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Note

Analysis of crocetane in crude oils and sediments: novel stationary phases for use in GC–MS

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Abstract

Cyclodextrin GC stationary phases, well documented for their superior isomeric separation capabilities, have been investigated in the separation of crocetane (2,6,11,15-tetramethylhexadecane) and phytane (2,6,10,14-tetramethylhexadecane). Although crocetane has been attributed to methane oxidizing archaea, its general occurrence in crude oils and sediments is yet to be fully established due to difficulties associated with the GC–MS analysis since crocetane co-elutes with phytane when chromatographed on most liquid phase coated capillary columns. A method is described for the routine GC–MS analysis of crocetane and phytane in crude oils and sediments, using cyclodextrin stationary phases. Numerous derivatised cyclodextrin columns were investigated. A 50 m permethyl-β-cyclodextrin was found to give the best resolution ($R = 0.8$ using hydrogen as the carrier gas and $R = 0.7$ using helium).

Keywords: Crocetane; Phytane; Cyclodextrin stationary phases

1. Introduction

Acyclic isoprenoids are biomarkers widely utilised in organic geochemistry. Studies of the lipid components of archaea continue to extend the range of acyclic isoprenoid skeletons known in organisms, many biomarkers of which have been identified in sediments and crude oils. Irregular head-to-head and tail-to-tail linked isoprenoids in particular, are compounds which appear to be uniquely synthesised by archaea (Chappe et al., 1979; Ward et al., 1985; Stefanova, 2000). Compounds such as 2,6,10,15,19-pentamethylicosane (PMI) (I) (see Appendix) and 2,6,15,19-tetramethylicosane (TMI) (II) have been identified in recent and ancient sediments, the natural product precursors of which are known components of methanogenic bacteria (e.g. Schouten et al., 1997; Vink et al., 1998). The presence of these compounds in geochemical samples clearly indicates the presence of methanogenesis and hence, anoxia in depositional environments. Squalane (**III**) and the regular C_{21}–C_{25} isoprenoids have been suggested to be markers for halophilic archaea (Grice et al., 1998) and hence molecular indicators of hypersaline environments of deposition (ten Haven et al., 1988; Sinninghe Damsté et al., 1993). Isoprenoid compounds have thus been established to have excellent potential as specific biomarkers of archaeal input to geochemical samples, the distribution and stable carbon isotopic composition of which are used to reconstruct palaeoenvironments of deposition.

Crocetane (**IV**) is a C_{20} tail-to-tail linked acyclic isoprenoid first identified in modern sediments from

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Kattegat (Bian, 1994) and more recently in a Miocene Marmorito limestone (Thiel et al., 1999), in methane-rich volcanic sediments of the Eastern Mediterranean Ridge (Pancost et al., 2000) and in deep sea sediments at cold seeps of the eastern Aleutian subduction zone (Elvert et al., 2000). Although a discrete source organism is yet to be identified, crocetane has been recognized as a marker for methane oxidizing archaea based on its highly depleted ^{13}C value (ca 100 per mil) in the Miocene limestone (Thiel et al., 1999). These organisms feed on isotopically light biogenic methane and are consequently typically significantly depleted in ^{13}C (Summons et al., 1994).

Studies concerning the GC elution behaviour of crocetane and phytane have been previously reported. Robson and Rowland (1993) reported partial resolution of the two compounds using OV-1 type stationary phases, although this separation could not be replicated in the present study. Thiel et al. (1999) achieved partial resolution ($R=0.6$) of crocetane and phytane when a mixture was examined by GC isothermally at 170°C on a CpSil 2CB (squalane) column, using hydrogen as the carrier gas. In this investigation we sought to overcome the co-elution of crocetane with phytane and establish an analytical method suitable for the routine identification of crocetane in crude oils and sediments by GC–MS. A range of stationary phases, in particular derivatised cyclodextrin phases (α, β and γ), were examined for their ability to resolve the two diastereomeric isoprenoids.

2. Experimental

2.1. Preparation of crocetane and phytane

The synthesis of crocetane has been reported previously (Robson and Rowland, 1993). Briefly, 1-bromo-3,7-dimethyloctane was prepared from geraniol (3,7-dimethylocta-2,6-dienol). The reaction of sodium metal on the alkyl bromide via a Wurtz-coupling reaction afforded crocetane (92% yield). Phytane was produced by hydrogenolysis (PtO_2 catalyst) of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) in ethanol. Mass spectra were obtained for both phytane and crocetane and were in accordance with their structures.

Isomerization reactions were performed on both crocetane and phytane in order to produce compounds more closely resembling those present in crude oils, to ensure that any separation of phytane isomers in crude oils would not be mistaken for the presence of crocetane. Crocetane (10 mg) and phytane (10 mg) were heated in a sealed vessel under vacuum (300°C, 24 h) with Pt/C (10 mg) and *meso*-pristane as an internal standard, such that the isomerization reaction could be monitored. The all-isomer mixtures of crocetane and phytane were then utilised in all chromatographic analyses.

2.2. Gas chromatography (GC) of crocetane and phytane

GC analyses of the synthetic crocetane and phytane were performed on a Hewlett-Packard 5890 instrument, fitted with a vapourizing injector and flame ionization detector. The injector was operated with a range of head pressures and heated at various rates to the maximum usable temperatures of the various stationary phases. Table 1 shows the range of stationary phases, column dimensions, heating rates, carrier gas pressures and the resolution (R) for the separation of crocetane and phytane.

2.3. Gas chromatography–mass spectrometry (GC–MS) of crocetane and phytane

GC–MS analyses were performed on a Hewlett-Packard 6890 instrument fitted with a fused silica open tubular column (50 m, 0.22 mm i.d., 0.25 μm film thickness (f.t.), SGE, Australia). Temperature program: 1 min at 50°C; 50 to 220°C at 3°C/min; 10 min at 220°C. Carrier gas: helium at a constant pressure of 25 psi. Samples for analysis were injected splitless (200°C) using a HP6890 autosampler. Typical MSD conditions were ionisation energy 70 eV, source temperature 230°C, electron multiplier voltage 2200 V.

3. Results and discussion

3.1. Separation of crocetane and phytane by GC

Chromatographic results from this study are summarised in Table 1. For each capillary column investigated the head pressure and temperature program were altered with each consecutive run to optimise the resolution. Regardless of the GC conditions, resolution of both compounds could not be achieved using a BP-1 stationary phase. Similar co-elution was observed using slightly more polar BP-5 and BP-20 stationary phases. Maximum resolution of crocetane and phytane (Fig. 1) was achieved with a β-CYDEX column (50 m, 0.22 mm i.d., 0.25 μm f.t., SGE, Australia), consisting of 10% permethyl-β-cyclodextrin using a constant head pressure of 15 psi.

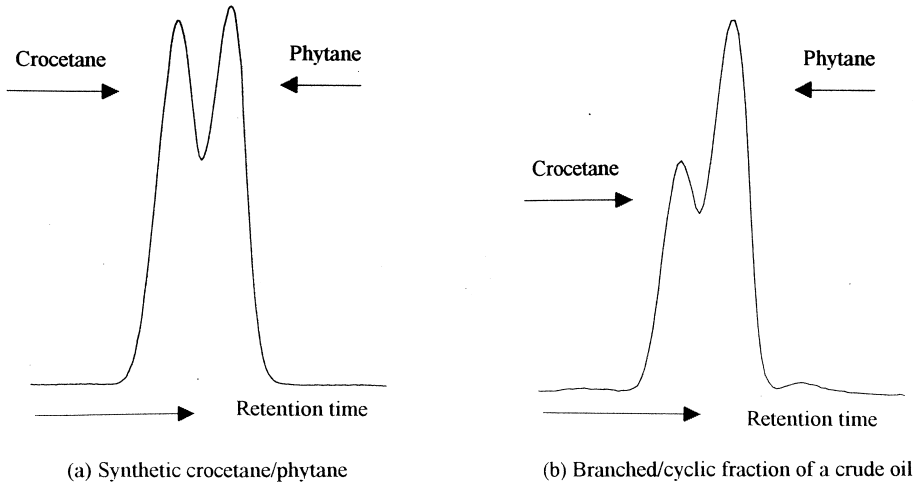
3.2. Separation of crocetane and phytane by GC–MS

For mass spectrometric analysis helium was employed as the carrier gas and a maximum resolution of 0.7 achieved with the β-CYDEX column, using a heating gradient of 3°C/min and constant pressure of 24 psi. Similar resolution was achieved with a 10% permethyl-γ-cyclodextrin column of similar length and internal diameter.

Table 1
Capillary columns investigated and resolution achieved

GC stationary phase	Carrier gas	Head pressure	Heating rate	Final temperature	Resolution (R)[a]
Permethyl-β-cyclodextrin (β-CYDEX)	Hydrogen	15 psi	4°C/min	220°C	0.8
(50 m, 0.22 mm i.d., 0.25 μm f.t.)	Helium	24 psi	3°C/min	220°C	0.7
Permethyl-γ-cyclodextrin (50 m, 0.22 mm i.d., 0.25 μm f.t.)	Helium	30 psi	3°C/min	220°C	0.6
Permethyl-α-cyclodextrin (10 m, 0.25 mm i.d., 0.25 μm f.t.)	Helium	2-7 psi	1-3°C/min	220°C	N/R[b]
6-TBDM-β-cyclodextrin[c] (30 m, 0.25 mm i.d., 0.25 μm f.t.)	Helium	12-20 psi	1-3°C/min	220°C	N/R
BP-1 (25 m, 0.32 mm i.d., 0.50 μm f.t.)	Helium	7-20 psi	1°C/min	310°C	N/R
BP-1 (40 m, 0.18 mm i.d., 0.40 μm f.t.)	Helium	35-45 psi	1°C/min	310°C	N/R
BP-5 (40 m, 0.8 mm i.d., 0.40 μm f.t.)	Helium	32-40 psi	1°C/min	310°C	N/R
BP-20 (50 m, 0.22 mm i.d., 0.25 μm f.t.)	Helium	20-35 psi	1°C.min	270°C	N/R

[a] Conventional chromatographic resolution (R), calculated according to the equation: $R = \frac{2(t_{R,X} - t_{R,Y})}{W_{b,X} + W_{b,Y}}$, where t_R is the retention time of components X and Y and W_b is the peak width at the baseline.
[b] N/R = no resolution achieved.
[c] 6-TBDM = tributyldimethyl.



(a) Synthetic crocetane/phytane (b) Branched/cyclic fraction of a crude oil

Fig. 1. Partial m/z 169 mass chromatograms of (a) synthetic crocetane/phytane and (b) branched/cyclic fraction of a crude oil. The GC was fitted with a 50 m×0.22 mm i.d. fused silica capillary column with β-CYDEX stationary phase (SGE, Australia). Temperature program: 1 min at 50°C; 50–220°C at 3°C/min; 10 min at 220°C. Carrier gas: helium.

The resolution reported here using hydrogen is slightly better than that achieved by Thiel et al. (1999) when a mixture of crocetane and phytane was examined, with hydrogen as the carrier gas, isothermally at 170°C on a 25 m CpSil 2CB (squalane) column. Although the use of hydrogen as a carrier gas results in improved sensitivity and slightly higher separation efficiency, the use of a higher molecular weight gas (e.g. helium) ensures that the solute diffusivity is minimised. In addition, programmed temperature operation provides for better separation of complex mixtures and improved detection limits, peak shapes and precision. Thus the method described in this study, which produces comparable resolution to that achieved by Thiel et al. (1999), allows for routine GC–MS analysis of complex geochemical samples including screening for crocetane, using helium as the carrier gas.

The mass spectra of crocetane and phytane are identical to those reported in the literature (Robson and Rowland, 1993). The two compounds produce mass spectra with almost identical fragmentation patterns (m/z 85, 99, 113, 127, 141 etc.) as expected for two isomers. The mass spectrum of crocetane is, however, characterised by an enhanced ion abundance at m/z 169 compared with that of phytane, thus allowing the two compounds to be distinguished from one another.

Although the level of separation achieved in this investigation is suitable for routine GC–MS analysis, the use of cyclodextrin phases cannot as yet be applied in the field of

compound specific isotope ratio mass spectrometry to determine the stable isotopic composition of crocetane in crude oils and sediments due to the requirement for baseline resolution of eluting components. The potential for these phases has, however, been recognised for use in the field of organic geochemistry and the range of derivatised cyclodextrin stationary phases available for investigation has been far from exhausted.

4. Conclusions

(i) The resolution capabilities of cyclodextrin columns have been investigated in order to separate the two isomeric C_{20} isoprenoids, crocetane and phytane, which co-elute on most GC capillary columns.

(ii) The permethyl-β-cyclodextrin stationary phase was found to be the most suitable column of those investigated to resolve crocetane and phytane for the routine GC–MS analysis of crude oil and sediment samples using helium as the carrier gas.

(iii) The cyclodextrin stationary phases cannot as yet be utilized for routine irm-GC–MS analysis to determine the isotopic composition of crocetane; however the potential for these stationary phases in organic geochemistry has been recognised.

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Appendix