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Determination of porphyrin carbon isotopic composition using gas chromatography–isotope ratio monitoring mass spectrometry

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Abstract

Carbon isotopic compositions of aetio I occurring in the form of free-base, nickel, demetallation, dihydroxysilicon(IV) and bis(*tert.*-butyldimethylsiloxy)silicon(IV) [(*t*BDMSO)$_2$Si(IV)] have shown that it has experienced no obvious isotope fractionation during the synthesis of [(*t*BDMSO)$_2$Si(IV)] porphyrin from aetio I. Here, aetio I porphyrin species such as free-base, nickel, demetallated and dihydroxysilicon were analyzed by the conventional method, namely it is combusted in sealed system, and followed by isotope ratio monitoring mass spectrometric analysis. [(*t*BDMSO)$_2$Si(IV)] aetio I was assayed by gas chromatography–isotope ratio monitoring mass spectrometry (GC–IRMS). A porphyrin mixture of [(*t*BDMSO)$_2$Si(IV)] aetio I and octaethylporphyrin was also prepared. Their carbon isotopic compositions measured by GC–IRMS indicate that no isotope exchange took place between the porphyrins during the synthesis of [(*t*BDMSO)$_2$Si(IV)] porphyrins. This method is employed for δ^{13}C determination of geoporphyrins from the Maoming and Jianghan oil shales.

Keywords: Isotope ratio mass spectrometry; Mass spectrometry; Porphyrins

1. Introduction

Geoporphyrins, which are widely present in sediments and crude oils, represent the "molecular fossils" of chlorophylls, bacteriochlorophylls and haems in the organisms from which organic matter is derived [1–3]. Previous studies suggested [4–7] that the carbon isotopic composition of porphyrin has the capability to selectively record effects in the process of primary production (which led to the biosynthesis of the molecular precursors of the geoporphyrins), but not secondary process (those mediating the transformation of precursor into geoporphyrin). δ^{13}C values of porphyrins have the potential to provide information about oil–oil and oil–source rock correlations, calculate the paleo P_{CO_2} [8], reconstruct the pathway of the carbon cycle in the paleoenvironment with other carbon isotope compositions of biomarkers [9].

The conventional method [9–11] to determination porphyrin carbon isotopic composition is: (1) isolation of metalloporphyrin from oil and rock by column chromatography and thin-layer chromatography (TLC); (2) isolation of individual porphyrins by high-performance liquid chromatography (HPLC); (3) conversion of individual porphyrins to CO$_2$ by way of off-line combustion; (4) δ^{13}C measurement of CO$_2$ by isotope ratio monitoring mass spectrometry (IRMS). This procedure is time-

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consuming and requires several mg of individual porphyrin. Therefore, limited work has been done on geoporphyrin carbon isotope because of the complication of measurement.

With the introduction of gas chromatography (GC) combined with GC–IRMS to geoscience [12,13], a wider range of low-molecular-mass hydrocarbons and functional precursors have been analyzed because of its simplicity and effectiveness [14–18]. But porphyrin has never been analyzed by GC–IRMS because of its low volatility, not readily amenable to GC analysis. Bis(*tert*.-butyldimethylsiloxy)silicon(IV) [(tBDMSO)$_2$Si(IV)] derivatives of porphyrins can be analyzed by routine GC–MS which is not directly applicable to alkyl porphyrins [19,20]. The bulky *t*BDMSO groups reduce the aggregation effects, making porphyrins sufficiently volatile for this technique (temperature up to ca. 300°C). Our study aims at establishing a method for the determination of porphyrin carbon isotopes by GC–IRMS. Before (tBDMSO)$_2$Si(IV) porphyrins are analyzed by GC–IRMS, it must be verified that each step involved in the synthesis of (tBDMSO)$_2$Si(IV) porphyrin should be free from obvious isotope fractionation, or even if isotope fractionation occurs, a certain approach could be developed for calibration. In this paper, aetio I and octaethylporphyrin (OEP) are used to attest the whole synthesis procedure.

2. Experimental

2.1. Reagents

Aetio I and OEP free-base porphyrins were obtained from Porphyrin Products (UT, USA); methanesulfonic acid, hexachlorodisilane and *N*-methyl-*N*-*tert*.-butyldimethylsilyltrifluoroacetamide (MTBSTFA) came from Aldrich (Milwaukee, USA).

2.2. Preparation of (tBDMSO)$_2$Si(IV) aetio I (Fig. 1)

2.2.1. Nickel porphyrin

Aetio I (ca. 20 μmol) and methanolic nickel(II) acetate (ca. 3.3 mmol) were dissolved in chloroform (ca. 3 ml) and refluxed for 2–3 h under the protection of purified nitrogen gas. The mixture was diluted (CH_2Cl_2, 10 ml) and washed with water (3×15 ml) to remove the salts. After drying (Na_2SO_4), the organic layer was evaporated and purified by TLC (silica gel G, toluene, R_F ca. 0.8). Their UV–Vis spectra are consistent with the characteristic of nickel porphyrins. The yield was quantified by the UV–Vis spectra.

2.2.2. Demetallation of nickel porphyrin

Methanesulfonic acid (2 ml) was added to nickel aetio I (19 μmol) in a sealed vial under the protection of purified nitrogen gas. The mixture was heated for 2 h at 100°C. After cooling, the mixture was diluted with water (10 ml) and extracted with dichloromethane (3×15 ml). After washing with aqueous $NaHCO_3$ (sat., 3×10 ml), the CH_2Cl_2 solution was evaporated to dryness. The resultant free-base aetio I was purified by TLC (silica gel G, CH_2Cl_2, R_F ca. 0.5). The yield was quantified by the UV–Vis spectra.

2.2.3. Insertion of silicon

Free-base porphyrin (6 μmol) was dissolved with dry toluene, then excessive hexachlorodisilane (0.1 ml) was added under the protection of purified nitrogen gas and allowed to stand at room temperature for 24 h. Aqueous KOH (sat.) was added until all solids were dissolved, and the products were extracted with dichloromethane (3×15 ml). After CH_2Cl_2 the solution was evaporated to dryness, The resultant dihydroxysilicon(IV) aetio I was purified by TLC (alumina, CH_2Cl_2, R_F ca. 0.6). The silicon porphyrin was quantified by the UV–Vis spectra.

2.2.4. Silylation of silicon porphyrin

Dihydroxysilicon aetio I was dissolved with dry pyridine, MTBSTFA was added under the protection of purified nitrogen gas. After heating for 12 h at 50°C, the mixture was kept at room temperature for 48 h. The derivative was quantified by MS (Fig. 2). The yield was near-quantitative [20].

2.3. Preparation of (tBDMSO)$_2$Si(IV) mixture porphyrins

The mixture porphyrins were composed of aetio I and OEP. Nickel insertion, demetallation, silicon

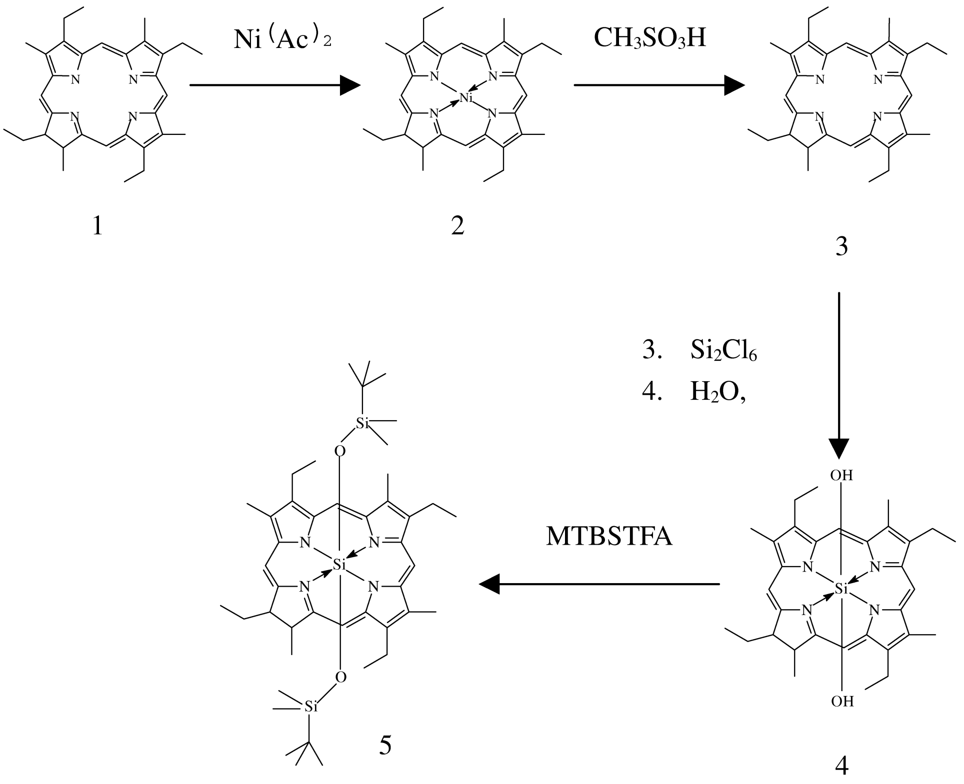

Fig. 1. Synthesis of (tBDMSO)$_2$Si(IV) aetio I porphyrin.

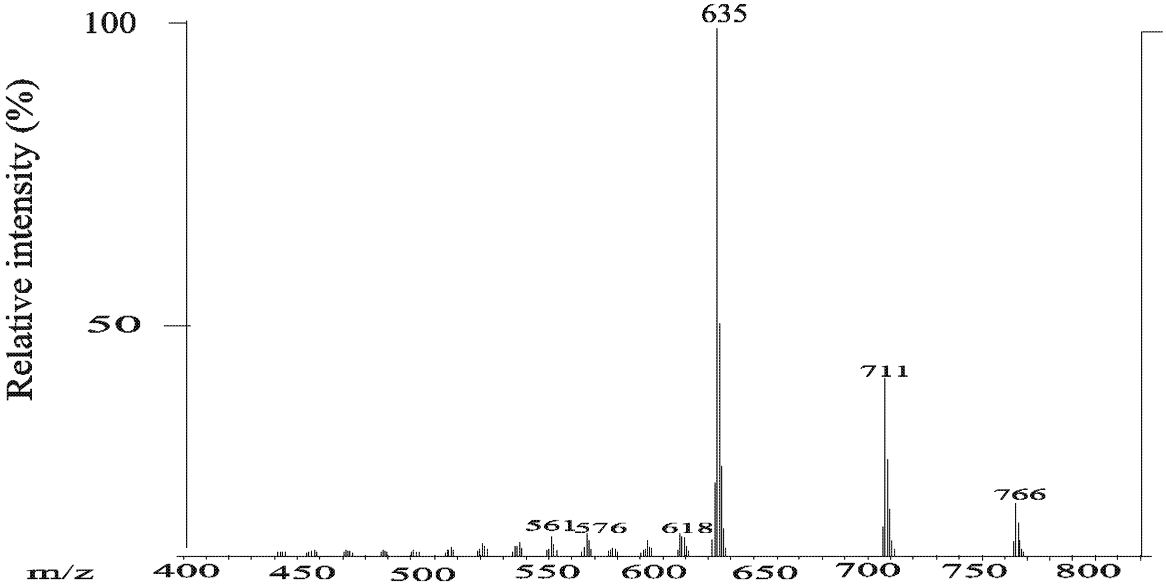

Fig. 2. Mass spectra of (tBDMSO)$_2$Si(IV) aetio I porphyrin.

insertion and silylation were performed using an analogous procedure for aetio I.

2.4. Preparation of (tBDMSO)$_2$Si(IV) geoporphyrins from Maoming and Jianghan oil shales

Powdered oil shales were Soxhlet extracted with methanol–toluene (58:42) for 3 days. After flocculation of asphaltene (light petroleum), the meltene was applied to a silica gel column for isolation of nickel porphyrin. The wash solvent was CH_2Cl_2–hexane (1:1, v/v). Demetallation, silicon insertion and silylation were carried out using the same procedure for aetio I.

2.5. Measurement of porphyrin carbon isotopes

Porphyrin compounds 1, 2, 3, 4 (Fig. 1) and free-base OEP porphyrin are off-line combusted in a sealed system at 950°C, the CO_2 derived from combustion procedure was transferred to the mass spectrometer MAT251 for isotopic analysis.

All derivative (tBDMSO)$_2$Si(IV) porphyrins are measured by GC–IRMS on a VG Isochrom II mass spectrometer.

2.6. Equipment

2.6.1. UV–Vis spectrophotometry

UV–Vis spectral analyses were done on a Shimadzu UV-3000 spectrophotometer using 1-cm pathlength cells. Typical scanning range was 700–350 nm. Porphyrin was dissolved with dichloromethane.

2.6.2. Gas chromatography–mass spectrometry

GC–MS analysis was performed on the Finnigan GC8000-Voyager equipped with an on-column injector. The following conditions were employed for analysis of (tBDMSO)$_2$Si(IV) porphyrins.

GC conditions: crosslinked methylsilicone-coated flexible silica capillary column (HP-5, 25 m×0.32 mm I.D.; 0.17 μm film thickness); helium carrier, flow-rate: 1.2 ml min^{-1}. 35–250°C at 15°C min^{-1}, 250–300°C at 3°C min^{-1}, and 300°C for 30 min.

MS conditions: ionizer temperature: 250°C. Ionization energy: 70 eV, emission current: 350 μA. Mass range: 50–900, full scan.

2.6.3. Isotope ratio monitoring mass spectrometry

IRMS analysis was performed on a MAT251 mass spectrometer.

MS conditions: ionization energy: 95.29 eV; trap current: 1.000 mA. Accelerating voltage: 10 kV, resolution: 200 (10% valley).

2.6.4. GC–IRMS

GC–IRMS analysis was carried out on a VG Isochrom II mass spectrometer linked with an HP 5890 gas chromatograph. The following conditions were employed for the analysis of (tBDMSO)$_2$Si(IV) porphyrins.

GC conditions: crosslinked methylsilicone-coated flexible silica capillary column (HP-5, 25 m×0.32 mm I.D., 0.17 μm film thickness); helium carrier, flow-rate: 1.2 ml min^{-1}; 35–250°C at 15°C min^{-1}, 250–300°C at 3°C min^{-1}, 300°C for 30 min; cooled on-column injection.

MS conditions: electrospray ionization (EI) source: ionization energy: 99.51 eV, trap current: 400 μA. Mass analyzer: accelerating voltage: 4.2 kV, resolution: >100 (10% valley).

2.6.5. Elemental analyzer

A Heraeus CHN-O rapid analyzer was used, combustion temperature: 950°C.

3. Results and discussion

3.1. Synthesis procedure

From nickel porphyrins (which are ubiquitous in sediments and crude oils) to (tBDMSO)$_2$Si(IV) porphyrins (which can be detected by GC–MS or GC–IRMS), three reaction steps were involved (Fig. 1). Taking aetio I as the standard, we measured the reaction yields for these steps using the UV–Vis spectrophotometer. Each of the steps was repeated five times. As can be seen in Table 1, demetallation and silicon insertion give an average yield of >90%. Quantification of silylation was not run since the (tBDMSO)$_2$ silicon derivatives are sensitive to mois-

Table 1
Yield (%) of derivative reaction

	No. 1	No. 2	No. 3	No. 4	No. 5	Yield average (%)
Ni insertion	95.6	95.7	97.3	96.2	95.4	96.0
Demetallation	90.2	88.3	92.4	88.7	91.5	90.2
Si insertion	89.6	90.7	91.3	90.7	90.2	90.5

ture. But Gill reported that the yield in this step is almost quantitative [20].

Great efforts have been made to enhance the yield of reaction (Table 1). All the reactions have to proceed in darkness to prevent photodegradation of porphyrins. In the step involving demetallation, reaction under a nitrogen gas atmosphere is very important to avoid destruction of porphyrins by oxygen at high temperature.

3.2. Measurement of porphyrin carbon isotopic composition

Table 2 lists the carbon isotopic composition of porphyrin compounds 1–4. They are off-line combusted in a sealed system, the CO_2 derived from combustion procedure was transferred to the mass spectrometer MAT251 for isotopic analysis. In the step pertaining to nickel(II) insertion and demetallation, the isotope values of porphyrin compounds 1–3 vary within the limit of measurement errors, and no sign of obvious isotope fractionation was observed.

However, in the step of silicon insertion, the isotope values of aetio I (compounds 3 and 4) vary remarkably, as reflected by the reduction of $\delta^{13}C$ by −1.8‰ on average. According to a previous study [21], silylation, which adds an alkylated silyl group to a hydroxy group, seems not to have a kinetic isotope effect associated with the derivatization reaction which will affect the $\delta^{13}C$ value of a carbon position contained in the final derivative. In our case, ^{13}C depletion of compound 4 is due to the incomplete combustion. We analyzed the carbon content of compound 4 on an elemental analyzer (Table 3). The combustion temperature (950°C) is similar to that used for off-line combustion. The percent content of carbon in compound 3 is close to the theoretical value (81.01%). But compound 4 only contains 58.06–63.83% carbon, that means 7–13% of the carbon remains incombusted. The polarity of compound 4 enhances as compared with compound 3, so aggregation effect is increased, making the compound difficult to gasify and the combustion of dihydroxysilicon porphyrin might be incomplete.

It has even been reported [22] that an organic compound containing silicon, is converted to CO_2 by way of combustion over a high frequency stove. The temperature may reach up to 1300°C under which the compound will be decomposed completely. In this study, the highest oven temperature is 950°C. Therefore, the incomplete combustion of dihydroxysilicon

Table 2
Carbon isotope values of aetio I and its derivatives

Compound[a]	No. 1	No. 2	No. 3	No. 4	No. 5	Average	SD
1[b]	−24.2	−24.2	−24.1	−24.3	−24.4	−24.2	0.1
2[b]	−24.4	−24.6	−24.4	−24.1	−24.3	−24.4	0.2
3[b]	−24.5	−24.7	−24.3	−24.1	−23.8	−24.3	0.3
4[b]	−26.1	−26.1	−26.6	−26.0	−25.8	−26.1	0.3
5[c]	−26.5	−26.5	−26.5	−26.3	−26.1	−26.4	0.1
Aetio I[d]	−24.6	−24.6	−24.6	−24.4	−24.1	−24.5	0.34

[a] See Fig. 1.

[b] Measured by an offline procedure (see Section 2.5).

[c] Measured by GC–IRMS (see Section 2.6.4).

[d] Carbon isotope value is calculated from $\delta^{13}C$ of $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ aetio I, the standard deviation (SD) calculation with the equation given by Rieley [21].

Table 3
C, H, N contents of free-base and dihydroxysilicon aetio I porphyrins

	Free-base aetio I porphyrin				Dihydroxysilicon aetio I porphyrin		
	C (%)	H (%)	N (%)		C (%)	H (%)	N (%)
No. 1	80.14	7.21	12.12	No. 1	59.10	2.91	7.14
No. 2	80.79	6.18	11.40	No. 2	63.83	3.17	8.14
No. 3	80.38	6.30	12.10	No. 3	61.66	3.92	9.55
No. 4	80.23	6.55	11.84	No. 4	61.31	4.08	8.56
No. 5	80.73	7.39	12.26	No. 5	58.06	4.26	8.87
Average	80.45	6.73	11.94	No. 5	60.79	3.67	8.45
T.V.[a]	81.01	7.17	11.81	T.V.[a]	71.61	6.71	10.45

[a] T.V.: Contents of C, H, N calculated from the structural formula.

porphyrin will result in a lighter δ^{13}C for compound 4.

The final product (tBDMSO)$_2$Si(IV) aetio I porphyrin (compound 5) was measured on the Isochrom II for determination of δ^{13}C (Fig. 3), and the original carbon isotopic composition of aetio I calculated using the following expression:

$$\delta^{13}C_3 = \frac{n_1\delta^{13}C_1 + n_2\delta^{13}C_2}{n_1 + n_2}$$

where: n_1 = carbon number of aetio I, namely 32; n_2: = 12, the carbon number of (tBDM)$_2$ in (tBDMSO)$_2$Si(IV) aetio I, $\delta^{13}C_1$ = carbon isotope value of aetio I; $\delta^{13}C_2$: = carbon isotope value of the tBDM portion in the (tBDMSO)$_2$Si(IV) aetio I molecule, −31.5‰, which is calculated from δ^{13}C of (*tert*.-butyldimethylsilyl)butyl ether resulting from the reaction of MTBSTFA with *n*-butanol (Table 4); $\delta^{13}C_3$: carbon isotope value of (tBDMSO)$_2$Si(IV) aetio I.

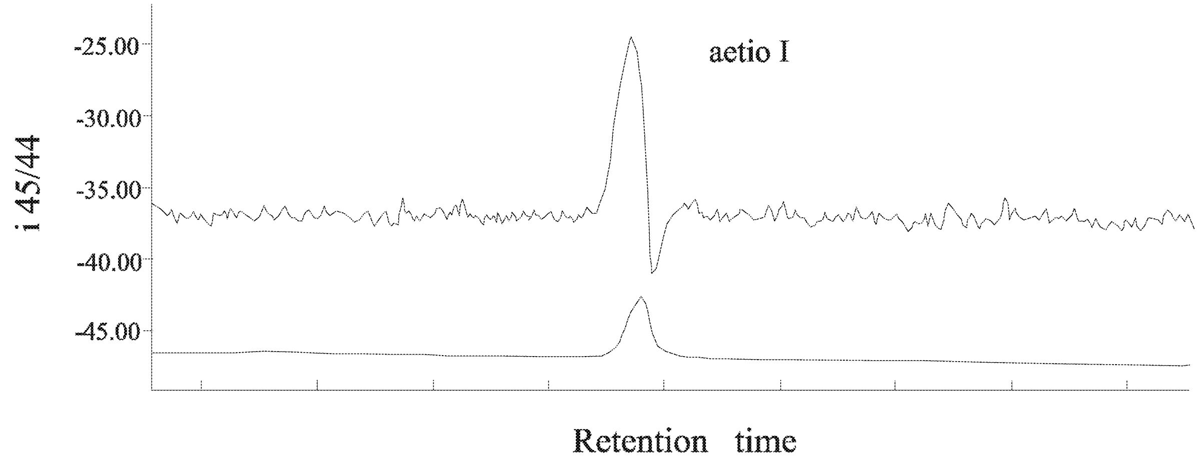

Fig. 3. GC–IRMS spectra of (tBDMSO)$_2$Si(IV) aetio I porphyrin.

Table 4
Carbon isotope values of *n*-butanol and its derivatives[a]

	No. 1	No. 2	No. 3	No. 4	No. 5	Average	SD
(*tert*.-Butyldimethylsilyl)butyl ether	−30.76	−31.03	−31.29	−31.17	−30.74	−31.00	0.2
n-Butanol	−30.29	−30.50	−30.47	−30.02	−30.34	−30.32	0.2

[a] Measured on the Isochrom II.

The calculated carbon isotope values (Table 2) have shown that the carbon skeleton of aetio I in $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ aetio I is similar to that of the original aetio I, indicating that no obvious isotope fractionation has occurred in the whole synthesis procedure. Although $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ aetio I contains three Si atoms per molecule, the bulky *t*BDMSO group will make its polarity decrease, showing differences from the results for off-line combusted compound 4. The combustion of compound 5 on the Isochrom II seems complete.

Our hypothesis, complete combustion of compound 5 in GC–IRMS, is supported by some studies. As we known, carboxylic acids and alcohols were usually derivatized by bis(trimethylsilyl)trifluoroacetamide (BSTFA) before GC–IRMS analysis. Though the derivatized compounds contain silicon, and in calibration a slight isotopic shift caused by derivatising groups has been found, as reported by Jones et al. [23] and Abrajano et al. [24]. But no obvious isotope fractionation has been detected when the compound is measured by GC–IRMS.

From Tables 1–3 and Fig. 1, it is concluded that this method can be used to measure the carbon isotopic composition of single porphyrins.

3.3. Carbon isotope values of mixture porphyrins

Earlier studies stated that porphyrins in geological samples are mixtures. It is necessary to verify whether there is any carbon isotope exchange between porphyrins during the whole synthesis procedure, especially in the step of demetallation with a strong acid at 100°C. The porphyrin mixtures of aetio I and OEP were chosen. The reaction steps and measuring approaches are the same as those for aetio I (Fig. 4). The calculated δ^{13}C of aetio I and OEP from $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ derivatives in mixture porphyrins are −24.3‰ and −27.8‰, respectively, which are approximate to that of the individual aetio I and OEP porphyrins measured by off-line combustion (−24.5‰, −27.5‰). Carbon isotope exchange among porphyrin mixtures is not observed in the whole synthesis procedure.

3.4. Application to the measurement of geoporphyrins

Before $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ geoporphyrins are submitted to GC–IRMS analysis, GC–MS analysis is necessary as the GC–MS spectra can identify their GC peaks, i.e., to distinguish the single porphyrin from a mixture of porphyrins.

Listed in Table 5 is the carbon isotopic composition of geoporphyrins in the Jianghan and Maoming oil shales (Fig. 5). Every geoporphyrin represents a single molecular structure. In spite of a slight difference, major porphyrins, C_{32} DPEP in the Maoming oil shale and C_{31} DPEP, C_{32} DPEP in the Jianghan oil shale, are similar in carbon isotopic

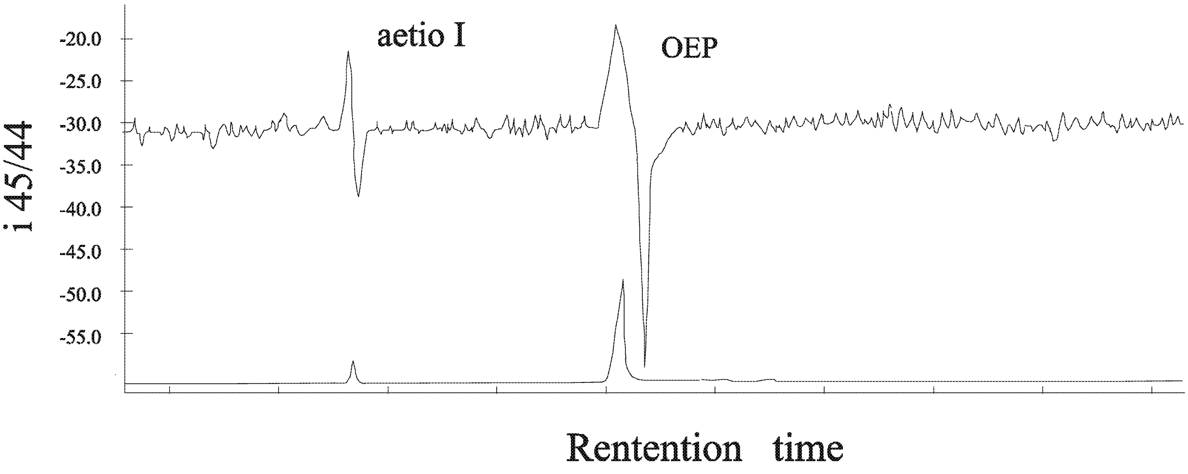

Fig. 4. GC–IRMS spectra of $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ mixture porphyrin of aetio I and OEP.

Table 5
Carbon isotope values of geoporphyrins determined by GC–IRMS

	C_{32} aetio	C_{31} DPEP^{1a}	C_{31} DPEP^{2a}	C_{32} DPEP
Jianghan oil shale		−23.5	−23.1	−23.9
Maoming oil shale	−26.9	−26.7		−22.1

[a] The structure of C_{31} DPEP^{1} is 13,15-ethano DPEP; C_{31} DPEP^{2} is the isomeric compound of C_{31} DPEP^{1}, the structure is not known yet. See Ref. [28].

compositions (−22.1~23.9‰). Phytane in both samples, which is thought to be derived from the phytol side chain of chlorophyll, has the same $\delta^{13}C$ value of −22.2‰. These lines of evidence are consistent with the conclusion that they are possibly derived from a common precursor, i.e., chlorophyll [25]. But the precursor of C_{31} DPEP in the Maoming oil shale seems different. Its $\delta^{13}C$ value is close to that of C_{32} aetio (−26.00‰).

It was original proposed by Triebs (1936) [29] that the aetio porphyrins, which lack the exocyclic ring the characteristic of photosynthetic pigment, are derived from heme, which predominates in cytochromes and various carrier proteins. Also, some researchers pointed out that the exocyclic ring on chlorophyll might be opened in response to various diagentic reactions, such as oxidation and thermal degradation, thus giving rise to the formation of aetio porphyrins [26,27]. Our oil shale samples from Maoming are immature (vitrinite reflectance $R^{\circ}=0.38$), with high organic carbon (6.27%), implying that these samples underwent weak oxidation and thermal degradation. Therefore, the carbon isotopic composition of C_{32} aetio in the Maoming oil shale is different from that of the major porphyrin C_{32} DPEP, suggesting that this compound derived from a distinct primary source, presumably hemes. Previous studies also showed that heme originated C_{32} aetio has a $\delta^{13}C$ value varying between −26.6‰ and −26.9‰ [6].

4. Conclusions

$(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ derivatives enable the determination of alkyl porphyrins by GC–IRMS. The reaction yield at each synthesis step is quantitative. Analysis of the carbon isotopic composition of intermediates and final product in the synthesis procedure has shown that no obvious isotope fractionation occurred.

Isotope exchange between porphyrin mixtures was not observed in the preparation of $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ derivatives of porphyrins.

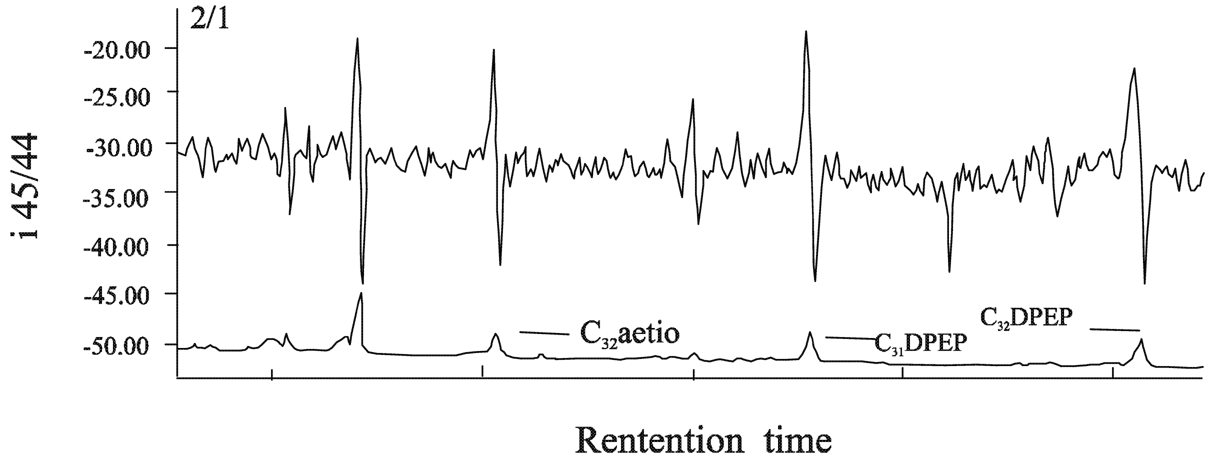

Fig. 5. GC–IRMS spectra of $(t\mathrm{BDMSO})_2\mathrm{Si(IV)}$ geoporphyrin in the Maoming oil shale.

This GC–IRMS method was also applied to the analysis of carbon isotopic composition of geoporphyrins from the Maoming and Jianghan oil shales. The $\delta^{13}C$ values of C_{31} DPEP, C_{32} DPEP and C_{32} aetio porphyrins in the Maoming oil shale are −26.7‰, −23.1‰ and −26.9‰, respectively, whereas C_{31} DPEP^{1},C_{31} DPEP^{2} and C_{32} DPEP porphyrins in the Jianghan oil shale give $\delta^{13}C$ values of −23.5‰, −23.1‰ and −22.1‰, respectively.

Acknowledgements

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