the retention behaviour observed for the range of metal porphyrins studied, have not been detailed in terms of specific mechanisms of interaction. However phenomena usually ascribed to adsorption in GC of metal chelates, such as peak asymmetry[24], have not been identified for any of the porphyrin compounds. Since most previous work in metal chelates has been packed-column GC, it may be that improved deactivation of the capillary columns employed here overcomes peak tailing.

Resolution of porphyrin standards of different metals

Separation numbers (Trennzahl, TZ) may be used in column characterisation[25,26] as an indication of the separation power of a column. TZ may be calculated by the equation

$$TZ = \frac{\Delta t_R}{W_{h1} + W_{h2}} - 1$$

where Δt_R = retention difference between solutes 1 and 2, of peak widths at half peak height of W_{h1} and W_{h2} respectively. Example TZ values for some porphyrins are presented in Table II, which lists values for the aetio-I and OEP components of particular metals. It should be pointed out that direct comparison of these values cannot be made since standard conditions have not necessarily been employed. Whilst porphyrin peaks are generally of comparable widths to those of *n*-alkanes of similar retention volumes, and also the Kovats index differences for aetio-I and OEP pairs are similar for the different metals, it is apparent that silicon derivatisation results in more favourable separation (by perhaps a factor of 2) of the two porphyrins due to the relatively narrower peak widths of the silicon compounds. This agrees with the generalisation that TZ values decrease between members of an homologous series as the molecular weight (or retention volume or temperature of analysis) increases for a temperature-programmed run[27]. Grob[27] indicates that generally TZ is only influenced by the temperature of analysis for a particular column. The inverse proportionality of TZ with molecular weight is not applicable for different types of metalloporphyrin derivatives (*e.g.*, different metals) because the metal types represent completely different homologies.

A major factor which governs the separation of compounds is column efficiency (ability to produce narrow peaks) and it seems that if an apolar column produces a higher TZ value for alkanes, then the porphyrins will likewise exhibit better resolution.

Aetio-I and OEP are macrocycles of 32 and 36 carbons, respectively. Separation numbers for Si(IV), Al(III) and Ga(III) complexes of these are all sufficiently large to permit resolution to baseline of homologues differing by one carbon (*i.e.*, C_{32}, C_{33}, C_{34} ... will all be separable from one another). However, within certain of these carbon numbers, structural isomers are possible and are encountered in porphyrin fractions isolated from geological materials[12]. For a fully alkylated C_{32} porphyrin with one methyl and one ethyl group on each pyrrole (C_{32} aetio) there are four possible structures (2 and 3 are just two of these). The steric differences between these may not be particularly large, and hence on apolar columns resolution of such isomers may not be possible [the aetio-I and -III were not resolved as their Si(IV) derivatives]. Polar phase columns may effect isomer resolution.

Detection limits

Detection limits of silicon(IV) porphyrins as trimethylsiloxy derivatives, are similar to those of normal alkanes. For example, the peak produced by *ca.* 10 ng of $(OTMS)_2Si$(aetio-I) was 52% full scale deflection at ×32 attenuation, whilst that for *ca.* 4 ng of *n*-C_{32} alkane was 18.8% full scale deflection (ratio of peak heights = 10:3.6). This was on a Chrompack CPSil 5 glass column. However, experience with some flexible silica columns (coated with apolar phases also) suggests that with these columns, detectability of the porphyrins may not be so good as for the alkanes — possibly due to the inherent acidity of the silica surface causing some decomposition of the porphyrin derivative as it traverses the column (the soft glass of the CPSil 5 capillary is, by contrast, slightly basic).

The *tert.*-butyldimethylsiloxy derivative proved to be an excellent compound to chromatograph, with good detection limits and adequate thermal stability under splitless injection techniques. Significantly, this bulky, sterically-hindered derivative could be chromatographed on almost any of the capillary columns investigated —

even when the trimethylsiloxy derivatives were irreversibly adsorbed (these conditions sometimes occurred after prolonged use of a column, when active sites become prevalent at the injection end of the column —porphyrin adsorption being identified by the orange fluorescence observed in the first column coil when it was placed under a UV lamp).

The detectability of aluminium porphyrins is similar to that of the silicon analogues; however, the gallium porphyrins appear to have a worse detection limit (by a factor of 10 or more), although the reasons for this are not clear.

Gas chromatography–mass spectrometry of porphyrins

The chromatographic elutions of all the complexes reported here have been confirmed by GC–MS analysis. Limited GC–MS results for some silicon porphyrins have been reported previously[4,9]. MS data for (OTMS)Al(III)OEP has also been tabulated[15].

Fig. 7 illustrates the reconstructed ion chromatogram for GC–MS of $(OTBDMS)_2Si(IV)OEP(F)$, along with selected mass chromatograms (fragmentograms) of major ions resulting from the electron impact ionisation of F, displayed over the range of scans 680 to 780.

Fig. 8 shows OTMS derivatives of some alkyl porphyrins run under the same conditions as Fig. 7, with coinjection of the alkane mixture and displayed from scan numbers 600 to 760. It can be seen that the porphyrin peak widths are similar to those of the alkanes.

Relative intensity values quoted are for an RIC value of 100 for the peak of maximum total ion current in the RIC. For example, in Fig. 7, peak F has a relative RIC of 100 for scan number 727. This corresponds to the summation of the individual ion intensities of all ions at this scan number. The plot of *m/z* 691, with a relative intensity of 23.4, indicates that the ion of mass 691 carries 23.4% of the total ion current of scan 727. These figures serve to illustrate the favourable MS monitoring of the silicon porphyrins, and the absence of any appreciable loss in sample resolution attributable to the interface design.

The elution profile of (OTMS)Al(III)(aetio-I) (H), Fig. 9, tends to suggest that some overloading of the column occurred, although the alkane peaks of comparable ion intensity maintain good shapes. However, this compound also shows good behaviour under GC–MS analysis.

Most of the MS information is contained in the molecular ion (M) or in the M − L ion, with one of these usually forming the base peak. Some ion intensities are listed in Table III. Other main fragmentation features are the benzylic cleavages of alkyl groups on the pyrrole ring which account for the series of losses of 15, 30, 45, etc. mass units observed in the spectra. Doubly-charged ions are a feature of porphyrin mass spectra[28]. The only other notable ion is that at *m/z* 75, which arises from fragmentation of the trimethylsiloxy ligand. Plotting out mass chromatograms of any of the diagnostic ions resulted in peak displays which matched very closely that obtained from the summed mass chromatogram of significant ions.

The mass spectrometer scan times (*ca.* 3 sec for full scan cycle) were rather slow when compared with compound elution times, and often only three or so scans contained the required data on the compound, and hence relative ion intensities obtained from a GC–MS run may not necessarily match those obtained from a probe analysis where compound sublimation into the source is over a greater time interval.

CONCLUSIONS

This paper has reported the first GC results obtained for a number of metalloporphyrin derivatives and discussed some of their chromatographic properties. Their retention behaviour is consistent with the anticipated volatility changes resulting from changes in alkyl substitution of the macrocycle and, more markedly, changes in the type of axial trialkylsiloxy ligand. Different types of derivative may be distinguished on the basis of their retention volumes, *i.e.* those species in which the porphyrin is unshielded, partly shielded or fully shielded by bulky axial ligands, in which the retention volumes decrease substantially with increased shielding.

The porphyrins appear to be a unique class of compound in their interest for gas chromatographic studies. The tetrapyrrolic macrocycle permits the study of a range of metal species in various oxidation states, since many metals can be complexed, with charge balance, if necessary, provided by axial ligation.

Volatile aluminium and gallium chelates have already been gas chromatographed. These M(III) species are of type $M(L)_3$, where L is a bidentate, univalent ligand such as those modelled on β-diketonates[29].

There are few reports of GC of silicon chelated into a molecular complex, though many organosilicon and inorganic silicon compounds may be amenable to GC. Incorporation of silicon into compounds as a derivatising agent[30] for functionalities such as hydroxy groups, as used in this work, is well known in GC.

Perhaps the most challenging area for application of GC to porphyrins will be the development of GC and GC–MS techniques for the rapid analysis of complex natural mixtures of porphyrins[3–5]. GC–MS would be a powerful addition to the high-performance liquid chromatography techniques widely applied to such analyses[11,12]. In the geochemical field, porphyrins are being investigated for possible use as marker compounds for geological maturation processes[31]. Alkylporphyrin mixtures in geological materials appear to be complex and to contain two major types, the aetio- and DPEP-types. The latter are related to deoxophylloerythroetioporphyrin and contain an isocyclic ring[32]. The aetio-I and OEP standards used herein are representative of aetio-types (though OEP is not geologically significant), at least as far as consideration of GC analysis of a natural sample is concerned.

From the resolution obtained between the C_{31} aetio, aetio-I and OEP standards, it is apparent that porphyrins of different carbon number should be adequately separated by capillary GC. Separation of positional (type) isomers was not achieved in the current work, *e.g.* aetio-I and aetio-III (Fig. 8, C and D). DPEP-type porphyrins are known to GC with slightly greater retention volumes than the equivalent carbon number aetio-types[5]. Lack of suitable standards prevented confirmation of this on capillary columns. It is expected that, as with the aetio-types, DPEPs of different carbon number should be easily resolved.

The strong ions (M^+ for TMS species, $M - 131^+$ for TBDMS species) obtained with electron-impact MS in the GC–MS experiments will be of benefit in the study of complex mixtures, allowing easy derivation of the molecular weight of components.

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