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CAPILLARY GAS CHROMATOGRAPHY AND GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF SILICON(IV) DERIVATIVES OF PORPHYRINS WITH POLAR SUBSTITUENTS

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SUMMARY

The successful gas chromatography (GC) of a number of standard polar porphyrins (with ester side chains) is reported for the first time. This is effected by preparation of their bis(trimethylsiloxy)silicon(IV) derivatives which show considerably greater volatility than the free base porphyrin precursor. Results for the mass spectrometric (MS) analysis (as GC–MS) are presented, thus facilitating identification of some of the components which almost coelute, and establishing the presence of a homologue (presumably a mixed ester) of one of the porphyrins. The GC retention volumes are correlated with structural features of the compounds, and this enables a superficial prediction of anticipated retention indices for other biologically significant polar porphyrins.

INTRODUCTION

Recently, the capillary gas chromatography (GC) of a number of alkyl porphyrins with various central metal species has been reported[1–3] and an account of some main features of their chromatography may be seen in ref. 3. A number of reports of packed column GC of silicon porphyrins have also appeared[4–6]. To date, successful GC of porphyrins with polar substituents has not been achieved although the preparation of the silicon complex of one such porphyrin (mesoporphyrin-IX dimethyl ester) by Games and co-workers[6,7], evidently with an aim to studying its GC potential, was carried out some years ago.

GC and GC–mass spectrometric (MS) techniques have, however, been used in relation to analysis of porphyrins with polar substituents, employing degradative methods[6] to produce, for example, monopyrrole subunits which aid in structural identification[6]. Similarly, oxidative degradation to maleimides has been employed for alkyl porphyrin identification using GC–MS[8].

The apparent difficulties associated with porphyrin GC, such as irreversible

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adsorption on packed columns[5] (although recent results suggest that these can be overcome with capillary column technology[1–3]) have prompted analysts to evaluate alternative methods for high resolution fractionation of porphyrins. In the field of porphyrins with polar substituents (referred to herein as "biological" porphyrins), the advent of high-performance liquid chromatography (HPLC) has proved to be of major importance and there are many reports of HPLC of porphyrin free acids on normal and reverse phases[9,10], and using ion-pairing techniques[11]. The most favoured procedure appears to be HPLC of the ester (usually methyl ester) derivatives[7,12–14], and some examples of HPLC of their metal chelates have been presented[15,16].

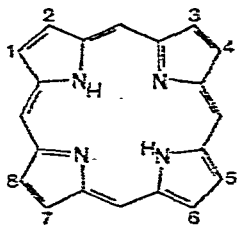
Other chromatographic techniques (thin-layer chromatography, paper chromatography and column LC) in general are widely employed in porphyrin analysis for both purification and separation[17], however, GC remains an as yet unestablished tool for routine analysis of porphyrin pigments. In this paper, a new method for the analysis of porphyrins with polar ester substituents based upon capillary GC and GC–MS of their volatile silicon derivatives is presented, and its potential application to the analysis of porphyrins with increasing numbers of polar substituents (such as those isolated from biological sources) is discussed.

EXPERIMENTAL

Preparation of silicon derivatives

The preparative procedure was based upon that of Boylan *et al.*[5] and Alturki[18] and has been described elsewhere[3]. Briefly, hexachlorodisilane was added to a solution of free base porphyrin in dry toluene in a two-necked round-bottom flask, with a flow of dry nitrogen flushing through the reflux condenser/flask system. The solution was refluxed for about 30 min, and its UV–visible spectrum was checked for absence of free base absorption bands. The required product was isolated as the dihydroxy species, which was derivatised to the bis(trimethylsiloxy) compound for GC studies.

Free base starting materials were obtained from Professor P. S. Clezy (rhodo-



Structures of free base porphyrins:

1 Deuteroporphyrin-IX dimethyl ester (deutero-IX)
1 = 3 = 5 = 8 = Me; 2 = 4 = H; 6 = 7 = p^{Me}

2 Mesoporphyrin-IX dimethyl ester (meso-IX)
1 = 3 = 5 = 8 = Me; 2 = 4 = Et; 6 = 7 = p^{Me}

3 Rhodoporphyrin-XV dimethyl ester (rhodo-XV)
1 = 3 = 5 = 8 = Me; 2 = 4 = Et; 6 = p^{Me}; 7 = –COOMe

4 Rhodoporphyrin-XV homologue (rhodo-XV homologue)
Structure as rhodo-XV, but with an additional methylene unit at one substituent position

5 Aetioporphyrin-I (aetio-I)
1 = 3 = 5 = 7 = Me; 2 = 4 = 6 = 8 = Et.

(Me = methyl; Et = ethyl; p^{Me} = $-CH_2CH_2COOMe$)

porphyrin-XV dimethyl ester*, rhodo-XV, 3) and Dr. O. T. Jones (mesoporphyrin-IX dimethyl ester, meso-IX, 2 and deuteroporphyrin-IX dimethyl ester, deutero-IX, 1).

GC and GC–MS instrumentation

The gas chromatograph used was a Carlo Erba FTV 4160 instrument. This was fitted with an on-column injector as standard, and was used for all the work described. Flame ionisation detection and hydrogen carrier gas were employed.

Gas chromatographic-mass spectrometric analyses were carried out using the same GC as above interfaced to an AEI MS 30 mass spectrometer. Details of this system have already been given[3]. Hydrogen carrier was used for GC–MS work, with source temperature *ca.* 200°C.

A glass capillary column, 20 m × 0.34 mm I.D., manufactured by Chrompack and coated with CPSil 5, was used for most of the results reported here. An OV-1 coated flexible silica column (Phase Separations), 18 m × 0.3 mm I.D. was used for one example presented here. Conditions for the GC experiments are indicated for individual chromatograms.

RESULTS AND DISCUSSION

Fig. 1a, b and c represent chromatographic traces for the deutero-IX (peak A), meso-IX (peak B) and rhodo-XV (peak C) compounds, respectively. The normal alkanes were coinjected in order to obtain retention indices for the porphyrins. The alkane suite in Fig. 1a and b was obtained from an oil pipe sludge whilst those in Fig. 1c were pure alkane standards (used to show peak D which would otherwise coelute with the n-C_{39} alkane). The retention indices of the porphyrins are tabulated in Table I [including the index of the aetioporphyrin-I (structure 4) derivative used for comparative purposes] along with their total carbon numbers and molecular weights. Retention indices were calculated by the approximate linear interpolation method, between successive n-alkanes whose retention times spanned that of the porphyrin of interest.

Rhodoporphyrin-XV "homologue" occurrence

The direct insertion probe MS analysis of the trimethylsiloxy derivative of the silicon rhodo-XV product indicated the presence of a second component of 14 mass units greater molecular weight than that of rhodo-XV. This corresponded to peak D in Fig. 1c, and will be referred to as the rhodo-XV homologue since its structure was not established. It evidently was generated during the preparative procedure used for silicon insertion/hydrolysis since the probe MS of the free base reactant indicated rhodo-XV only. Other observations were that probe MS of the reactant after attempted derivatisation of any remaining carboxylic acid groups gave only rhodo-XV, and also a "control" reaction procedure without the addition of the silane gave only rhodo-XV, and no homologue. The homologue probably arises as a result of the

* Rhodoporphyrin-XV dimethyl ester (rhodo-XV) refers to the structure as given by 3, and is not to be confused with the rhodo-type porphyrins (so named because they have the rhodo-type electronic spectrum) which occur in geological samples and which are believed to possess a fused benzene ring substituent on one of the pyrrole rings.

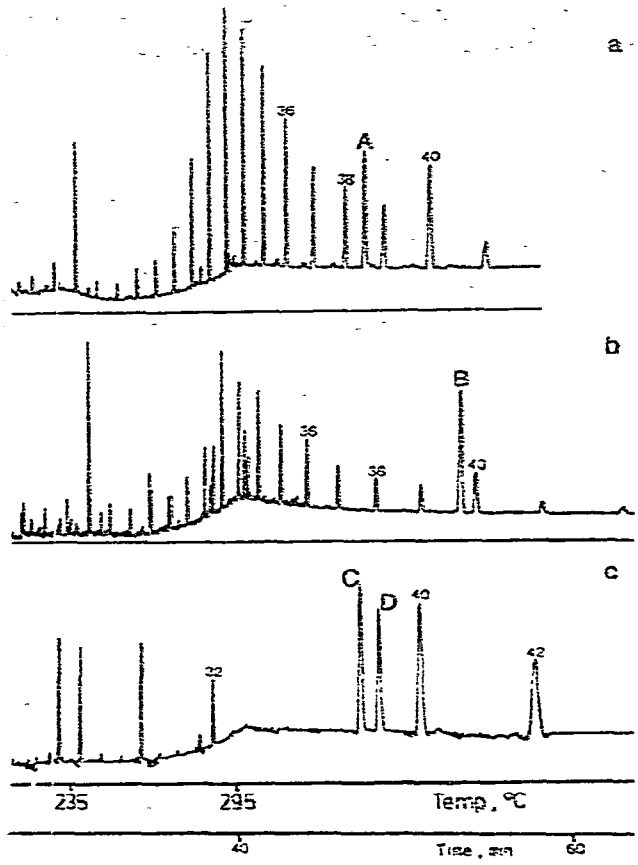


Fig. 1. Capillary gas chromatograms of $(OTMS)_2Si(IV)$ derivatives of porphyrins, coinjected with normal alkanes. Chrompack CPSil 5 glass column, 20 m × 0.34 mm I.D., temperature programmed from 60°C to 295°C at 6°C min^{-1}. Hydrogen carrier gas average linear flow velocities ($\bar{u}$) were: (a) 45 cm sec^{-1}; (b) 36 cm sec^{-1}; (c) 53 cm sec^{-1}. Peaks A, B, C and D correspond to the derivatives of this same designation in Table I. Some coinjected *n*-alkanes are identified by their carbon numbers (*i.e.* 40 = n-C_{40} etc.).

ethanolic hydrolysis step, with subsequent formation of an ethyl ester. Since this would entail hydrolysis of a methyl ester (the initial free base porphyrin reactant was supposedly the dimethyl ester), it is more likely that the ethyl group is incorporated on the $-CH_2CH_2COO-$ group rather than the $-COO-$ group, since the methyl ester of the former is more easily hydrolysed[19]. The occurrence of the homologue is rather unusual, if the above rationale is correct, since both deutero-IX and meso-IX did not produce a second compound after the silicon insertion/hydrolysis work up. It has been reported that trace quantities of ethanol in solvents can result in the formation of mixed esters during the preparation of the methyl esters of uroporphyrin[14]. The relative chromatographic retentions of rhodo-XV and its assumed homologue are reasonably consistent with the suggestion that they are homologues.

Relative retention indices of porphyrins

Comparison of the retention index of aetio-I and those of the other porphyrins

TABLE I

GC RETENTION DATA FOR $(OTMS)_2Si(IV)$ DERIVATIVES OF SOME PORPHYRINS ON A CPSil 5 COATED GLASS CAPILLARY COLUMN

Porphyrin	*Structure of free base*	*GC peak*	*No. of carbons**	*Molecular weight***	*Retention index****
Deutero-IX	1	A	38	742.31	3850
Meso-IX	2	B	42	798.38	3975
Rhodo-XV	3	C	40	770.35	3855
Rhodo-XV "homologue"	4	D	41	784.37	3905
Aetio-I	5	E	38	682.37	3330

* Including trimethylsiloxy groups.
** Calculated for the most abundant isotopes.
*** Determined by linear interpolation between alkanes; quoted to nearest 5 index units.

reveals that the polar porphyrins have significantly longer retentions than the alkyl porphyrin (aetio-I). Aetio-I and meso-IX differ in retention index by 515 index units, whilst the structural difference is the presence of two 2'-carbomethoxyethyl groups in meso-IX compared with ethyl groups in aetio-I. Clearly the two ester groups will influence (decrease) volatility to a much greater extent than two ethyl groups substituted on the pyrrole β positions, since they protrude out more from the periphery of the molecule. These ester groups might have an effect upon chromatographic retention to a similar extent to those usually obtained for ester compounds in GC. Methyl esters of acid groups (R–$COOCH_3$) usually add 360–370 retention units[20,21] to that obtained for a compound without the –$COOCH_3$ functionality (R–H), on apolar columns. This was confirmed here on an OV-1 capillary column by chromatographing pristane (a C_{19} isoprenoid alkane) and the methyl ester of phytanic acid (a C_{20} isoprenoid acid), with a resultant ΔI (retention index difference) of 370.

The above generalisations allow prediction of the retention indices of the polar porphyrins from those of the alkyl porphyrins. Since meso-IX has two carbomethoxy groups, addition of 2(360–370) *i.e.* 720–740 retention units may be expected from this contribution. Prediction of the meso-IX retention index on the basis of that for aetio-I would give 3330 + (720–740) = 4050–4070, and this compares with an observed index of 3975. Hence, the retention index of the polar porphyrin is a little less than that predicted.

The retention difference between deutero-IX and meso-IX is $\Delta I = 125$. This is for a structural difference of 4 carbons, and is in close agreement with the ΔI value of aetio-I and octaethylporphyrin ($\Delta I = 130$)[3], which also differ by 4 carbons. The above discussion does not take into account absolute structure differences such as position of substituents around the macrocyclic porphyrin ring, and the effects which these might have are not yet established.

Rhodo-XV and deutero-IX almost coelute, although their structures are quite different. Deutero-IX has two unsubstituted positions on the pyrrole rings (compared with two substituent ethyl groups in rhodo-XV) and rhodo-XV has a –$COOCH_3$ group instead of the –$CH_2CH_2COOCH_3$ group of deutero-IX. Evidently, these structural variations are almost counterbalanced when their contributions to gas chromatographic properties are compared.

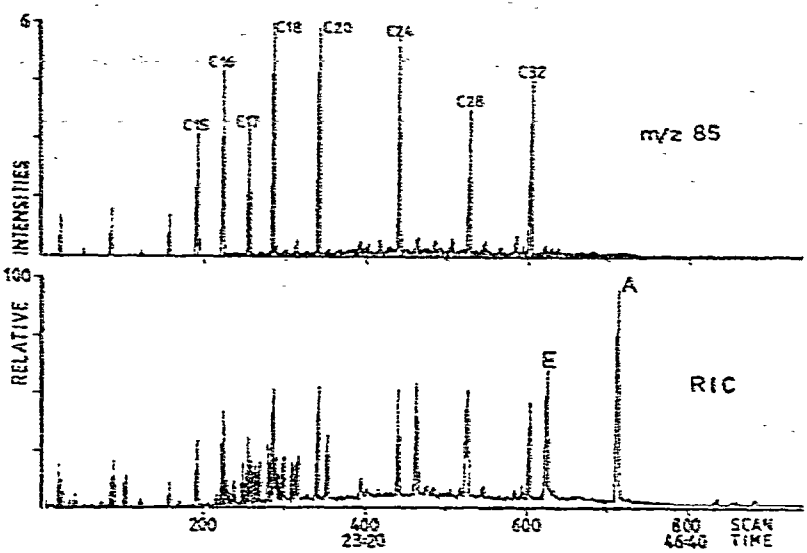


Fig. 2. GC–MS traces for $(OTMS)_2Si$(deutero-IX) (A) and $(OTMS)_2Si$(aetio-I) (E), with a coinjected standard alkane mixture. Lower trace, reconstructed ion chromatogram (RIC); upper trace, plot of diagnostic ion *m/z* 85, which arises from fragmentation of the alkanes. Phase separations OV-1 flexible silica column, 18 m × 0.3 mm I.D., temperature programmed from 60°C to 320°C at 6°C min^{-1}. 320°C isothermal hold commenced at scan 700. Total scan time (per cycle) = 3.5 sec (scanning from *m/z* 800 to 50).

The above observations may allow prediction of retention indices of porphyrins with an increased number of polar substituents (ester groups). Therefore, coproporphyrin tetramethyl ester (4 × methyl, 4 × $-CH_2CH_2COOCH_3$ groups) might be expected to elute at about *n*-C_{46} and this is approaching the limits for routine capillary GC. Uroporphyrin octamethyl ester would elute in the region of *n*-C_{58}, which is rather an impractically high retention volume for routine analysis. Other important porphyrin classes which may be of interest include protoporphyrin (structure as mesoporphyrin, except with two vinyl groups instead of ethyl groups and hematoporphyrin, and these are currently being investigated.

Gas chromatography–mass spectrometry

GC–MS analyses were routinely carried out on the prepared samples on a GC–MS system developed for this work. GC–MS data should prove indispensable when complex porphyrin samples derived from natural sources are to be analysed. The results of a few selected examples of the standard porphyrins follow.

In Fig. 2, two traces indicate the reconstructed ion current (RIC) chromatogram and *m/z* 85 chromatogram (lower and upper traces, respectively). The alkane diagnostic ion (*m/z* 85) plot allows identification of the peaks on the RIC which correspond to alkanes. The two porphyrin peaks, at scan numbers 626 and 714, are aetio-I and deutero-IX, respectively, and both exhibit excellent peak shapes. Relative intensities are indicated in this figure, and also in Figs. 3 and 4. These are quoted relative to an RIC value of 100, which corresponds to the intensity of the sum of all ions in the particular scan which produces the largest peak on the RIC. Thus, in Fig.

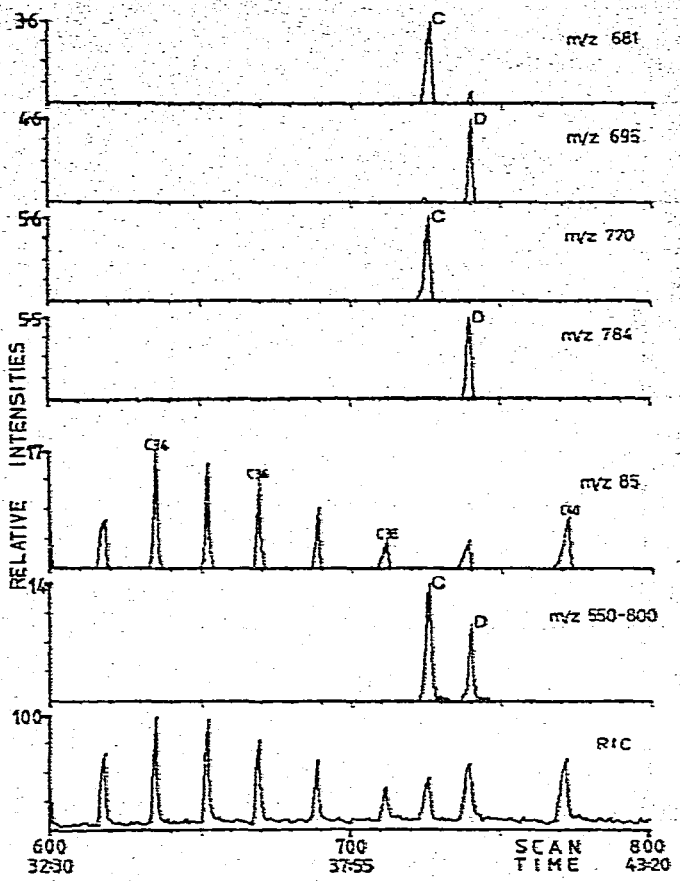


Fig. 3. GC–MS traces for $(OTMS)_2Si$(rhodo-XV) (C) and its presumed homologue (D), with coinjected alkanes (alkane sample as used in Fig. 1a and b). Scans 600 to 800 only illustrated. Trace *m/z* 550–800 is a summation of all ions within mass range *m/z* 550 to *m/z* 800 and includes the major ions for the porphyrins, thus presenting a display of the two individual porphyrin peaks. Mass chromatograms of single masses *m/z* 784, 770, 695 and 681 correspond to displays of the molecular ion for D, molecular ion for C, loss of axial ligand from D and loss of axial ligand from C, respectively. Column as in Fig. 1, using the same temperature programme conditions as in Fig. 1. 295°C isothermal hold, commenced at scan 670. Total scan time (per cycle) = 3.25 sec (scanning from *m/z* 850 to 35).

2, the relative intensity of the *m/z* 85 ion for normal alkane C_{18} is 6% of that of the total sum of ions giving rise to peak A.

Rhodo-XV (C) and its homologue (D) are shown in Fig. 3, with three traces indicating the RIC, the mass chromatogram of *m/z* 85 and an averaged mass chromatogram obtained by summing all ions between *m/z* 550 and 800 (which plots out the porphyrin peaks). As can be seen, the homologue elutes just after *n*-C_{39} (not easily identified in the RIC or GC traces). In Fig. 3, mass chromatograms of individual ions of masses *m/z* 681, 695, 770 and 784 are illustrated for the same data. The masses 770 and 784 correspond to molecular ions for rhodo-XV and its homologue, with 681 and 695 being their major fragment ions, respectively (for loss of axial trimethylsiloxy ligand).

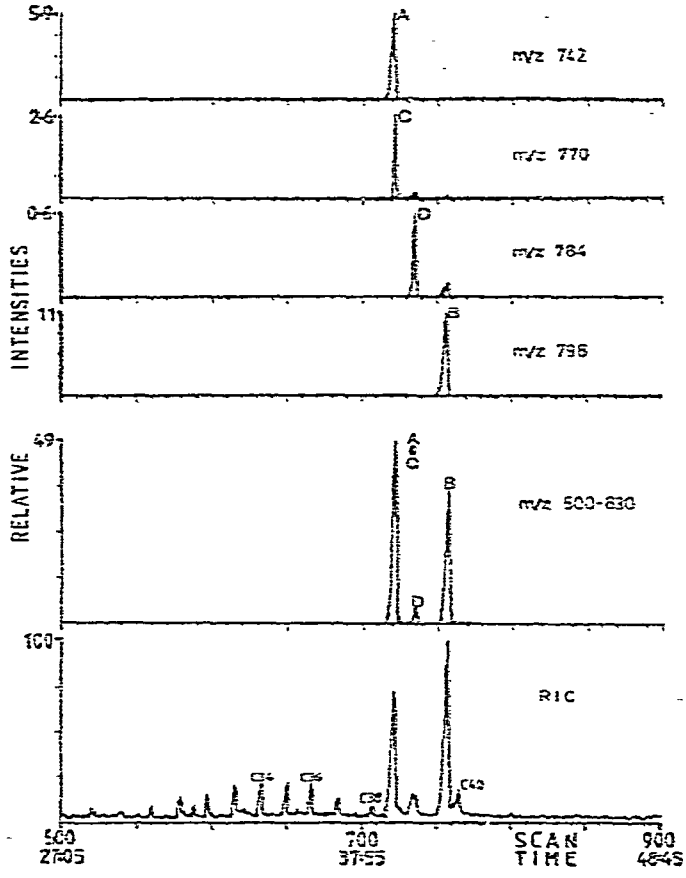


Fig. 4. GC–MS analysis of a mixture of all 4 porphyrin derivatives (A, B, C and D), coinjected with the alkane sample used in Fig. 3. Trace *m/z* 500–830 includes all the major ions for the porphyrins. Ions of *m/z* 798, 784, 770 and 742, displayed in the mass fragmentograms, correspond to the porphyrin molecular ions (see Table I). Column and conditions as Fig. 3, with 295°C isothermal hold commencing at scan 660.

A mixture of all three porphyrins produced the result in Fig. 4, again with the summed mass chromatogram (*m/z* 500–830) displaying the porphyrin peaks. The mass chromatograms of the ions of *m/z* 742, 770, 784 and 798 (Fig. 4) correspond to the compounds of the same molecular weights in Table I. The mass chromatogram displays can be used to separate deutero-IX from rhodo-XV, which overlap severely on this column. Meso-IX (*m/z* 798) also contributes a small amount of ion current to the *m/z* 784 mass chromatogram, due to fragmentation resulting in loss of 15 mass units, which could either be ascribed to β-cleavage of a methyl group of one of the ethyl side chains[22] or to loss of a methyl from the trimethylsiloxy ligand[23], according to general observations.

Figs. 3 and 4 are partial chromatograms, plotted from just before the isothermal hold region of 295°C, after programming up to this temperature at 6°C/min.

Some portions of the mass spectra of these compounds are presented in Fig. 5. As a comparison, ion intensities for $(OTMS)_2Si$ (aetio-I) (molecular weight 682)

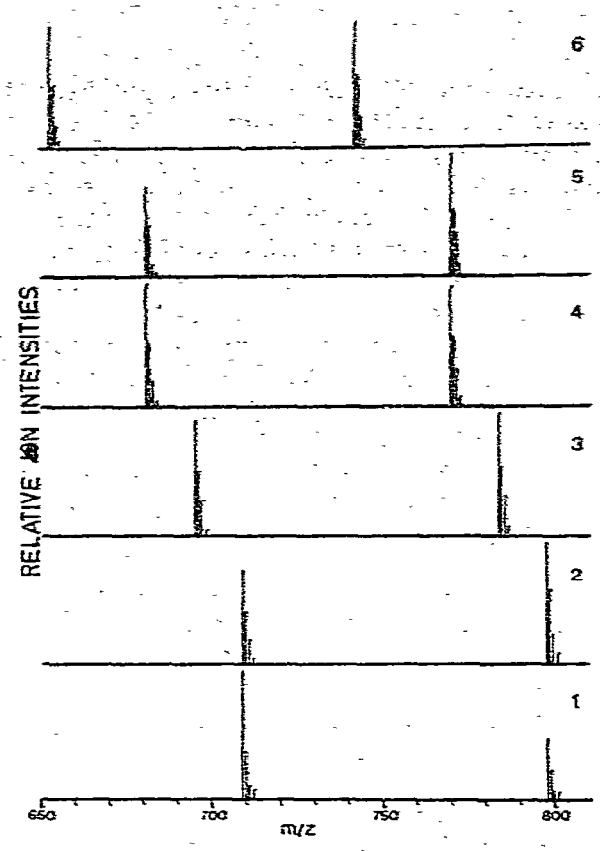


Fig. 5. Isotopic patterns for molecular ions and M − 89 ions for the $(OTMS)_2Si$ porphyrin derivatives, obtained under the following experimental conditions: Examples 1 and 4 = probe analysis data; examples 2, 3, 5 and 6 = GC–MS analysis data, obtained by summation of individual scans across the peak. 1 and 2 refer to compound B (Table I), 3 refers to D, 4 and 5 refer to C, and 6 refers to compound A.

obtained by summing ions over the solute elution peak in a GC–MS experiment, were [in terms of mass (intensity)] 593 (87), 594 (42), 595 (17), 596 (5.5), 682 (100), 683 (57), 684 (28), 685 (9). Mass 682 corresponds to the most intense molecular ion, and 593 is the ion for loss of one trimethylsiloxy group. The other ions listed are those for the isotopic contributions to the mass, and they arise primarily from carbon and silicon. The isotopic patterns for the porphyrins in Fig. 5, obtained under the conditions in the legend, generally resemble the expected patterns calculated for the silicon and carbon isotope abundances for these compounds.

In the GC–MS analyses, fragmentations of polar side chains from the molecular ion were generally not very intense (*ca.* 1 % or less), with the spectrum dominated by the loss of one axial ligand. This reduced intensity, compared with the usually observed fragment ion intensities seen for the free base analogues, has been previously noted by Budzikiewicz[24]. For the $-CH_2CH_2COOCH_3$ side chains, losses of 31, 59 and 73 (cleavage β to the pyrrole ring) were observed, in accordance with expectations for such substituents on porphyrins[22,25].

CONCLUSIONS

This paper has illustrated the first successful GC analysis of polar porphyrins (those with ester side-chains). The apparently favourable GC and GC–MS results suggest that a wide range of similar tetrapyrroles should be evaluated for their GC suitability. The main constraints placed upon such analyses appear to be the molecular weight of the derivatised sample and its degree of functionalisation which thereby influence retention volumes in the GC experiment. Mesoporphyrin-IX dimethyl ester, with a molecular weight of 798 after derivative formation, has a retention index of almost 4000 (*i.e.*, elutes close to n-C_{40}), and by prediction of retention volumes of other polar porphyrins (using simple structure–retention arguments) it is probable that GC methodologies should be suitable for porphyrins with about four carbomethoxyethyl substituents, in addition to alkyl groups on the other pyrrole positions (although the use of higher temperature-stable columns could extend this limit).

The retention behaviour of polar porphyrins seems to be consistent with expected retention when contributions to retention volume from structural features of the side chains are considered. Peak shapes in general compare very favourably with those of alkanes with similar retention volumes.

The significance of these results lies in the fact that a technique utilising a sensitive, high-resolution analytical tool has been shown to have great potential for the analysis of porphyrins containing polar substituents. Advantages include the ability to fractionate complex samples, to obtain information on the molecular weight of components, and to analyse data using computerised processing capabilities. Studies involving isotopic labelling of porphyrins should also be amenable to GC–MS.

The proven HPLC technique offers certain attractions, such as relatively simple compound handling prior to analysis, but HPLC–MS requires considerable development before complex mixtures can be effectively studied.

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