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# Biomarkers Reveal Diverse Microbial Communities in Black Smoker Sulfides from Turtle Pits (Mid-Atlantic Ridge, Recent) and Yaman Kasy (Russia, Silurian)

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**The steep biogeochemical gradients near deep sea hydrothermal vents provide various niches for microbial life. Here we present biosignatures of such organisms enclosed in a modern and an ancient hydrothermal sulfide deposit (Turtle Pits, Mid-Atlantic Ridge, Recent; Yaman Kasy, Russia, Silurian). In the modern sulfide we found high amounts of specific bacterial and archaeal biomarkers with $\delta^{13}$C values between −8 and −37‰ VPDB. Our data indicate the presence of thermophilic members of the autotrophic Aquificales using the reductive tricarboxylic acid (rTCA) cycle as well as of methanogenic and chemolithoheterotrophic Archaea. In the ancient sample, most potential biomarkers of thermophiles were obscured by compounds derived from allochthonous organic matter (OM), except for an acyclic $C_{40}$ biphytane and its $C_{39}$ breakdown product. Both samples contained high amounts of unresolved complex mixtures (UCM) of hydrocarbons. Apparently, OM in the sulfides had to withstand high thermal stress, indicated by highly mature hopanes, steranes, and cheilanthanes with up to 41 carbon atoms.**

**Keywords** Massive sulfides, hydrothermal, black smoker, Aquificales, methanogenic Archaea

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## INTRODUCTION

Black smokers at deep sea hydrothermal vents are unique geobiologic settings offering microorganisms to gain energy from steep redox gradients (e.g., Corliss et al. 1979; Fisher et al. 2007). In addition, the massive metal sulfide deposits forming at these settings contain a long-lasting energy source for phylogenetically and metabolically diverse microorganisms. Due to these conditions, and because similar settings might have already existed billions of years ago, deep sea hydrothermal vents are excellent candidate sites for the origin of life on Earth. Indeed, microbiological studies revealed that Bacteria and Archaea branching deep in the phylogenetic tree of life form a considerable fraction of microorganisms in these environments (Reysenbach and Cady 2001 and refs. therein).

Several studies reported on hydrocarbons and other biomarkers in deep sea hydrothermal deposits (Simoneit et al. 1990, 2004; Lein et al. 1998, 2003) but only very few focused on autochthonous microbial communities by using structures and stable isotope signatures of those biosignatures (Blumenberg et al. 2007; Bradley et al. 2009a, 2009b). However, these studies, and others aimed at terrestrial hot spring settings (e.g., Jahnke et al. 2001) revealed specific biomarkers and demonstrated an unparalleled phylogenetic and metabolic diversity. Chemoautotrophic Bacteria and Archaea that gain their energy from hydrogen and/or sulfide are at the base of the food chain.

The uses of these metabolisms are evidenced by organic compounds with $^{13}$C-enrichments with $\delta^{13}$C values close to dissolved inorganic carbon (DIC) indicating DIC as the carbon substrate. In some settings this was related to carbon limitation (Bradley et al. 2009b), however, in other settings showing a broader range of $\delta^{13}$C values (0 to −50‰), an autotrophic $H_2$-using bacterial source using the reversed tricarboxylic acid (rTCA) pathway was suggested to be more likely than carbon limitation (Blumenberg et al. 2007; Naraoka et al. 2010).

In addition, in a sulfide from the Mid-Atlantic Ridge biomarkers from methanogenic Archaea were strongly depleted in $^{13}C$ ($\delta^{13}C = -48‰$; Blumenberg et al. 2007), which has been described to be characteristic for a thermophilic $CO_2$-reducing subgroup (Takigiku et al. 1996; Summons et al. 1998). A thermophilic life style of Bacteria and Archaea is also reflected by the lipid structures. Very common are glycerol ether lipids occurring either as conventional dialkyl glycerol diethers (DAGEs; e.g., Jahnke et al. 2001; Bradley et al. 2009b) or as macrocyclic analogues (Pancost et al. 2006; Blumenberg et al. 2007). The latter were found to be particularly stable under high temperatures (Comita et al. 1984).

Nevertheless, knowledge of biomarkers of microbial communities in recent hydrothermal sulfides is still fragmentary, and the preservation potential of these biomarkers in ancient black smoker settings is as yet unknown. Therefore, our study aimed at getting insights into microbial life entrapped in a sulfide rock sampled at the top of an active black smoker (Mid-Atlantic Ridge, Turtle Pits). Furthermore, we focused on the preservation of biomarker imprints of respective microorganisms in an ancient hydrothermal sulfide rock of Late Ordovician to Early Silurian age (Yaman Kasy; Russia; ~445 million years; Little et al. 1997; Buschmann and Maslennikov 2006).

## MATERIALS AND METHODS

### Sampling

The recent sulfide (code ROV GTV 114-7) was obtained during a cruise with RV Meteor (M64/1) in April 2005, using a remotely operated vehicle (ROV "Quest 4000m" MARUM, University of Bremen). The sample was collected at 2998 m water depth from the flank of the active black smoker Southern Tower within the Turtle Pits hydrothermal field at 4°48.573′S and 12°22.424′W (Haase et al. 2007). The sulfide comprised an outer and an inner porous part with the core consisting entirely of pyrrhotite covered by a crust dominated by pyrite and marcasite (Fig. 1).

The fossil sulfide was sampled at the Yaman Kasy volcanic hosted massive sulfide deposit during mine operation in 1999 (Orenburg district, southern Urals, Russia). The Yaman Kasy deposit displays hydrothermal altered footwall sections, clastic ore textures, vent chimney relics, sedimentary reworked sulfides, and fossilized hydrothermal vent fauna recording a seafloor hydrothermal vent origin of the massive sulfide (Zaykov et al. 1995; Little et al. 1997, 1999; Herrington et al. 1998, 2005; Maslennikov 1999; Buschmann and Maslenikov 2006).

Fluid inclusion data are interpreted to record a water depth of about 1600 m during hydrothermal venting (Herrington et al. 1998). The ancient sulfide sample represents a quartz-cemented hydrothermal chimney fragment composed of marcasite-pyrite wall material and chalcopyrite lining of hydrothermal fluid conduits that were subsequently filled by clastic marcasite-pyrite sand (Fig. 1).

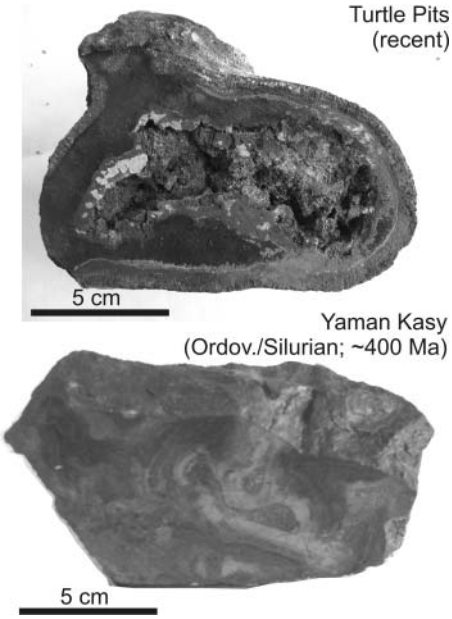


FIG. 1. Images of the recent Turtle Pits (Mid-Atlantic Ridge) and Silurian Yaman Kasy massive hydrothermal sulfides.

### Sample Preparation for Lipid Analyses, Extraction, Derivatization, and Fractionation

Crushed small pieces of the massive sulfides from Turtle Pits and Yaman Kasy (408 g and 254 g, respectively) were repeatedly washed with distilled water and acetone and subsequently ground using a mortar (Turtle Pits) and a Retsch MM301 vibrating tube mill (Yaman Kasy).

An aliquot of powdered Yaman Kasy sample was subjected to X-ray diffraction (XRD) analysis, which was performed on a Philips X'Pert MPD equipped with a PW3050 Goniometer, CuK$\alpha$ radiation 40 kV, 30 mA, soller slit 0.04 rad, graphite secondary monochromator, and proportional counter. Scans were performed in the range between 4° and 70° 2$\theta$ and steps of 0.02° 2$\theta$, 3s counting time per step, continuous mode. The divergence slit and antiscatter slit were both set to 0.5°, the receiving slit to 0.2 mm, a 10 mm mask was used. Sample diameter was 16 mm. The sample was spinning 1 rps during analysis. Qualitative phase analyses were performed with X'Pert HighScore (Version 2.2.1). For extraction, two procedures were applied.

### Procedure 1 (Turtle Pits)

The procedure was described in detail in (Blumenberg et al. 2007). Briefly, the powder was saponified in 6% KOH in methanol (75°C, 3 h). The alkaline solution/sample mixture was centrifuged and the supernatant was extracted with *n*-hexane to yield the neutral lipids. Carboxylic acid methyl esters were obtained by acidification of the residual phase to pH 1, extraction with $CH_2Cl_2$ and subsequent methylation using trimethylchlorosilane reagent in methanol (1/8; v/v; for 1 h at 80°C). An aliquot of the neutral lipids was fractionated by column chromatography using silica gel. The first fraction was

eluted with a hexane/$CH_2Cl_2$-mixture (98/2, v/v), the second with $CH_2Cl_2$, and the third with methanol.

An aliquot of the third fraction was silylated with (N,O-bis[trimethylsilyl]trifluoroacetamide; BSTFA; 1 h at 80°C). Another aliquot was subjected to ether cleavage through HI treatment (2 h at 110°C). Subsequently the resulting iodides were reduced using $LiAlH_4$ in dry tetrahydrofurane under an Argon atmosphere (modified after Kohnen et al. 1992).

### Procedure 2 (Yaman Kasy)

The rock powder was extracted with $CH_2Cl_2$/methanol (9/1, v/v; 2x) and *n*-hexane (3x) using ultrasonication. Elemental sulfur was removed by adding activated copper. The combined extracts were dried on minute amounts of silica gel under vacuum and a nitrogen stream to dryness. Then the extract was fractionated by column chromatography using 7 g silica gel (column inner diameter 15 mm). For elution of hydrocarbons the column was rinsed with 1.5 dead volumes (dv) of *n*-hexane, for aromatics with 2 dv *n*-hexane/$CH_2Cl_2$, (1/1, v/v) and for the polar fraction with 2 dv $CH_2Cl_2$/methanol (1/1, v/v). For the separation of cyclic and branched hydrocarbons, an aliquot of the hydrocarbon fraction was rinsed with 2 dv *n*-pentane over silicalite powder (UOP S-115; adapted from West et al. 1990).

### Gas Chromatography-Mass Spectrometry (GC-MS) and GC-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS)

Biomarkers were analyzed using a Varian CP3800 GC coupled to a VARIAN 1200L MS. The analytical conditions for GC-MS measurements were as follows: DB-5MS or a ZB-5MS column (30 m length; 0.32 mm inner diameter, 0.25 $\mu$m film thickness); electron impact ionization [EI], electron energy 70 eV; mass range m/z 50–750; scan time 0.7 s; selected ion monitoring (SIM) width 0.7 amu; source temperature 200°C. Compounds were identified by comparison of mass spectra and retention times with published data and/or reference compounds. Quantifications of individual compounds were achieved from the total ion chromatograms by comparison of peak areas of individual components with an internal standard (5$\alpha$(H)-cholestane) of known concentration.

$\delta^{13}$C-values of individual biomarkers were analyzed (min. of two replicates) using either a THERMO Trace GC equipped with a split/splitless injector and coupled to a Finnigan MAT 252 IRMS (Turtle Pits sample) or a THERMO Trace GC equipped with a split/splitless injector and coupled to a Delta Plus IRMS (Yaman Kasy sample). Combustion of the components to $CO_2$ was performed with a CuO/Ni/Pt-furnace operated at 940°C.

The stable carbon isotope compositions are calculated from comparisons with a $CO_2$ reference gas with known $\delta^{13}$C and are reported in the delta notation ($\delta^{13}$C) vs. the V-PDB standard (standard deviation was usually less than 0.5‰). Isotopic compositions of alcohols and fatty acids were corrected for addition of TMS and the addition of the carbon atom during the preparation of methyl esters. GC-C-IRMS precision and linearity was checked daily by using an external *n*-alkane isotopic standard provided by A. Schimmelmann (Indiana University). The typical GC-program used for GC-MS and GC-C-IRMS analyses was 80°C (3 min) –6°C/min –310°C (30 min).

## RESULTS

### Rock Composition

XRD analysis of the Yaman Kasy sulfide revealed about 48% pyrite, 20% chalcopyrite, 18% marcasite, and 15% quartz. XRD-measurements of the recent Turtle Pits sulfide were not performed, but macroscopic observations suggested a similar predominance of metal sulfides (i.e., pyrite, chalcopyrite, marcasite). A prevalence of these minerals was also reported from another hydrothermal precipitate from the Turtle Pits field (Blumenberg et al. 2007) (Fig. 1).

*Biomarkers.* Preliminary biomarker studies revealed the absence of biomarkers in the inner part of the Turtle Pits sulfide (see Fig. 1; most likely due to very high formation temperatures as indicated by the mineral composition). Therefore, for further lipid analyses only the outer part was used. The recent sulfide from Turtle Pits contained apolar and polar lipids, while the Yaman Kasy sulfide contained almost entirely hydrocarbons.

*Hydrocarbons.* The majority of freely extractable compounds in the Turtle Pit sulfide consisted of hydrocarbons (hydrocarbon fraction of neutral lipids). Most of these structures are part of an unresolved complex mixture (UCM) hump and could not be assigned to distinct structures due to massive co-elutions affecting mass spectra. The UCM of the Turtle Pits sulfide had its maximum at $C_{25}$ (total range $C_{16}$ to $C_{35}$) whereas that of the Yaman Kasy sample showed two maxima at $C_{37}$ and $C_{44}$ (total range $C_{14}$ to $>C_{48}$), respectively (Fig. 2). However, several isoprenoids were identified, including pristane (Pr; 2,6,10,14-tetramethylpentadecane) and phytane (Ph; 2,6,10,14-tetramethylhexadecane) with the latter predominating by far (Fig. 2).

The most abundant hydrocarbon was the acyclic $C_{40}$-isoprenoid biphytane (3,7,11,15,18,22,26,30-octamethyl dotriacontane). Other features of hydrocarbons in the Turtle Pits sulfide were revealed by SIM-GC-MS runs (summarized in Table 1), which demonstrated a prevalence of (regular) $C_{27}$ and $C_{29}$-steranes, fully isomerized hopanes ($C_{31}S/(C_{31}S + R) = 0.6$), and mature *n*-alkanes (CPI = 1.04) showing a maximum at $C_{24}$. The $\delta^{13}$C values of pristane, phytane and biphytane were −27.8‰, −27.4‰, and −21.6‰, respectively (Table 2).

Like the Turtle Pits sample, the Yaman Kasy sulfide contained a strong UCM. *N*-alkanes showed a bimodal distribution with maxima at $C_{22}$ and (minor) at $C_{35}$. The latter *n*-alkane co-eluted with $C_{40}$-biphytane. SIM-analyses revealed that about 40% of the peak consisted of biphytane. Other abundant isoprenoids included norpristane, pristane, phytane, and a $C_{39}$-biphytane derivative (nor-biphytane). To minimize co-elution effects during stable carbon isotope analyses, isoprenoids were isolated using silicalite (West et al. 1990).

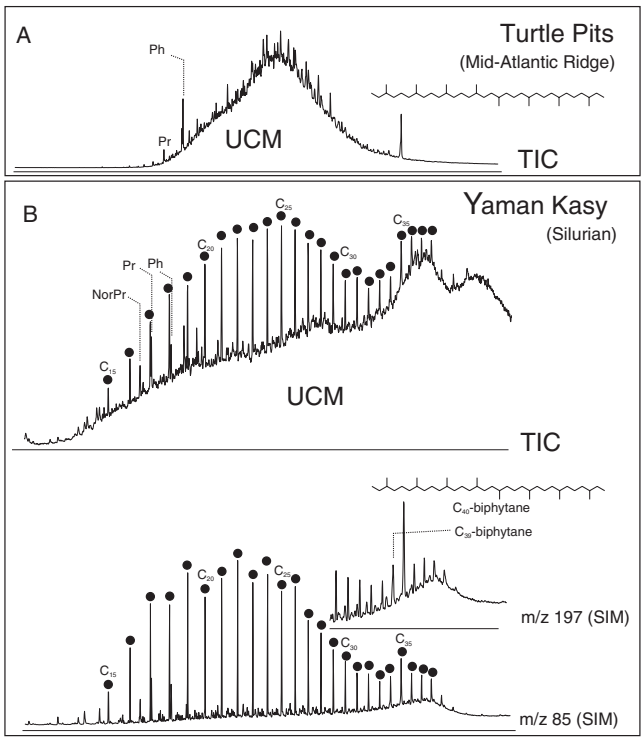


FIG. 2. Chromatograms of the hydrocarbon fractions obtained from the massive hydrothermal sulfides. A: Total ion current (TIC) chromatogram of the recent Turtle Pits sulfide. B: TIC chromatogram of the hydrocarbons of the Silurian Yaman Kasy sulfide (top) and SIM traces of m/z 85 and 197 showing mainly aliphatic hydrocarbons and isoprenoids (bottom). UCM = unresolved complex mixture, NorPr = norpristane, Pr = pristane, Ph = phytane. Numbers refer to carbon length.

For the resulting branched and cyclic fractions and the untreated hydrocarbon fraction, $\delta^{13}C$ values were between −29 and about −33‰ for isoprenoids and *n*-alkanes (Table 2). SIM-mass spectrometry revealed the absence of regular steranes, but a suite of $C_{27}$ to $C_{29}$ diasteranes ($C_{29}$ predominating) was observed (Table 1). Hopanes were almost absent from the Yaman Kasy sample and superimposed by cheilanthanes with carbon numbers up to 41 (Fig. 3).

*Fatty acids.* Considerable amounts of fatty acids were found in the Turtle Pits sulfide. As expected, fatty acids were observed in the extract after acidification but also, and unexplained, in the polar fraction of the alkaline neutral lipid fraction. However, concentrations were higher in the first with 3-hydroxytetradecanoic acid (3-OH 14FA), hexadecanoic acid (16FA), octadecanoic acid (18FA), eicosanoic acid (20FA), and heneicosenoic acid (21:1FA) as most prominent structures (Table 3 and Fig. 4). A slightly different fatty acid distribution was found for the polar fraction of the neutral lipids. All fatty acids were enriched in $^{13}C$ with respect to normal marine lipids, with 3-OH 14FA having the highest $\delta^{13}C$ value (−8.7‰; Table 2).

*Polar neutral lipids (incl. (di)alcohols).* The polar fraction of the neutral lipids contained a suite of *n*-alcohols, phytanol, fatty acids and long-chain dialkyl glycerol diethers (DAGEs; Fig. 4). The highest amounts were found for archaeol. A macrocyclic archaeol was identified by comparison with published mass spectra (Comita et al. 1984). In addition, three DAGEs were found with molecular masses of 668 (DAGE 1), 682 (DAGE 2), and 694 (DAGE 3). Comparisons with published mass spectra of related compounds (Pancost et al.

TABLE 1
Hydrocarbon biomarker indices of the recent and ancient massive hydrothermal sulfides

| | Turtle Pits (Mid-Atlantic Ridge) | Yaman Kasy (Silurian) |
|---|---|---|
| Steranes | | |
| $C_{27}$ (in %) | 45.3 | 30.7 |
| $C_{28}$ (in %) | 15.0 | 21.2 |
| $C_{29}$ (in %) | 39.7 | 48.1 |
| $C_{30}$ sterane index ($C_{30}/(C_{27}–C_{30})$ | 0.05 | 0 |
| Diasterane/regular sterane ($C_{27}$) | reg. nd | 3.6 |
| Hopanes | | |
| $C_{31}S/(C_{31}S + R)$ | 0.60 | 0.60 |
| Others | | |
| CPI | | 1.04 |
| *n*-alkane distribution | modal | (bi)modal |
| *n*-alkane max. | 24 | 22 (+35) |
| Pristane/Phytane | 0.35 | 1.4 |
| Pristane/(Pristane+Phytane) | 0.26 | 0.73 |
| Pristane/*n*-$C_{17}$ | −(17 nd) | 0.89 |
| Phytane/*n*-$C_{18}$ | 2.34 | 0.46 |
| $C_{18}/C_{18}$Blank | — | >1000 |

TABLE 2
$\delta^{13}$C values [‰ versus VPDB] of hydrocarbons, fatty acids and (dialkyl glycerol) alcohols found in the recent and ancient massive hydrothermal sulfides with the tentative source organism

| | $\delta^{13}$C [‰] Turtle Pits | $\delta^{13}$C [‰] Yaman Kasy | Potential source organisms |
|---|---|---|---|
| Hydrocarbons | | | |
| Norpristane | — | −32.9* | Phototrophs; Archaea |
| Pristane | −27.8 | −32.7* | Phototrophs; Archaea |
| $C_{18}$ | −25.2 | −33.2 | Various |
| Phytane | −27.4 | −30.5* | Phototrophs; Archaea |
| $C_{19}$ | — | −32.6 | Various |
| $C_{20}$ | — | −31.8 | Various |
| $C_{21}$ | — | −31.6 | Various |
| $C_{22}$ | — | −31.1 | Various |
| $C_{23}$ | — | −30.9 | Various |
| $C_{24}$ | — | −29.4 | Various |
| $C_{25}$ | — | −30.3 | Various |
| $C_{26}$ | — | −30.5 | Various |
| $C_{27}$ | — | −29.3 | Various |
| Biphytane ($C_{40:0}$) | −21.6 | −29.1* | Archaea |
| Fatty acids, (dialkyl glycerol) alcohols, and glycerol ether-derived hydrocarbons | | | |
| 3-Hydroxytetradecanoic acid (3OH-14FA) | −8.7 | — | Bacteria (Thiobacilli?)[1] |
| Hexadecanoic acid (16FA) | −21.8 | — | Various |
| Phytanol | −14.6 | — | S- or Fe-oxidizing Archaea[2] |
| Octadecanol | −24.2 | | Various |
| Octadecanoic acid (18FA) | −11.1 | — | Bacteria (Aquificae)[3] |
| Eicosanoic acid (20FA) | −10.5 | — | Bacteria (Aquificae)[3] |
| Heneicosenoic acid (21:1FA) | −10.5 | — | Bacteria (Aquificae)[3] |
| Archaeol | −11.4 | — | S- or Fe-oxidizing Archaea |
| DAGE 1 ($C_{18}/C_{18}$) | −11.1 | — | Bacteria (Aquificae)[3] |
| Macrocyclic archaeol | −36.7 | — | $CO_2$-reducing methanogenic Archaea[4] |
| DAGE 2 ($C_{18}/C_{19[7\text{-}Me18]?}$) | −11.5 | — | Bacteria (Aquificae)[3] |
| DAGE 3 ($C_{18:1}/C_{20}$?) | −9.0 | — | Bacteria (Aquificae)[3] |
| Octadecane (ex. DAGE 1 to 3?) | −16.6 | — | Bacteria (Aquificae)[3] |
| Phytane (ex. archaeol?) | −9.7 | — | S or Fe-oxidizing Archaea[2] |
| 7-Methyloctadecane (ex. DAGE 2?) | −12.5 | — | Bacteria (Aquificae)[3] |
| Eicosane (ex. DAGE 3?) | −23.8 | — | Various |
| Biphytane (ex. macrocyclic archaeol?) | −37.3 | — | $CO_2$-reducing methanogenic Archaea[4] |

FA = fatty acid.



2001), and elution characteristics (Jahnke et al. 2001) indicated $C_{18}/C_{18}$, $C_{18}/C_{19}$, and $C_{18:1}/C_{20}$ alkyl chains for DAGEs 1 to 3, respectively. These identifications are further supported by the release of octadecane, 7-methyloctadecane ($C_{19[7me\text{-}18]}$) and eicosane after ether-bond cleavage of this fraction with HI.

Except for eicosane and octadecane the hydrocarbons—and their potential DAGE precursors—were enriched in $^{13}$C, showing $\delta^{13}$C values between −9 and −12.5‰. In addition, cleavage of ether bonds released high amounts of phytane and biphytane with $\delta^{13}$C values of −9.7 and −37.3‰, respectively. In both samples, cyclic biphytanes were neither occurring in the free nor in ether-bond form.

## DISCUSSION

A recent and an ancient hydrothermal massive sulfide of Silurian age were analyzed for the presence of biomarkers and their stable carbon isotope signatures. Both rocks consisted mainly

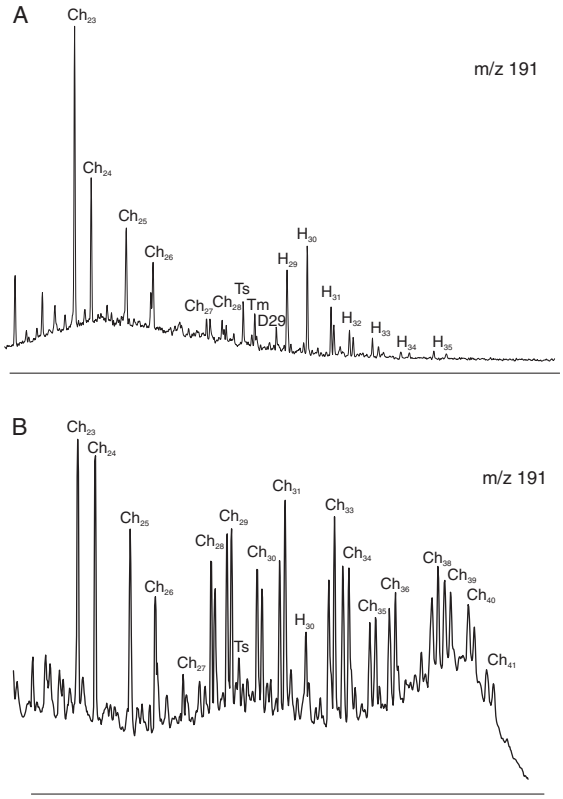


FIG. 3. SIM traces of m/z 191 showing mainly cheilanthanes (carbon numbers with prefix Ch) and hopanes (carbon numbers with prefix H) of (A) Turtle Pits 114 and (B) Yaman Kasy. Numbers refer to carbon numbers of the molecules.

of pyrite, chalcopyrite and marcasite indicating genesis under extreme hydrothermal conditions. At the recent Turtle Pits hydrothermal field hot and acidic fluids escape from the sea floor (407°C, pH 3.1; Haase et al. 2007), favoring particularly thermoacidophilic Bacteria to live in this environment. Respective information about the ancient hydrothermal environment is lacking.

Distinct distributions of compounds with a presumably biological origin were observed in both samples. In the ancient Yaman Kasy sulfide, however, unambiguous biomarkers for former microbial, thermophilic life were low in abundance, most likely because they were obscured by thermally altered, allochthonous OM from algae and other photic zone organisms and/or abiogenically formed Fischer-Tropsch derived hydrocarbons (see below). This feature was also observed for the recent sulfide from Turtle Pits, but in that sample structurally and isotopically specific functionalized lipids of diverse autotrophic Bacteria and Archaea were still clearly distinguishable from these hydrocarbons. The low abundances of biosignatures of hydrothermal microorganisms are most likely related to the low biomasses as well as to the low preservation potential of these compounds due to thermal alteration reactions.

## Sources of Biomarkers in Hydrothermal Sulfide Deposits

### Bacterial Biomarkers

Relatively high amounts of bacterial fatty acids were found in the recent Turtle Pits sulfide. The highest amounts were

TABLE 3
Concentrations of selected biomarkers (hydrocarbons, (glycerol) alcohols, fatty acids) in the recent Turtle Pits and the Silurian Yaman Kasy sulfides

| | Conc. [ng/g] Turtle Pits | Conc. [ng/g] Yaman Kasy |
|---|---|---|
| Neutral lipids (hydrocarbons and alcohols) | | |
| Octadecane | 50.1 | 54.9 |
| Phytane | 84.5 | 23.4 |
| Biphytane ($C_{40:0}$) | 114.6 | 12.1 |
| Phytanol | 3.5 | — |
| Octadecanol | 6.3 | — |
| Archaeol | 47.7 | — |
| DAGE 1 ($C_{18}/C_{18}$) | 0.8 | — |
| Macrocyclic archaeol | 2.4 | — |
| DAGE 2 ($C_{18}/C_{19[7\text{-}Me18]}$?) | 8.2 | — |
| DAGE 3 ($C_{18:1}/C_{20}$?) | 1.8 | — |
| Fatty acids | | |
| 3-Hydroxytetradecanoic acid (3OH-14FA) | 5.5 | — |
| Hexadecanoic acid (16FA) | 9.5 | — |
| Octadecanoic acid (18FA) | 48.2 | — |
| Eicosanoic acid (20FA) | 5.5 | — |
| Heneicosenoic acid (21:1FA) | 4.7 | — |

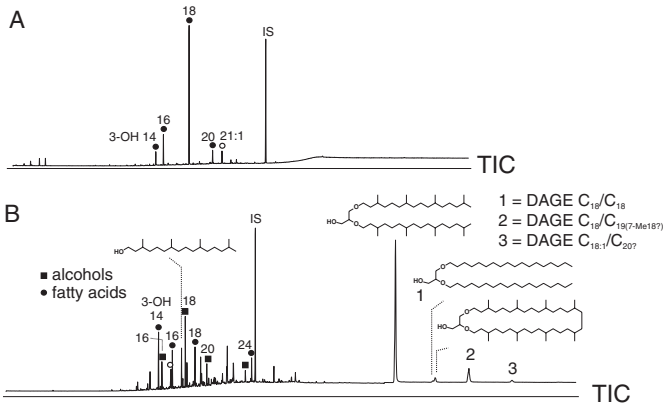


FIG. 4. Total ion current (TIC) chromatograms of the fatty acid fraction (A) and the polar fraction (B) obtained from the neutral lipids of the recent Turtle Pits sulfide. For shorthand notations, see Table 3. IS = internal standard.



observed for 16FA and 18FA. Concentrations were in the range of fatty acids recently reported from another altered sulfide rock from Turtle Pits (Blumenberg et al. 2007). 16FA and 18FA are ubiquitous and thus non-specific for the (bacterial) source. However, high amounts of 3-OH 14FA were found, a fatty acid belonging to 3-hydroxylated ($\beta$-functionalised) fatty acids, which are less common in Bacteria (Table 2, Fig. 4). 3-Hydroxylated fatty acids are known from various thermophilic and mesophilic Bacteria (e.g., Cytophage; Fautz et al. 1979). However, they are also widely distributed among *Thiobacillus* and *Acidobacillus* (Kerger et al. 1986). These Bacteria are known to be involved in the oxidation of reduced sulfur compounds, which is an important microbiological process at hydrothermal vents (Reysenbach and Cady 2001; Fisher et al. 2007). Further, the $\delta^{13}$C value of the 3-OH 14FA ($-8.7$‰) is also typical for autotrophic Bacteria living in hydrothermal settings (Takai et al. 2006). Among other biomarkers in the Turtle Pits sulfide, 3-OH 14FA was strongly enriched in $^{13}$C, indicating that the source bacterium used the reversed tricarboxylic acid pathway (rTCA) for carbon fixation, which leads only to minor isotopic fractionation (Preuß et al. 1989; Jahnke et al. 2001), or feeds on $^{13}$C-enriched biomass. Both autotrophic and heterotrophic growth have been reported among the genus *Thiobacillus* (e.g., Mason and Kelly 1988). However, autotrophically grown Thiobacilli as the source for $^{13}$C-enriched lipids are unlikely since these Bacteria use ribulose-1,5-bisphosphate (Rubisco) for carbon fixation (Levicán et al. 2008), a pathway fractionating strongly against $^{13}$C (about 22 to 30‰; Hayes 2001). Consequently, only heterotrophically grown Thiobacilli and/or other yet unknown Bacteria using rTCA or a similar pathway are the sources of the $^{13}$C-enriched 3-OH 14FA as well as of 18FA, 20FA and 21:1FA ($-10.5$‰ to $-11.1$‰).

In lower concentrations than fatty acids, but still in considerable amounts, three DAGEs ($C_{18}/C_{18}$-, $C_{18}/C_{19[7\text{-me}18]}$—and the tentatively identified $C_{18:1}/C_{20?}$) occur in the recent sulfide. DAGEs were also reported from other hydrothermal settings (Jahnke et al. 2001; Pancost et al. 2006; Bradley et al. 2009a) including another Turtle Pits sulfide (Blumenberg et al. 2007). Interestingly, in the latter, DAGE distributions strongly differed from the patterns observed here. Terminally methylated ($C_{i15}/C_{i15}$)-structures were predominating, together with non-isoprenoidal macrocyclic DAGEs, which are both lacking in the present study. This indicates that the microbial communities can be highly heterogeneous among individual vent fields of a given hydrothermal area. However, the distribution of DAGEs in the Turtle Pits sulfide resembles that of a siliceous sinter from a 92°C hot Octopus Spring vent pool (Jahnke et al. 2001). These authors assigned the DAGEs, as well as long-chain fatty acids, to the predominating microorganisms, members of the Aquificales, a phylogenetically deeply branching class of Bacteria. Indeed, these Bacteria are well known to use the rTCA pathway for carbon fixation leading to relatively $^{13}$C-enriched lipids. Since DAGEs in our sample exhibited high $\delta^{13}$C values and have distributions similar to those of the Aquificales-dominated Octopus Hot Spring, these or related Bacteria are plausible sources of DAGEs and isotopically $^{13}$C-enriched fatty acids in the Turtle Pits sulfide.

In the ancient Yaman Kasy sulfide we observed neither DAGEs, fatty acids, nor any other $^{13}$C-enriched biomarkers. We believe this is most likely due to the relatively high amounts of allochthonous organic matter and abiogenically formed hydrocarbons, superimposing the biosignatures of vent microbiota. Support for the importance of allochthonous input to our samples came from the occurrence of bacteriogenic homohopanes. These pentacyclic triterpenoids were found in the Yaman Kasy and the recent Turtle Pits sulfide, but they were not accompanied by GC-amenable functionalized precursors (e.g. hopanols, hopanoic acids). We therefore assume hopanes in both sulfide samples to derive from Bacteria thriving in the overlying water column and not representing biomarkers of Bacteria indigenous to the hydrothermal vent systems.

*Archaeal biomarkers.* Lipids with a presumably archaeal origin were found in both sulfides. In the recent Turtle Pits sulfide highest amounts were observed for archaeol and an extractable $C_{40}$-biphytane (Table 3; Figs. 4 and 2). $C_{40}$-biphytane (in addition to a $C_{39}$ homologue, which is most likely a degradation product; Ventura et al. 2007) was also abundant in the ancient Yaman Kasy sample and appears to be a feature of hydrothermal biosignatures. Support for an indigenous origin of these structures in both samples comes from the absence of cyclic biphytanes, including those derived from crenarchaeol. These structures are constituents of ubiquitous pelagic crenarchaeotes and are often the predominant archaeal biomarkers in marine sediments (Sinninghe Damsté et al. 2002).

Biomarkers distinguish two distinct archaeal communities in the Turtle Pits sulfide. The presence of a macrocyclic archaeol with a $\delta^{13}$C value of about $-37$‰ is consistent with a thermophilic $CO_2$-reducing methanogenic source (Comita and Gagosian 1983; Takigiku et al. 1996; Summons et al. 1998). Archaeol, on the other hand, had a very

different $\delta^{13}C$ value (−11‰). Blumenberg et al. (2007) suggested Fe/S-oxidizing Archaea (e.g., Ferroplasmales) to be an important source of biomarkers in a strongly altered sulfide sampled close to the Turtle Pits sulfide of this study. Some groups of these Archaea live chemolithoheterotrophically (Golyshina and Timmis 2005). Hence, feeding on $^{13}C$-enriched microbial biomass at Turtle Pits (e.g., from Aquificales) would explain the observed $\delta^{13}C$ value. However, the tentative biomarker distributions of *Ferroplasma* relatives in both Turtle Pits samples are different. Blumenberg et al. (2007) reported a monocyclic biphytane, which was not found in our sample, suggesting either distinct populations for both samples/(micro)environments or different archaeal sources for these compounds.

*Other biogenic and abiogenic sources for hydrocarbons.* Hydrothermal environments are well known for "instantaneous" petroleum generation (Simoneit 1988) as well as for the genesis of hydrocarbons via Fischer-Tropsch synthesis. Fischer-Tropsch synthesis involves the conversion of CO into organic compounds through sequential reduction and polymerization of carbon on the surface of a solid catalyst such as specific minerals (McCollom et al. 2007). Moreover, it must be considered that the resulting aliphatic structures may have $\delta^{13}C$ values similar to biogenic structures (McCollom and Seewald 2006), but lack their complexity and isomeric configurations. Hence, it is possible that a considerable portion of the abundant hydrocarbons in our samples is derived from Fischer-Tropsch synthesis. UCMs are often present in hydrocarbon fractions from hydrothermal settings (Simoneit 1988).

UCMs are, however, most likely not abiogenetically sourced, since Fischer-Tropsch synthesis generates almost exclusively aliphatic and no complex structures (McCollom and Seewald 2006). In fact, it is known that in marine sub-surface hydrothermal settings hydrocarbons are "instantaneously" formed from organic matter (Simoneit 1988). Such newly produced hydrocarbons include aliphatic and aromatic structures, but due to thermal alteration reactions complex mixtures of structurally and isomerically diverse hydrocarbons are also generated. These latter compounds form the building blocks of UCM at hydrothermal settings (Ventura et al. 2008).

Cheilanthanes are diagenetically stable tricyclic terpanes having extended side-chains and total carbon numbers up to at least $C_{45}$ (Moldowan and Seifert 1983; Brocks and Summons 2003). Cheilanthanes are and were highly abundant in both, the modern Turtle Pits and the ancient Yaman Kasy sulfides (Fig. 3). But, the cheilanthane distributions in the recent and ancient sulfides were different. Although relative amounts were highest for the $C_{23}$ and $C_{24}$ homologues in both, only the Yaman Kasy sulfide contained considerable portions of extended cheilanthanes with up to 41 carbon atoms (Fig. 3).

Moreover, hopanes were reduced in abundance and overlain by cheilanthanes, which is often observed in severely biodegraded oils or oils with a high thermal maturity (Connan 1984). We suggest either thermal stress or a high biological production of these compounds (or their lipid precursors) to be responsible for the predominance of cheilanthanes in the ancient sulfide. Unfortunately, the biological origin for cheilanthanes is as yet unknown (Brocks and Pearson 2005), although some data hint to an algal source (Greenwood et al. 2000).

Norpristane, pristane, and phytane in our samples may also at least partly derive from phototrophic algae. Likewise, the predominance of $C_{29}$ pseudohomologues among the steranes of both samples suggests important contributions by Eukaryota (e.g., algae; Huang and Meinschein 1979). Apparently, these biomarkers have an allochthonous origin and were strongly altered due to thermal stress in the hydrothermal sulfides, indicated by the high diasterane/sterane ratio (Turtle Pits) or the absence of regular steranes (Yaman Kasy).

## CONCLUSIONS

Our study on two massive sulfides (Turtle Pits [recent; Mid-Atlantic Ridge]; Yaman Kasy [Silurian; Russia]) revealed the presence of allochthonous and autochthonous biosignatures of Eukaryota, Bacteria, and Archaea. In the recent sample, ample biomarkers for a phylogenetically and metabolically diverse microbial fauna were identified. High amounts of dialkyl glycerol diethers with strong $^{13}C$-enrichments suggest $H_2$-oxidizing members of the Aquificales as sources.

Among Archaea two prominent groups were observed. $CO_2$-reducing thermophilic methanogens appear to be the origin of a macrocyclic archaeol, while Fe/S-oxidizing chemolithoheterotrophs were most likely the source of abundant $^{13}C$-enriched archaeol. The ancient Yaman Kasy sulfide also contained high amounts of microbial hydrocarbons. However, many biomarkers could not be assigned to distinct groups of extremophilic microorganisms, most likely due to thermal alteration and/or high amounts of accompanying allochthonous OM in the samples. Nevertheless, our work shows that deep-sea hydrothermal sulfides, including fossil materials as old as ∼445 million years, harbor a high diversity of structures with a broad range of $\delta^{13}C$ values, almost unparalleled in any other setting on Earth and worth to be further explored in future studies.

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