Note

C_{16}–C_{29} homologous series of monomethylalkanes in the pyrolysis products of a Holocene microbial mat

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Abstract

Preparative open pyrolysis products of kerogen isolated from a Holocene microbial mat collected in Abu Dhabi (United Arab Emirates) contain homologous series of monomethylalkanes (MMAs, C_{16}–C_{29}). The isomer and carbon number distributions of these branched alkanes closely match those of MMAs in ancient sediments and oils. It is proposed that monomethylalkanes in oils and in thermally mature ancient sediments derive from non-extractable organic matter produced by one or more members of the cyanobacterial assemblage, eubacterial primary producers and/or heterotrophic bacteria. © 2000 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Since the identification of monomethylalkanes (MMAs) in the extractable lipid fraction of cyanobacterial cultures (Han et al., 1968; Gelpi et al., 1970), cyanobacteria have been considered the possible source of methyl-branched alkanes in sediments and oils. However, cyanobacterial cultures and modern cyanobacterial assemblages generally contain extractable MMAs with a carbon range and isomer distribution (C_{17}–C_{21}; see review by Shiea et al., 1990; Kenig, 1991; Kenig et al., 1995) incompatible with those observed in ancient sediments and oils (C_{15}–C_{33}; i.e., Jackson et al., 1986; Fowler and Douglas, 1987; Summons et al., 1988a,b). This discrepancy casts doubt on a direct relationship between free cyanobacterial hydrocarbons and the homologous series of MMAs identified in ancient sediments and crude oils.

Organisms other than cyanobacteria also produce MMAs. Although C_{24}–C_{40} MMAs were identified in the free hydrocarbon fraction of Abu Dhabi Holocene microbial mats, these compounds originate from insects feeding on the microbial mats, not from the microbial assemblage (Kenig et al., 1994; 1995). In these insect-derived MMAs, the methyl groups are located exclusively at odd-numbered carbon atoms, and the linear carbon skeletons almost exclusively have an odd number of carbon atoms. In oils and ancient sediments, methyl groups are located at both even and odd-numbered carbon atoms.

Recently, Thiel et al. (1999) identified homologous series of C_{15}–C_{25} mid-chain branched alkanoic acids in eubacteria living symbiotically with demosponges. This supports the hypothesis of Summons (1987) and Summons et al. (1988a) that MMAs could be diagenetic products of functionalized lipid precursors. Thiel et al. (1999) showed that these mid-chain branched acids could be precursors of some of the MMAs identified in sponge-containing sediments, but could not account for the carbon number range of the MMAs frequently identified in ancient sediments and crude oils.

All published data on MMAs in cyanobacterial cultures and in modern and Holocene cyanobacterial assemblages were obtained from extractable organic fractions. In this report, the open pyrolysis products of

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a Holocene cyanobacterial assemblage sampled from the Abu Dhabi sabkha (United Arab Emirates) are analysed for MMAs. The carbon number distribution and methyl-branch location of MMAs in the hydrocarbon fraction of the pyrolysis products are determined and compared to those of ancient sediments and petroleum.

2. Samples

The Holocene microbial mat was collected in Abu Dhabi (United Arab Emirates) along a section of the Ras al Khaf channel (Kenig, 1991). This microbial mat formed at the end of the Holocene transgression. Based on the ^{14}C age obtained from the overlying aragonitic sediments, the sample is estimated to be older than 5110 ± 170 y BP (Kenig, 1991). The sample is also estimated to be younger than 8000 year BP, the time at which the Holocene transgression reached Abu Dhabi (Evans et al., 1969). The sedimentological setting and location are described in detail by Kenig et al. (1990) and Kenig (1991). This microbial mat was deposited in the intertidal zone and exhibits polygons of desiccation. Original sedimentary structures are preserved well and are disrupted only by rare gypsum crystal growth. This sample contains 1.7 wt% total organic carbon (TOC). Rock-Eval pyrolysis of the microbial mat yielded a Hydrogen Index (HI) of 620 mg HC/gTOC. Biomarkers in the hydrocarbon extract of this microbial mat do not suggest any contribution from petroleum to the sample (see Kenig, 1991; Kenig et al., 1995).

The exact microbial assemblage forming this Holocene mat could not be determined. However, since its structure and its growth environment are similar to modern microbial mats of Abu Dhabi, it can be speculated that these mat-forming communities are probably similar. Modern Abu Dhabi microbial mats are dominated by the cyanobacteria *Lyngbya aestuarii* (Mertens) Liebman and *Microcoleus chthonoplastes* Thuret which overlie laminae of unidentified pink filamentous bacteria and purple sulphur bacteria including *Chromatium okenii* (Ehrenberg) Perty and *Thiocystis violacea* Winogradsky (Kinsman and Park, 1976; Cardoso et al., 1978). The base of the mat is inhabited by heterotrophic bacteria, including sulfate reducers and methanogens (Kenig, 1991).

3. Methods

The kerogen in the Abu Dhabi sample was prepared following the procedure described by Durand and Monin (1980). A sample, pre-extracted with chloroform at 55°C for 1 h (Kenig et al., 1995), was decarbonated with HCl (6 N) and then treated with hydrofluoric acid. The residue was sonicated twice with chloroform to remove free lipids.

Preparative open pyrolysis with a micro-pyrolyser (Vinci-Technologies; Vandenbroucke and Behar, 1988; Vandenbroucke et al., 1988) was performed on 47 mg of kerogen under a flow of Argon. The sample was deposited on a gold rod and introduced in the pyrolysis unit. The oven was preheated at 150°C for 2 min to evaporate residual hydrocarbons. The oven temperature was then raised at 60°C/min. to 550°C and held at 550°C for 5 min. During the temperature rise and plateau at 550°C, pyrolysis eluents were recovered on-line in a closed trap cooled by liquid nitrogen. After pyrolysis, the trap was disconnected from the pyrolysis unit, removed from liquid nitrogen and opened. The pyrolysate was recovered using chloroform and weighed (14.92 mg). The saturate hydrocarbon fraction was isolated using microcolumns, following the method described by Behar et al. (1989), and yielded 0.2 mg.

Identification of monomethylalkanes was performed by mass chromatography using the the characteristic even mass fragment ions formed by cleavage of the carbon–carbon bond adjacent to the tertiary carbon atom (McCarthy et al., 1968; Klomp, 1986; Fowler and Douglas, 1987; Summons, 1987). Identification was confirmed by comparison of mass spectra of MMAs with those of published standards (Pomonis et al., 1978; Summons, 1987), by comparison of retention indices with literature data (Kenig et al., 1995, and references therein) and with retention indices calculated following Kissin and Feulmer (1986).

4. Results and discussion

The gas chromatogram of the saturated hydrocarbon fraction of the pyrolysate of the Abu Dhabi Holocene microbial mat is dominated by *n*-alkanes with a maximum at *n*-C$_{19}$ (Fig. 1a). In the *n*-C$_{16}$ to *n*-C$_{29}$ range, the trace exhibits clusters of peaks. Most of the compounds in these clusters have been identified as homologous families of monomethylalkanes (MMAs, C$_{17}$–C$_{29}$) coeluting with a family of *n*-alkylcyclohexanes (Fig. 1b). In each cluster, all isomers of the MMAs are present. However, the most centrally branched isomer, for example 9-methylheptadecane (C$_{18}$), is only present in trace amount in the monomethylheptadecane cluster. Similarly, 11-methylheneicosane (C$_{22}$) is only present in trace amounts in the monomethylheneicosane cluster. Although the dominating isomers, in each cluster, are the 3-methyl- (anteiso-) and 2-methyl- (iso-) alkanes, coelution of centrally branched MMAs results in an enhanced peak. For example, 10-, 9-, and 8-monomethylheneicosane coelute to form one large peak on the FID trace (Fig. 1b).

The monomethylalkane clusters show a maximum intensity between C$_{18}$ and C$_{28}$, and are clearly visible in the FID trace up to C$_{29}$. For carbon numbers larger
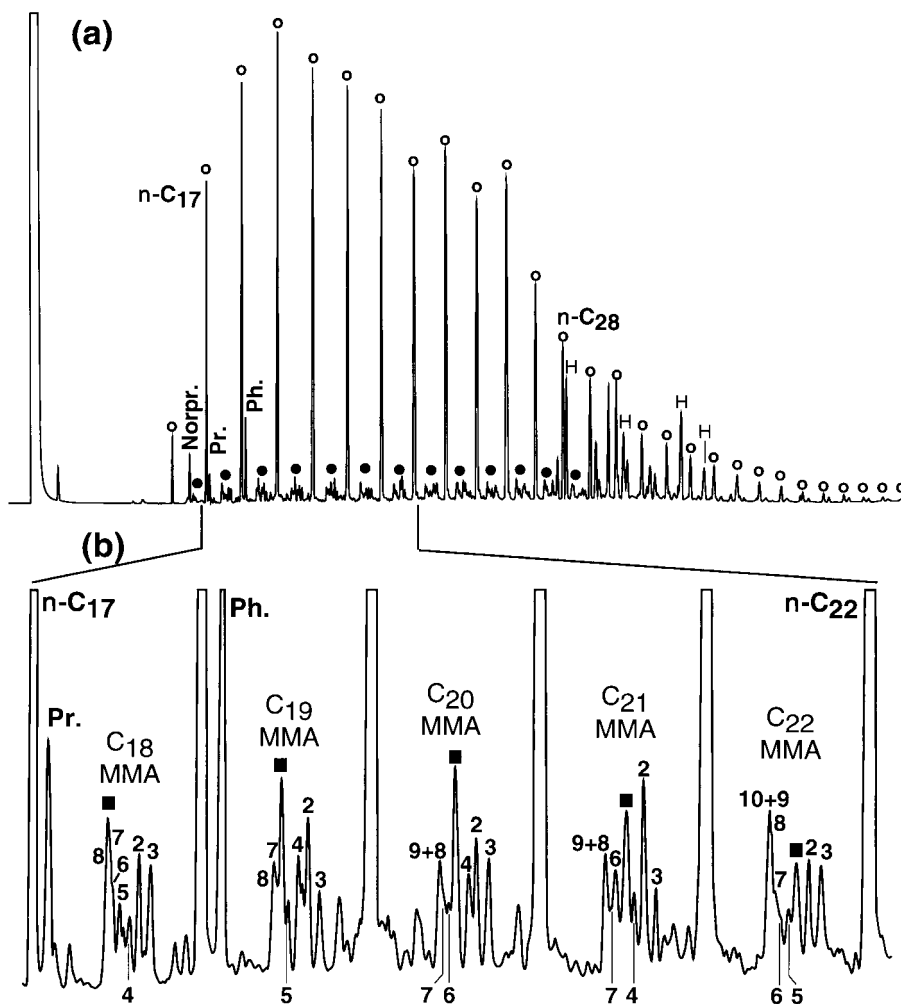
Fig. 1. (a) FID chromatograms of the saturated hydrocarbon fraction of the pyrolysate of the kerogen of a Holocene Abu Dhabi microbial mat. Pr.: pristane; Ph.: phytane; Norpr.: Norpristane; H: 17β(H),21β(H)-hopanes; open circles indicate *n*-alkanes, solid circles indicate clusters of monomethylalkanes. Inset (b) shows enlargement of the *n*-C$_{17}$–*n*-C$_{22}$ region of the FID trace (a). Solid squares indicate *n*-alkylcyclohexanes. Numbers refer to the location (carbon number) of the methyl substituents of monomethylalkanes.

than C$_{29}$, low concentration of MMAs and coelution with large peaks of tetra- and pentacyclic triterpenoids dominated by 17β(H),21β(H)-isomers of C$_{27}$–C$_{35}$ hopanes prevented formal identification of MMAs by GC–MS.

It could be speculated that the MMAs identified in the Holocene cyanobacterial assemblage are derived from contamination of the kerogen by asphaltenes. However, the biological configuration of hopanes, 17β(H),21β(H), both in the lipid extract (Kenig, 1991) and in the pyrolysis products (Fig. 1a), discount this possibility.

The distribution of MMAs in the pyrolysate of the Abu Dhabi Holocene microbial mat differs completely from that of MMAs in the extractable hydrocarbon fraction of the same sample analysed by Kenig et al. (1995). In the extractable hydrocarbon fraction, 7-methylheptadecane and 8-methylhexadecane, in trace amounts, were the only MMAs present in the C$_{16}$–C$_{23}$ range. Their small concentration is in contrast with what is observed in modern microbial mats of Abu Dhabi where MMA and dimethylalkanes (DMA) in the C$_{16}$–C$_{23}$ are major compounds (Kenig et al., 1995). A similar trend was previously observed by Boon et al. (1983) in the cyanobacterial mats of Solar Lake and was explained by preferential biodegradation of short chain hydrocarbons. In contrast, in the extract of Abu Dhabi Holocene microbial mats, numerous isomers of long-chain MMAs were detected in the C$_{24}$–C$_{40}$ range, but those were shown to originate from epicuticular waxes of insects feeding on the mat, based on carbon isotopic data (Kenig et al., 1994) and structural data (Kenig et al., 1995). However, insects are very unlikely to be the

source of the MMAs identified in the pyrolysate of the Abu Dhabi microbial mat. In insect-derived MMAs, the methyl substituents are located exclusively at odd-numbered carbon atoms, and linear carbon skeletons of MMAs mainly contain an odd number of carbon atoms. In the microbial mat pyrolysate (Fig. 1), methyl substituents are located at both even- and odd- numbered carbon atoms and there is no preference for linear carbon skeletons with an odd number of carbon atoms. Moreover, insect-derived MMAs are part of the readily extractable epicuticular waxes and should be found exclusively in the extractable organic fractions. MMAs were not observed in the pyrolysate of the non-extractable parts of insect cuticles (i.e. Stankiewicz et al., 1997). Therefore, MMAs of the microbial mat pyrolysate must be derived from non-extractable organic matter of the primary producing eubacterial population of the microbial mat and/or of a member of the bacterial heterotrophic population feeding on the microbial assemblage (eubacteria and archaebacteria).

Many studies report the presence of homologous families of MMAs in pre-Ordovician oils and oil-seeps (Eglinton et al., 1964; Hoering, 1976, 1981; Jackson et al., 1986; Klomp, 1986; Fowler and Douglas, 1987; Hieshima and Pratt, 1991) and sediments (Summons et al., 1988a,b; Summons and Walter, 1990). Hoering (1976) and Hieshima and Pratt (1991) showed the presence of C_{19}–C_{30} homologous series of MMAs in the Nonesuch Formation (Precambrian, USA). The distribution of MMA in the Abu Dhabi microbial mat is similar to that observed by Fowler and Douglas (1987) in Late Precambrian oils of Eastern Siberia. In their review of molecular fossils in Proterozoic sediments and oils, Summons and Walter (1990) show that homologous families of MMAs are common in sediments since 1.6 By in a carbon number range close to that observed for the Abu Dhabi Holocene microbial mat (C_{15}–C_{30}). All of these authors suggest a prokaryotic origin for these compounds. This hypothesis is consistent with the identification of homologous series of MMAs (C_{16}–C_{29}) in our pyrolysate of a Holocene microbial mat from Abu Dhabi.

It cannot be determined from the above results if the MMAs are derived from cyanobacteria, from other members of the microbial mat primary producers (unidentified pink filamentous bacteria and purple sulphur bacteria) or from heterotrophic bacteria living on microbial mat organic matter. Abundant and diverse microbial communities inhabit dead cyanobacterial assemblages (i.e. eubacterial sulfate reducers, archaebacterial methanogenic bacteria). However, the identification of homologous series of MMAs (C_{16}–C_{29}) in Abu Dhabi Holocene microbial mats indicates that precursor organisms of MMAs found in ancient sediments and oil are not extinct as hypothesized by Fowler and Douglas (1987).

5. Conclusions

Homologous families of monomethylalkanes (MMAs; C_{16}–C_{29}) identified in the pyrolysate of a Holocene microbial mat differ in carbon number and methyl-branch location from the extractable MMAs. This indicates that homologous families of MMAs in the C_{16}–C_{29} range are not present as free hydrocarbon precursors in the microbial mat and must, therefore, be part of non-extractable organic matter derived from one or more members of the microbial assemblage: eubacterial primary producers or associated heterotrophic eubacteria or archaebacteria.

It can be speculated that monomethylalkanes in ancient sediments and crude oils originated from similar non-extractable organic material produced by a member of cyanobacterial assemblages. Cyanobacteria are strong candidates, but other bacteria present in the microbial assemblage cannot be excluded. Further investigation of the pyrolysis products of monospecific cultures of cyanobacteria, other eubacteria and archaebacteria commonly found in Abu Dhabi microbial mats should help to more precisely determine the sources of homologous series of mid-chain branched monomethylalkanes.

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