

Petroleum biomarkers  
East Pacific Rise  
Hydrothermal sulfides  
Sediment trap

Pétrole  
Biomarqueurs  
Dorsale du Pacifique Est  
Sulfures hydrothermaux  
Piège à sédiment

# Trace petroliferous organic matter associated with massive hydrothermal sulfides from the East Pacific Rise at 13 and 21°N

Martine BRAULT <sup>a\*</sup>, Bernd R. T. SIMONEIT <sup>a+</sup>, Alain SALIOT <sup>b</sup>

<sup>a</sup> Petroleum Research Group, College of Oceanography, Oregon State University, Corvallis OR 97331, USA.

<sup>b</sup> Laboratoire de Physique et Chimie Marines, Université Pierre et Marie Curie, UA-CNRS n° 353, 4, place Jussieu, Tours n° 24-25, 75252 Paris Cedex 05, France.

\* Present address: Instituto Hidrografico, Rua das Trinas, 49, P-1296 Lisboa, Portugal.

+ to whom all correspondence should be addressed. .

Received 29/8/88, in revised form 16/2/89, accepted 27/2/89.

## ABSTRACT

Two active hydrothermal vent sites on the East Pacific Rise at 21° and 13°N have similar biogeochemical settings. They are characterized by large animal communities surrounding large porous sulfide edifices venting hot water (200-300°C) as black smokers. Samples from these sites were analyzed in terms of biological marker lipids such as *n*-alkanes, isoprenoid hydrocarbons, triterpanes, steranes and fatty acids to describe their sources, bacterially-mediated transformation products and alteration by hydrothermal fluids. A mixed sample containing interior and exterior portions of a chimney at 21°N shows a series of *iso*- and *anteiso*-alkanes without the presence of hopane and sterane biomarkers. This unusual distribution could be derived from high wax crude oil which crystallized from a cooling aqueous solution. The *iso*-alkanes could initially originate from a significant bacterial biomass which would metabolize the other organic residues during an increase in microbial activity. Chimney fragments with tube worm casts at 21°N contain products that are characteristic of immature biodegraded organic matter as indicated by microbial markers (*cis*-11-octadecenoic acid, branched C<sub>15</sub> and C<sub>17</sub> fatty acids, diploptene) and low thermal alteration which enhanced formation of cholestene isomers and diacholestenes. In comparison, a metalliferous sediment and sediment trap particles at 13°N contained biomarkers derived from early diagenetic transformations of biogenic compounds (sterols, squalene), and more mature components [ $5\alpha$ (H),  $14\beta$ (H),  $17\beta$ (H) steranes (20R + 20S), dominant  $17\alpha$ (H)-hopane series], resulting from the alteration of precursors by hydrothermal stress.

*Oceanologica Acta*, 1989. 12, 4, 405-415.

## RÉSUMÉ

Traces de matière organique pétrolière associée à des sulfures hydrothermaux de nature « massive » de la dorsale du Pacifique Est à 13 et 21°N

Deux sites d'événements hydrothermaux actifs de la dorsale du Pacifique Est à 13 et 21°N présentent des compositions biogéochimiques similaires. Ils sont caractérisés par de luxuriantes communautés animales environnant de grands édifices de sulfures d'où s'échappent des fluides très chauds (200-350°C) chargés de particules noires caractéristiques des « fumeurs noirs ». Des fragments de ces édifices hydrothermaux ont été échantillonnés et analysés en termes de traceurs lipidiques biogéochimiques comme

les *n*-alcanes, les hydrocarbures isoprénoïdes, les triperpènes, les stérènes et les acides gras afin de décrire les sources, les produits de transformations occasionnées par l'activité bactérienne et les processus d'altération par les fluides hydrothermaux. Un échantillon contenant des fragments des parties intérieure et extérieure d'une cheminée à 21°N présente une série d'*iso*- et *antéiso*-alcanes sans la présence de biomarqueurs de type hopane et stérane. Cette composition peu souvent rencontrée proviendrait de quelques cires d'huiles brutes qui auraient cristallisé au moment du refroidissement de l'eau hydrothermale. Ces compositions d'*iso*-alcanes pourraient initialement provenir d'une biomasse bactérienne importante qui, par augmentation de l'activité microbiologique, éliminerait les autres résidus organiques. Cependant l'abondance d'hydrocarbures aromatiques polycycliques dans ce même échantillon refléterait une origine pyrolytique lors du passage d'un fluide hydrothermal à haute température. Les fragments de cheminées avec des débris de tubes de vers à 21°N contiennent des produits caractéristiques d'une matière organique immature biodégradée comme l'indique la présence des traceurs d'origine microbiologique (acide *cis*-11-octadécénoïque, acides gras ramifiés à 15 et 17 atomes de carbone, diploptène) et d'une faible altération thermique qui accentue la formation des isomères du cholestène et des diacholestènes. Par contre, le sédiment métallifère et les particules contenues dans un piège à sédiment à 13°N contiennent des biomarqueurs dérivés des transformations diagénétiques précoces de composés biogéniques (stérols, squalène) et des composés plus matures [stéranes 5 $\alpha$ (H), 14 $\beta$ (H), 17 $\beta$ (H), avec les configurations (20R + 20S) et la série dominante des 17 $\alpha$ (H)-hopanes] qui résultent de l'altération de composés précurseurs par les effets hydrothermaux.

*Oceanologica Acta*, 1989. 12, 4, 405-415.

## INTRODUCTION

Submarine hydrothermal systems deposit heavy metal sulfides, and in sedimented areas also generate and migrate petroleum (*e. g.* Guaymas Basin, Escanaba Trough, Atlantis II Deep; Simoneit, 1984; 1985; 1988; Simoneit *et al.*, 1987). These petroleum products are derived primarily from hydrothermal pyrolysis of immature sedimentary organic matter (Simoneit, 1983). Massive sulfides are found in active areas along sediment-starved mid-oceanic ridges (*e. g.* East Pacific Rise at 21°N and Mid-Atlantic Ridge at 26°N; Ballard *et al.*, 1981; Spiess *et al.*, 1980; Rona *et al.*, 1984; Thompson *et al.*, 1985), and sulfide deposits of a submarine origin have also been described uplifted on land (*e.g.* Rona, 1973; Scott, 1985). These terrestrial ore bodies contain fossils of fauna typical of hydrothermal vent environments (*e.g.* tube worm casts; Haymon *et al.*, 1984; Oudin and Constantinou, 1984; Banks, 1985).

It is of interest to determine whether any molecular fossils (*i. e.* biomarkers) from vent biota are detectable and recognizable in submarine sulfides of mid-oceanic ridges, for they may be of utility in identifying the species contributing the organic matter. In addition, hydrothermal pyrolysis of suspended organic matter and biota entrained into the venting water from the ambient surroundings may also generate recognizable compounds. Can these pyrolytic compounds be distinguished from biomarkers attributable to the vent macrofauna? Massive chimney sulfides from the East Pacific Rise (EPR) at 21°N were analyzed to evaluate the questions. The results are also compared with data for metalliferous sediment and sediment trap particles from 13°N on the EPR.

## EXPERIMENTAL

Manually-collected samples were obtained with the DSV Alvin (dive number 1226) from different parts of a mound-shaped accretion/chimney: 21N-1 from the base of the chimney with tube worm casts; 21N-2 from the interior of the chimney with pieces of obsidian; 21N-3 from the top of the chimney; 21N-4 from an admixture of mainly the interior and some exterior surfaces of the chimney; 21N-5 CR4 from a dark-black part of the chimney; and 21N-6 CR1 from solid chips at the base of the chimney. Dive 1226 was in the "clam acres" site (20°50'N, 109°06'W) at 2616 m water depth (Spiess *et al.*, 1980). The chimney that was sampled was heavily colonized by filamentous bacteria forming a mat and actively seeping hot vent fluids (grey smoker; Baross, *priv. comm.*).

The bulk samples were sealed in polyethylene bags and stored frozen on board ship. The subsamples containing some water were immersed in chloroform/methanol, with the quantity of methanol dependent on the amount of water present but enough to azeotrope all water. Then the samples were sonicated twice for 10 minutes and filtered through a Whatman GF/A glass fiber filter. The organic and aqueous extract phases were centrifuged and the organic layers removed. Then the total organic extracts were evaporated to dryness at 30°C using a rotary vacuum evaporator and weighed.

Residual sulfur was removed by using an activated copper column (Blumer, 1957) and rinsing three times with *n*-hexane. The organic extracts were saponified using toluene and a 10% solution of KOH in methanol

for 2 hours at reflux under an argon stream. After addition of excess water, the mixtures were acidified to pH 2 with concentrated HCl, and the total neutrals were then extracted into hexane/diethyl ether (9 : 1 v/v). The organic extracts were fractionated using a chromatographic column (4 × 0.5 cm i.d.) filled with 300 mg of silica gel G (Merck, type 60). The following fractions were eluted and collected, using solvents of increasing polarity (hexane, ethyl acetate and methanol, respectively): alkanes + alkenes (*n*-hexane; F1) and acids, alcohols and sterols (ethyl acetate; F3). An aliquot of each polar fraction (F3) was derivatized with 14% BF<sub>3</sub> in methanol and after addition of hexane followed by water, the mixture was extracted 3 times with hexane. The fatty acid methyl esters (FAME) were isolated from this extract fraction on the previously described column and were eluted with 0.2% ethyl acetate in hexane, yielding F2.

The total hydrocarbon fraction of the metalliferous sediment used for comparison was separated by liquid-solid column chromatography using silica gel G (Merck, type 60) with 10% AgNO<sub>3</sub> into saturated and unsaturated hydrocarbons. The two fractions were eluted with hexane and ethyl acetate respectively. Further analyses using gas chromatography are as described below for the aliphatic hydrocarbon fractions.

Aliphatic hydrocarbons (F1) were analyzed with a Hewlett Packard 9630 GC instrument fitted with a flame ionization detector (FID) and splitless injector. The column was a 25 m × 0.25 mm i.d. fused silica capillary column, coated with DB-5, temperature programmed from 65–135°C at 25°C/min, then 135–300°C at 4°C/min, using helium as carrier gas. Computerized gas chromatography-mass spectrometry (GC-MS) was performed on a Finnigan 9610 GC interfaced directly

with a Finnigan quadrupole mass spectrometer and an INCOS Model 2300 data system. The GC had an identical column and was temperature programmed from 65–300°C at 4°C/min, with helium as carrier gas. The GC-MS conditions have been described previously (Kawka and Simoneit, 1987). Gas chromatographic analysis of the FAME (F2) fractions was carried out with a Girdel 3000 GC instrument equipped with a FID and on-column (Ross type) injector. A polar fused silica capillary column (25 m × 0.32 mm i.d.) coated with Silar 5 CP (CP index = 58) was used. This was temperature programmed from 100–195°C at 2°C/min, with helium as carrier gas at a flow rate of 2 ml/min.

Compounds were identified by consideration of GC retention times, coinjection of standards, interpretation of MS data and comparison of mass spectra with those of authentic compounds or with published spectra. Quantification of components was by measurement of the peak area of the GC response, compared to that of a known quantity of perdeuterated tetracosane (*n*-C<sub>24</sub>D<sub>50</sub>) and two perdeuterated fatty acids as methyl esters, methyl *n*-tetradecanoate (C<sub>15</sub>D<sub>27</sub>H<sub>3</sub>O<sub>2</sub>) and methyl *n*-eicosanoate (C<sub>21</sub>D<sub>39</sub>H<sub>3</sub>O<sub>2</sub>).

## RESULTS AND DISCUSSION

### Hydrocarbons and fatty acids

The sample collected from the base of the chimney (21N-1), which was colonized by animal communities, such as tube worms, had the highest content of aliphatic hydrocarbons and fatty acids (Tab. 1). The presence of low molecular weight *n*-alkanes between *n*-C<sub>16</sub> and *n*-C<sub>18</sub> and the occurrence of pristane and

Table 1

Yields and chemical compositions of extractable organic matter from hydrothermal sulfides of the EPR at 21°N.

Samples	21N-1 (base of chimney with tube worm casts)	21N-2 (interior)	21N-3 (top of chimney)	21N-4 (interior/ exterior)	21N-5 (bulk chimney)	21N-6 (base)
<b>Hydrocarbons</b>						
Total extract + S (μg/g)	45,700	1,010	1,180	270	430	750
Total <i>n</i> -alkanes (μg/g)	0.9	0.30	0.05	0.06	0.03	0.10
Total resolved hydrocarbons (μg/g)	2.2	0.04	0.06	0.10	0.04	0.14
Total aliphatic hydrocarbons (μg/g)	8.0	0.22	0.26	0.31	0.10	0.33
Pr/Ph <sup>a</sup>	0.59	n. d.	n. d.	n. d.	0.89	0.53
CPI ( <i>n</i> -C <sub>23</sub> to <i>n</i> -C <sub>35</sub> ) <sup>b</sup>	1.1	1.0	0.9	1.0	0.8	1.0
CPI ( <i>n</i> -C <sub>12</sub> to <i>n</i> -C <sub>35</sub> ) <sup>b</sup>	1.1	1.0	1.0	1.0	0.8	1.0
C <sub>max</sub> <sup>c</sup>	29	29	26	25,26	30	31
<b>Fatty acids</b>						
Total fatty acids (μg/g)	89.5	0.70	1.62	0.22	3.35	0.71
Total branched fatty acids (μg/g)	4.5	0.01	0.02	0.003	0.05	0.01
Total unsaturated fatty acids (μg/g)	61	0.53	1.1	0.16	2.4	0.3
Total saturated fatty acids (μg/g)	24	0.16	0.5	0.06	0.9	0.4
Unsaturated/saturated fatty acids	2.5	3.3	2.2	2.7	2.7	0.8
CPI ( <i>n</i> -C <sub>12</sub> to <i>n</i> -C <sub>35</sub> ) <sup>b</sup>	14.9	9.8	13.6	7.5	44.0	17.6
C <sub>max</sub> <sup>c</sup>	16:1, 18:1 Δ <sup>11</sup>	22:1	22:1	22:1	18:1, 16	16, 22:1
C <sub>18:1</sub> Δ <sup>11</sup> /C <sub>18:1</sub> Δ <sup>9</sup>	8.0	0.4	1.2	0.5	n. d.	2.1
C <sub>16:1</sub> /C <sub>16:0</sub>	1.8	0.9	0.6	0.3	0.5	0.3
C <sub>18:1</sub> /C <sub>18:0</sub>	4.4	1.1	0.6	10.9	10.0	0.6

<sup>a</sup> pristane to phytane ratio.

<sup>b</sup> CPI = (Σ odd/Σ even *n*-alkanes or Σ even/Σ odd *n*-alkanoic acids).

<sup>c</sup> the dominant homolog is italicized.

n. d. = not detected.

Figure 1

Gas chromatograms of hydrocarbon fractions from hydrothermal sulfide chimney fragments collected at 21°N, East Pacific Rise: Numbers = n-alkane chain length; Pr = pristane; Ph = phytane; Py = pyrene; b = cholest-4-ene; c = cholest-5-ene; d = diploptene; 27D = diacholest-13 (17)-ene (20R); i and a = iso- and anteiso-alkanes, respectively; IS = internal standard.

a) 21N-5, bulk dark-black chimney;  
b) 21N-1, base of chimney with tube worm cast;  
c) 21N-4, admixture of interior and exterior of chimney.

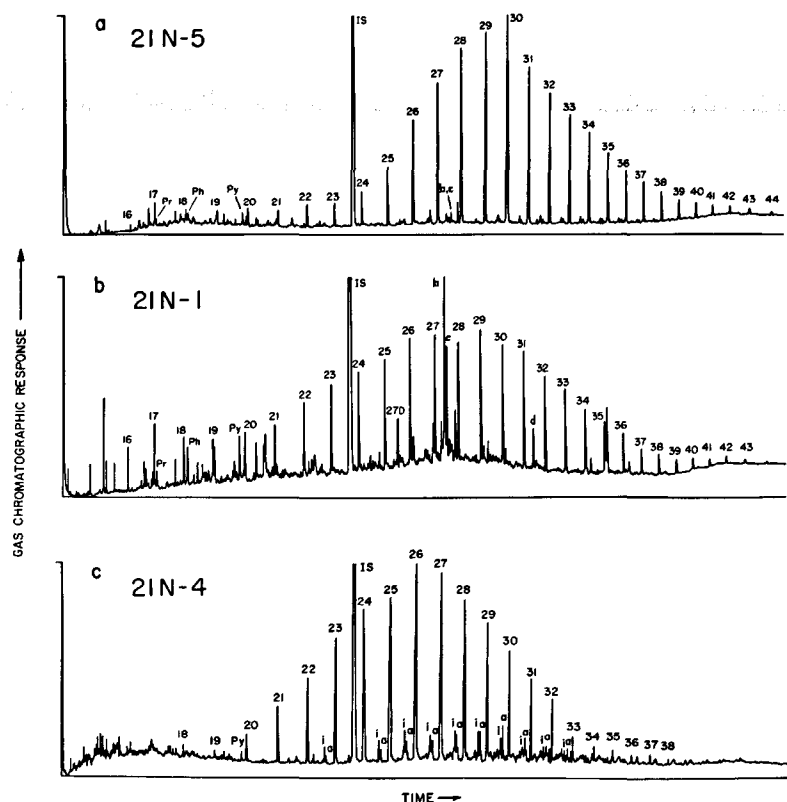


Table 2

Concentration of fatty acids from hydrothermal sulfides of the EPR at 21°N.

Fatty acids <sup>a</sup> (ng/g)	21N-1 (base of chimney with tube worm casts)	21N-2 (interior)	21N-3 (top of chimney)	21N-4 (interior/ exterior)	21N-5 (bulk chimney)	21N-6 (base)
<b>Saturated:</b>						
12:0	540	—	—	—	7.1	2.8
13:0	—	—	—	—	—	—
14:0	2 900	8.0	22	4.2	60	23
15:0	740	4.3	16	2.0	8.9	11
16:0	12 000	79	190	27	640	200
17:0	480	3.3	8.8	1.1	8.0	7.7
18:0	5 100	31	210	0.9	170	150
19:0	110	2.2	2.6	0.6	0.9	1.5
20:0	610	6.2	17	2.3	5.1	10
21:0	—	1.2	1.5	0.8	1.6	1.7
22:0	280	8.5	14	4.6	6.7	6.7
23:0	44	1.3	2.0	0.5	0.8	1
24:0	260	4.1	8.2	2.1	2.7	4.3
25:0	91	1.3	2.6	0.5	—	—
26:0	100	1.9	3.7	0.8	1.0	2.4
27:0	—	0.6	0.8	0.2	—	—
28:0	—	1.3	1.9	0.6	—	1.4
30:0 → 34:0 (even)	—	2.2	1.5	0.3	—	—
<b>Branched:</b>						
iso 14:0	210	0.3	—	—	1.8	0.6
anteiso 15:0	1 400	2.2	7.9	1.3	13	4.4
iso 15:0	1 500	1.0	3.5	0.4	15	2.5
anteiso 17:0	670	1.6	3.2	0.8	7.9	3.4
iso 17:0	700	2.0	4.0	0.4	9.5	2.6
<b>Monounsaturated:</b>						
14:1	1 800	0.9	5.7	0.2	19	3.8
16:1	22 000	68	120	9.3	300	60
17:1	1 100	1.3	5.7	—	8.2	2.1
Δ9 18:1	2 500	23	62	6.9	—	28
Δ11 18:1	20 000	10	72	3.3	1 700 <sup>b</sup>	60
20:1	3 700	28	60	6.2	110	7.9
22:1	8 700	390	740	130	300	120
24:1	250	8.0	14	2.9	6.8	3.4

<sup>a</sup> analyzed as methyl esters.

<sup>b</sup> coelution of fatty acids 18:1 Δ<sup>9</sup> + 18:1 Δ<sup>11</sup>.

phytane ( $Pr/Ph < 1$ ) in this sample indicates a biogenic origin from synthesis or decarboxylation/reduction reactions of fatty acids and alcohols (Han and Calvin, 1969; Gelpi *et al.*, 1970). Only the base of the chimney with the tube worms shows the presence of a broad unresolved complex mixture (UCM) of branched and cyclic hydrocarbons which could be attributed to a microbial origin ( $C_{max}$  at  $C_{27}$ , Fig. 1). Long-chain *n*-alkanes with carbon numbers ranging from  $C_{21}$  to  $C_{38}$  are found in all the samples without any significant predominance of odd or even carbon numbered hydrocarbons (Carbon Preference Index, CPI = 0.8 to 1.1; Tab. 1). The regular *n*-alkane distribution with a maximum at *n*- $C_{29}$  (21N-1 base and 21N-2 interior of the chimney, example Fig. 1b) has also been observed for dissolved *n*-alkanes in water column samples surrounding another hydrothermal vent system at 13°N on the East Pacific Rise (Brault *et al.*, 1988). Most deep ocean waters without petroleum contamination have this profile, which has been characterized as the oceanic background signal of deep sea waters (Saliot, 1981).

The samples collected at the top (21N-3) and the mixed interior/exterior (21N-4) of the chimney also have a smooth *n*-alkane distribution, but with a maximum at *n*- $C_{26}$  and no odd/even carbon-number preference. This pattern could be an indication of a *n*-alkane-enriched hydrothermal pyrolysate entrapped with the sulfides, analogous to that obtained from a sample of the interior of a mound-shaped accretion in the Guaymas Basin hydrothermal area (Simoneit, 1984).

On the other hand, the inside/outside chimney sample shows a series of *iso*- and *anteiso*-alkanes ranging from  $C_{25}$  to  $C_{34}$  (Fig. 1c) possibly derived from bacterial waxes (Tissot *et al.*, 1977) and/or formed by thermal and catalytic reactions. Various compounds with linear alkyl chains (*n*-alkanes, fatty acids, esters) could, when undergoing thermal cracking reactions, produce  $\alpha$ -olefins, which are then catalytically transformed in the presence of acidic clay minerals into monomethyl-substituted alkanes (Kissin, 1987). The occurrence of *n*-*iso*- and *anteiso*-alkanes, known constituents of high wax crude oils (Hedberg, 1968), could be due to crystallization of waxes from a cooling aqueous solution; while the absence of triterpanes and steranes indicates either complete biodegradation by a significant bacterial biomass, or an initial low level of biomarker precursors.

The dominant fatty acids for all samples are  $C_{16}$ ,  $C_{18}$  and  $C_{22}$ , most with one degree of unsaturation (Tab. 1 and 2). The significant amount of *cis*-11-octadecenoic acid ( $C_{18:1}$ ,  $\Delta^{11}$ ) in sample 21N-1 is probably derived mainly from bacterial input (Perry *et al.*, 1979; Volkman *et al.*, 1980). Branched  $C_{15}$  and  $C_{17}$  fatty acids present at levels of 5% in the total fraction of this sample, are also common in some bacteria (Perry *et al.*, 1979). The other major  $C_{18}$  isomer, oleic acid (*cis*-9-octadecenoic acid,  $C_{18:1}$ ,  $\Delta^9$ ) is a strong indicator of zooplanktonic material (Culkin and Morris, 1969). Additional monounsaturated fatty acids  $C_{16:1}$ ,  $C_{20:1}$  and  $C_{24:1}$  are also present in significant amounts and can

be related to bathypelagic organisms (Lee *et al.*, 1971). Also, the high amount of  $C_{22:1}$  may be derived from tube worms or other chemosynthetic organisms which could have unusual fatty acid compositions compared to other bathypelagic organisms. Unsaturated fatty acids in such deep-living organisms are largely present bound in wax esters (Lee *et al.*, 1971). Therefore, the larger amounts of lipid components in sample 21N-1 in comparison with those from the top or inside of the chimney are due to production by microbial and/or other biological processes from hydrothermal organisms colonizing the sulfide chimney fragments after their deposition (*e.g.* activities of tube worms, bebbiatoa, *etc.*). A high signature of microbial fatty acids has also been found for vent waters and particulates from the EPR at 13°N (Brault *et al.*, 1984).

## Biomarkers

### Base of chimney (21N-1) from 21°N EPR

Mass chromatograms of the hydrocarbons in a sample from the base of the chimney (21N-1) colonized by tube worms show the presence of sterenes and diasterenes with 27 carbon atoms and a low amount of a  $C_{24}$  ster-2-ene (Fig. 2, Tab. 3). Diasteranes and steranes were not detectable. Cholest-4-ene and cholest-5-ene are present at high concentrations compared with cholest-2-ene and cholest-6-ene (Fig. 2a). The major  $C_{27}$  diasterenes are found with the 20S configuration in lower amounts than the 20R counterpart (Fig. 2b). The triterpane distributions (Tab. 3) show the presence of very low amounts of  $17\alpha(H)$ ,  $21\beta(H)$ -30-norhopane,  $17\alpha(H)$ ,  $21\beta(H)$ -hopane and hop-17(21)-ene, with only  $17\beta(H)$ ,  $21\beta(H)$ -hop-22(29)-ene (diploptene) evident at a significant concentration and originating from marine bacteria and/or microorganisms (De Rosa *et al.*, 1971; Ourisson *et al.*, 1979). We also suggest that the sterenes are formed from biologically mediated reactions upon

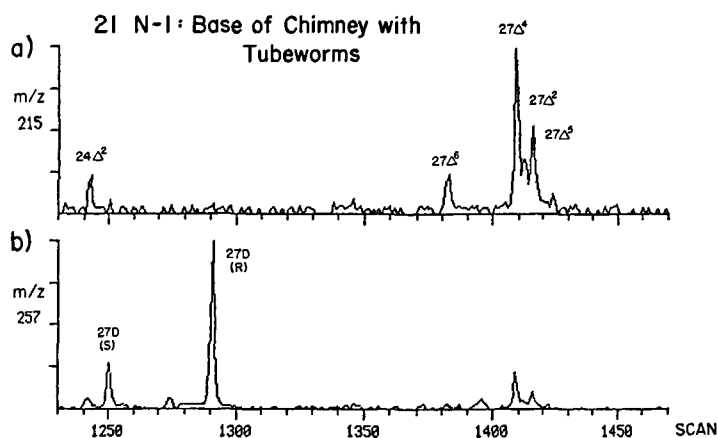


Figure 2  
Mass fragmentograms of (a)  $m/z$  215 and (b)  $m/z$  257, characteristic of sterene and diasterene fingerprints, respectively, for sample 21N-1 collected at the base of an active chimney with tube worm casts from 21°N, East Pacific Rise. Numbers refer to carbon skeleton size and  $\Delta^n$  refers to the carbon number location of the double bond; 27D(S) and 27D(R) = diacholest-13(17)-ene with the 20S and 20R configuration, respectively (GC-MS operating conditions as described in the text).

Table 3

Occurrence and relative abundance of biomarkers (triterpenoid and steroid hydrocarbons) for representative samples from 21° and 13°N, East Pacific Rise.

	21N-1 (base of chimney with tube worm casts)	21N-4 (interior/ exterior)	Metalliferous sediment (base of inactive chimney, 13°N)	Sediment trap (13°N)
Triterpanes	low	—	+	+
Triterpenes	+	—	+	+
Steranes	—	—	+	+
Sterenes	+	—	+	+
Diasteranes	—	—	—	+
Diasterenes	+	—	+	—
PAH's	tr.	+	low	tr.

+: present in significant amount.

low: present in low amount.

tr.: present in trace amount.

—: not detectable.

precursors (Gagosian *et al.*, 1980; Gagosian and Farrington, 1978) such as cholesterol, which was present in high amounts in a sediment trap moored above the hydrothermal vents. This compound can undergo subsequent dehydration and double-bond isomerization reactions (Mackenzie *et al.*, 1982). It is unlikely that marine organisms and bacteria are the direct source of these sterenes, and reports describing the presence of sterenes in such organisms are limited (Wakeham and Canuel, 1986). Since this sample contains high concentrations of triterpenoid compounds of a bacterial origin, it is quite probable that the sterenes have been formed microbiologically from sterols. For this locale, we suggest that the diagenetic pathway of sterene formation may be a combination of both bacterial and chemical reactions, enhanced by the hydrothermal activity.

Triterpenoid and steroid hydrocarbons were not detected in the other hydrothermal sulfide samples (Tab. 3). However, the bulk and base of the chimney (samples 21N-5 and 21N-6) contain low levels of polynuclear aromatic hydrocarbons (PAH) consisting mainly of phenanthrene, methylphenanthrenes and pyrene, and the interior/exterior of the chimney (sample 21N-4) contains significant amounts of PAH and thio-PAH compounds, confirming the pyrolytic origin of the organic matter (*see Aromatic hydrocarbon section*).

#### Metalliferous sediment from 13°N EPR

In comparison, a metalliferous sediment collected at the base of an inactive chimney at 13°N on the EPR shows the presence of the hopane series, and steranes, sterenes and diasterenes with 27 carbon atoms only, but no diasteranes (Fig. 3, Tab. 3). The two major

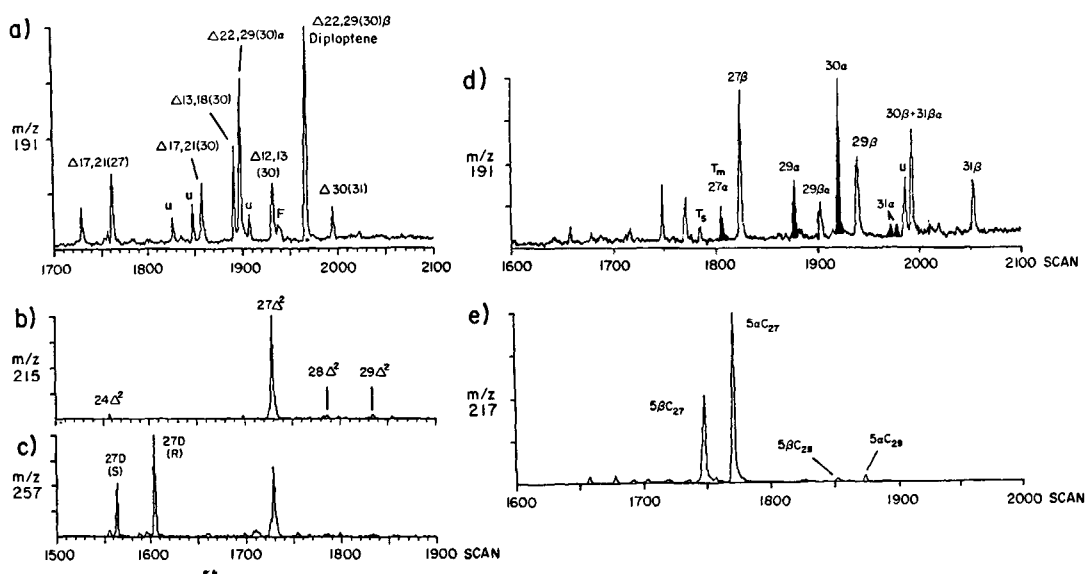


Figure 3

Mass fragmentograms for biomarker hydrocarbons from the metalliferous sediment collected at 13°N, East Pacific Rise, at the base of an inactive chimney (the same GC-MS operating conditions were used as for the analyses above, Fig. 2). The total hydrocarbon fraction was separated by liquid-solid column chromatography using silica gel with 10%  $\text{AgNO}_3$  into saturated (d, e) and unsaturated hydrocarbons (a-c).

a)  $m/z$  191:  $\Delta 17,21(27) = 22,29,30$ -trisorhop-17-(21)-ene;  $\Delta 17,21(30) = \text{hop-17(21)-ene}$ ;  $\Delta 13,18(30) = \text{isohop-13(18)-ene}$  (hopene II);  $\Delta 22,29(30)\alpha = 17\alpha(\text{H}),21\beta(\text{H})$ -hop-22(29)-ene (interpreted);  $\Delta 12,13(30) = \text{neohop-12(13)-ene}$ ,  $\Delta 22,29(30)b = 17\beta(\text{H}),21(\text{H})$ -hop-22(29)-ene (diploptene);  $\Delta 30(31) = \text{homohop-30-ene}$  (interpreted); u = unknown triterpene; F = fern-7-ene;

b)  $m/z$  215, characteristic sterene fingerprint: Numbers refer to carbon skeleton;  $\Delta^2 = \Delta^2$  sterenes;

c)  $m/z$  257, characteristic diasterene fingerprint: 27D(R) and 27D(S) = diacholest-13(17)-ene with the 20R and 20S configuration, respectively;

d)  $m/z$  191, characteristic triterpane fingerprint: Numbers refer to carbon skeleton while suffixes are their configuration:  $\alpha = 17\alpha(\text{H}),21\beta(\text{H})$ -hopane series;  $\beta = 17\beta(\text{H}),21\beta(\text{H})$ -hopane series;  $\beta\alpha = 17\beta(\text{H}),21\alpha(\text{H})$ -hopane series; u = unknown triterpanes;

e)  $m/z$  217, characteristic sterane fingerprint:  $5\beta\text{C}_{27} = \text{coprostane}$ ;  $5\alpha\text{C}_{27} = \text{cholestane}$ .

compounds are cholestane and copropane at very high concentrations, with  $C_{28}$  and  $C_{29}$  steranes in trace amounts (Fig. 3b). The triterpanes (Fig. 3d) consist of a series of hopanes ranging from  $C_{27}$  to  $C_{32}$  ( $C_{28}$  absent). The biologically derived  $17\beta(H)$ ,  $21\beta(H)$ -hopane series is dominant, as well as significant concentrations of the intermediate  $17\beta(H)$ ,  $21\alpha(H)$ - and the thermally most stable  $17\alpha(H)$ ,  $21\beta(H)$ -hopane configurations. This distribution reflects an elevated temperature of thermal stress as compared with the massive sulfide samples from  $21^\circ N$ , but it is still very immature.

The major triterpene encountered in the metalliferous sediment is diploptene (Fig. 3a). Other triterpenes present consist of isomers of  $C_{30}$  hopenes such as neohop-13(18)-ene, neohop-12(13)-ene,  $17\alpha(H)$ ,  $21\beta(H)$ -hop-22(29)-ene (tentative assignment), hop-17(21)-ene, unknown  $C_{30:1}$  triterpenes, fern-7-ene, 22, 29, 30-trisnorhop-17(21)-ene and homohop-30-ene (Fig. 3a). Some of these hopenes may result from a direct input by microorganisms (De Rosa *et al.*, 1971; Ourisson *et al.*, 1979; Howard *et al.*, 1984) or reflect a diagenetic conversion from diploptene (Ageta *et al.*, 1968; Ensminger, 1977; Brassell *et al.*, 1980).

The sterene distribution consists mainly of cholest-2-ene, with 24-methylcholest-2-ene, 24-ethylcholest-2-ene, a  $C_{24}\Delta^2$ -sterene and cholesta-3,5-diene (not shown on Fig. 3b) present in minor amounts (Fig. 3b). The occurrence of diasterenes with 27 carbon atoms and the 20R configuration more abundant than 20S (Fig. 3c) is similar to that observed for the sample collected at the base of the chimney at  $21^\circ N$  (*cf.* Fig. 2b), but cholest-4-ene and cholest-5-ene, which are more stable than cholest-2-ene (Mackenzie *et al.*, 1982), are absent. On the other hand, copropane and cholestane are the dominant steranes (Fig. 3e). This sediment collected at the base of an inactive chimney seems to show the superposition of two "types" of biomarkers having a similar origin but different maturity. "Recent" biomarkers may be derived from autochthonous marine sources in the vicinity of the hydrothermal area and are transported by the currents or turbulent hydrothermal waters, with subsequent redeposition. The more mature biomarkers also have an autochthonous origin, resulting from the alteration of the precursors by hydrothermal stress in active chimneys. Indeed, copropane and cholestane may also result from hydrothermal reduction of cholest-2-ene or other isomers (*e.g.* cholest-4-ene and cholest-5-ene). Sterenes arise from stanols by either microbially-mediated dehydration of the stanol or via microbial production of the stanol itself by reduction of a stenol (Gagosian and Farrington, 1978; Gagosian *et al.*, 1980). Sterane formation, by initial microbiological processes, seems to be enhanced, in less than 100 years (approximate age of the site), in the subsurface of the sediments by passage of hot fluids, or in biological detritus carried into and transformed by hot vent waters and then resettling.

#### Sediment trap from $13^\circ N$ EPR

Data from a sediment trap moored above the hydrothermal vents at  $13^\circ N$  indicate the presence of particles of a biological origin which were entrained and altered

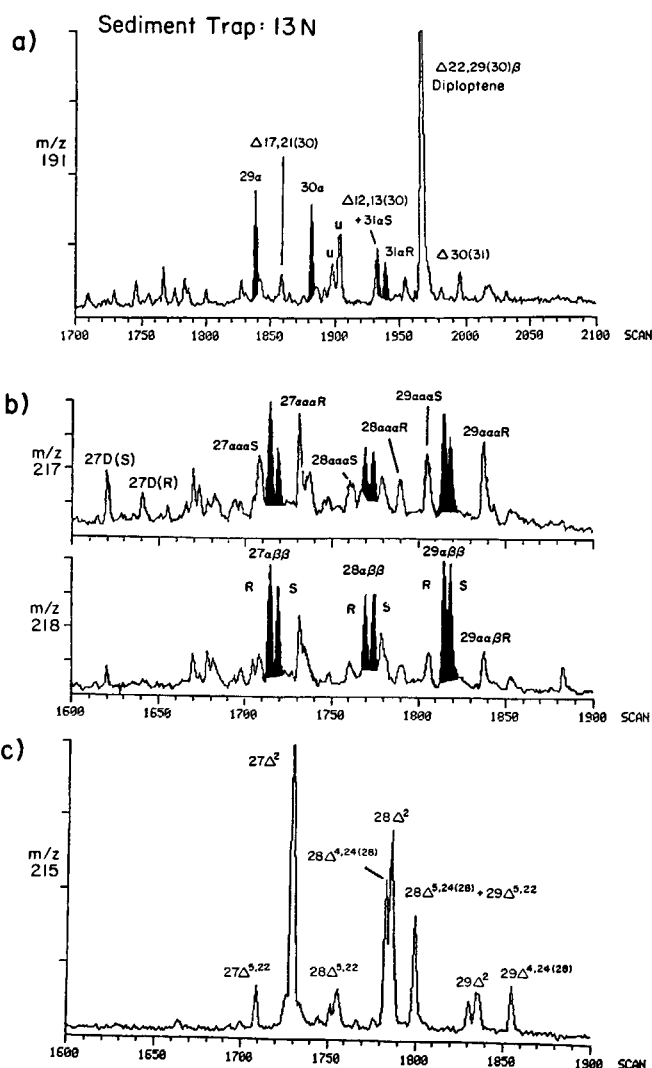


Figure 4

Mass fragmentograms for biomarker hydrocarbons from the sediment trap particles at  $13^\circ N$ , East Pacific Rise (GC-MS conditions were the same as for the previous samples).

a)  $m/z$  191, key as in Fig. 3d with the  $17\alpha(H)$ ,  $21\beta(H)$ -hopane series shaded;

b)  $m/z$  217 and 218, characteristic sterane fingerprints: Numbers refer to carbon skeleton,  $\alpha$  or  $\beta$ =hydrogen configurations at C-5, C-14, C-17, respectively; R or S=epimer at C-20;

c)  $m/z$  215, key as in Fig. 3b with  $\Delta^{5,22}$ =stera-15,22-diene;  $\Delta^{5,24}$ =stera-5,24-diene;  $\Delta^{4,24}$ =stera-4,24-diene;  $\Delta^{4,24(28)}$ =stera-4,24(28)-diene;  $\Delta^{5,24(28)}$ =stera-5,24(28)-diene

by hydrothermal fluids. The hopanoid distribution shows the presence of a major amount of diploptene (Fig. 4a) and the  $17\alpha(H)$ ,  $21\beta(H)$ -hopane series is also abundant, with the other triterpenes present in lesser amounts than in the metalliferous sediment (*cf.* Fig. 3a, Tab. 3).

In contrast with the metalliferous sediment, the sterane series ranges from  $C_{27}$  to  $C_{29}$ , dominated by  $5\alpha(H)$ ,  $14\beta(H)$ ,  $17\beta(H)$ -steranes with the 20R greater than the 20S configuration (Fig. 4b, Tab. 3). This confirms the greater maturity of those hydrocarbons (Seifert and Moldowan, 1978; Mackenzie *et al.*, 1980) possibly induced by the hot water emissions. This dominant sterane series was also found in the dissolved hydrocarbon fraction of hot water ( $T > 250^\circ C$ ) collected at  $13^\circ N$  (Brault *et al.*, 1988), which indicates entrainment of thermally-altered organic compounds in hydrothermal vent plumes.

On the other hand, sterenes ranging from  $C_{27}$  to  $C_{29}$ , with cholest-2-ene predominant, are present as observed for the metalliferous sediment (*cf.* Fig. 3*b*). But cholesta-5,22-diene, 24-methylcholesta-5,22-diene, 24-methylcholesta-4,24(28)-diene, 24-methylcholesta-5,24(28)-diene, 24-ethylcholesta-5,22-diene and 24-ethylcholesta-4,24(28)-diene are also present in significant amounts (Fig. 4*c*) with cholesta-3,5-diene at an intermediate concentration. In contrast with the metalliferous sediment, diasterenes are not formed because no suitably acidic conditions and clay-catalyzed rearrangements occur in the sediment trap particulate matter. The occurrence of steradienes could result from microbial alteration of biogenic sterols which are present in many genera of marine animals (Morris and Culkin, 1977), their fecal matter (Prahl and Eglinton, 1985; Wakeham and Canuel, 1986) and various species of planktonic algae (reviewed by Volkman, 1986). We do not yet know the steroidal composition of specific animals inhabiting hydrothermal vent environments, so these compounds could also originate in sinking particles from surficial productivity. Steroid distributions in the Vertex II and III particles described by Wakeham (1987) in the eastern tropical North Pacific (15-18°N, 107-109°W) are similar to our data. However, Wakeham (1987) did not report  $\Delta^4$ - or  $\Delta^5$ -sterenes, diasteranes or steranes which are present in these samples with the enhanced alteration effects due to hydrothermal activity.

As suggested for the metalliferous sediment, the sediment trap particles contain an admixture of "recent" biomarkers originating from marine organisms and bacteria (we are not yet able to differentiate input from surficial productivity and input from autochthonous hydrothermal environments) and more mature organic material rapidly altered by hot water emissions. It seems that most of the mature biomarkers initially come from precursors in ambient sea water, which — in the course of percolation through fissures in the basalts — have undergone high temperature hydrothermal alteration. It is also possible that biodegradation effects can change the sterane distributions by selective removal of  $C_{27}$  steranes (Goodwin *et al.*, 1983). However, "recent" biomarkers from autochthonous hydrothermal organisms are degraded rapidly at moderate temperatures, and with longer contact times by hot water and suitable quenching near the high heat flow, such compounds will undergo more extensive thermal alteration.

The fragment of massive sulfide chimney with tube worm casts does not contain mature biomarkers (*i. e.* steranes), as found in the metalliferous sediment and sediment trap particles, but intermediate compounds such as  $\Delta^4$ - and  $\Delta^5$ -cholestenes (absent in the sediment and sediment trap particles) and diacholestenes (Tab. 3). This suggests that the organic matter associated with massive sulfide chimneys has a shorter contact time with hot water or high heat flow than the metalliferous sediment (talus) and suspended particles in the sediment trap; and that mature biomarkers have not yet formed due to the lower temperature existing

at the base of the chimney and/or the biogenic input is too recent and in excessive amounts compared to the more mature compounds.

The vent/mound detritus from these two EPR areas at 13° and 21°N on fast spreading, sediment-starved ridges contain traces of thermogenic, petroleum-like hydrocarbons. However, their concentrations are extremely low compared to Guaymas basin for example, with its thick sedimentary cover of diatomaceous oozes and mud turbidites (Curry *et al.*, 1982), which provides more organic source material for alteration and transport by the hydrothermal fluids (Simoneit, 1988; Simoneit *et al.*, 1984; Kawka and Simoneit, 1987).

#### *Aromatic hydrocarbons*

Polynuclear aromatic hydrocarbons (PAH) present in the massive hydrothermal sulfides, metalliferous sediment and sediment trap particles are listed in Table 4. These are minor components compared to the alkanes, and represent a bitumen fraction derived from high temperature reforming processes (Simoneit, 1984). The PAH series consist mainly of phenanthrene and its alkylated homologs, pyrene and fluoranthene, with a minor amount of benzo-(*b*)-fluorene. The thio-PAH, dibenzothiophene and benzonaphthothiophene, are also present in significant amounts. The inside/outside chimney fragment (21N-4) has the most diverse PAH distribution compared to the other chimney fragments and sediment trap particles (Tab. 4). The additional PAH analogs consist mainly of peri-condensed aromatic structures such as coronene, dibenzoanthracene, benzopyrene, benzopyrenes, dibenzopyrene, benzo-fluoranthene and perylene, with a small amount of anthracene. Chrysene (and/or triphenylene) and benzoanthracene are also present in the base of the chimney with pyritized tube worm casts (21N-1). The abundances of various (PAH) with an alicyclic five-membered ring (*e. g.* fluoranthene and benzofluorene) and thio-PAH are additional evidence for a pyrolytic origin of this fraction by condensation from the passage of high-temperature hydrothermal fluids through the chimney (LaFlamme and Hites, 1978; Simoneit and Lonsdale, 1982; Simoneit, 1984).

The calculation of methylphenanthrene indices (MPI 1 and 2 of Radke and Welte, 1983) is problematic in the case of hydrothermal petroleum samples at the seafloor. Secondary effects such as water solubilization and/or biodegradation most likely affect these ratios and thus they are not considered here. It has been observed that the ratio of phenanthrene to all methylphenanthrenes more closely reflects the thermal stress experienced by hydrothermal petroleum (Kawka and Simoneit, unpublished results). This ratio has been calculated and indicates that the organic matter of sample 21N-1 has been exposed to lower temperatures than that of samples 21N-4 to 21N-6 and the samples from 13°N (Tab. 4), which are all approximately similar in terms of thermal maturation.

The base of the chimney with pyritized tube worm casts (21N-1) contains the highest levels of aromatic hydrocarbons, namely pyrene, thio-PAH (benzonaph-



Table 4

Relative abundance of polynuclear aromatic hydrocarbons in massive hydrothermal sulfides from the EPR at 21°N, metalliferous sediment and sediment trap particles from the EPR at 13°N.

PAH	Samples*						Sediment trap 13°N	Metalliferous sediment 13°N
	21N-1	21N-2	21N-3	21N-4	21N-5	21N-6		
Phenanthrene (MW : 178)	100	—	—	100	100	100	100	100
Anthracene (MW : 178)	—	—	—	38	—	—	—	—
C <sub>1</sub> -phenanthrenes (MW : 192)	276	—	—	50	80	104	55	73
3MP	80	—	—	13	13	31	10	12
2MP	77	—	—	12	15	17	19	25
9MP	65	—	—	14	39	41	11	18
1MP	54	—	—	11	13	15	15	18
Phenanthrene/methyl phenanthrenes	0.36	—	—	2.0	1.25	0.96	1.82	1.37
C <sub>2</sub> -phenanthrenes (MW : 206)	532	—	—	50	—	70	40	69
C <sub>3</sub> -phenanthrenes (MW : 220)	201	—	—	—	—	—	—	18
Fluoranthene (MW : 202)	402	—	—	53	8	42	30	152
Pyrene (MW : 202)	3285	—	—	80	73	111	15	512
Benzo-(b)-fluorene (MW : 216)	100	—	—	18	—	1.7	—	35
Benzo-(a)-anthracene (MW : 228)	42	—	—	59	—	—	—	—
Chrysene/triphenylene (MW : 228)	71	—	—	78	—	—	—	—
Benzo-(b+k)-fluoranthene (MW : 252)	—	—	—	52	—	—	—	—
Benzo-(e)-pyrene (MW : 252)	—	—	—	60	—	—	—	—
Benzo-(a)-pyrene (MW : 252)	—	—	—	48	—	—	—	—
Perylene (MW : 252)	—	—	—	68	—	—	—	—
Dibenzo-(a, e)-anthracene (MW : 278)	—	—	—	124	—	—	—	—
Benzo-(g, h, i)-perylene (MW : 276)	—	—	—	78	—	—	—	—
Anthanthrene (MW : 276)	—	—	—	54	—	—	—	—
Coronene (MW : 300)	—	—	—	238	—	—	—	—
Dibenzo-(a, d)-pyrene (MW : 302)	—	—	—	71	—	—	—	—
Dibenzothiophene (MW : 184)	44	—	—	68	53	38	34	46
Benzonaphthothiophene (MW : 234)	2387	—	—	39	—	132	—	82

\*Concentrations relative to phenanthrene.

thiophene), methyl and dimethylphenanthrenes (Tab. 4). This suggests that the base of the chimney received a higher input of more volatile/soluble pyrolytic aromatic compounds compared to the upper (inside/outside) chimney, which is in more direct contact with high temperature fluids. The sediment trap particles (13°N) do not contain high molecular weight PAH and pyrene is present in a lower amount than fluoranthene. It seems that the higher molecular weight PAH associated with hydrothermal mineral particles do not migrate or dissolve as easily as the lower molecular weight analogs (*i.e.* phenanthrene and its alkylated homologs present in metalliferous sediment (13°N) and all chimney fragments except 21N-2 and 21N-3, where no PAH are detected).

## CONCLUSION

Biomarkers from vent biota are detectable in sulfide chimneys at an oceanic fast-spreading center, even without an organic-rich sedimentary cover. In addition petroleum-like components are also formed during hydrothermal pyrolysis of suspended organic matter and biota. According to the conditions of formation and deposition of sulfide chimneys and the abundance of surrounding hydrothermal organisms and bacteria, these biomarkers will undergo various degrees of maturation.

Lipids extractable from the base of a young and active chimney at 21°N, recently colonized by animal communities (tube worms), are derived from immature, primarily marine components from microorganisms. This is confirmed by the presence of *cis*-11-octadecenoic acid (C<sub>18:1</sub>Δ<sup>11</sup>) and diploptene. The immature organic compounds rapidly undergo bacterial and/or chemical alteration reactions, enhanced by low temperature hydrothermal activity or by short term contact with high heat flow. This is supported by the presence of various isomers of C<sub>27</sub>-sterenes and diasterenes, 17α(H)-hopanes and hop-17(21)-ene. The inside/outside fragment of the active chimney shows an *n*-alkane-enriched hydrocarbon fraction, possibly derived from bacterial waxes and analogous to those observed in high wax crude oils. These compounds could be formed by thermal cracking reactions due to rapid passage of hydrothermal fluids, followed by intense microbial activity. The base of an inactive chimney and sediment trap particles from 13°N exhibit an admixture of lipids from recent autochthonous marine sources including bacteria and more mature organic matter rapidly altered by hydrothermal maturation. This is confirmed by the presence of diploptene, isomers of hopenes and sterenes for the immature biomarkers and by 17α(H), 21β(H)-hopanes, 5α(H), 14β(H), 17β(H)- >5α(H), 14α(H), 17α(H)-steranes and diasteranes for the mature biomarkers. The biomarkers matured by hydrothermal fluids are advected by the plumes and then resettle to the seafloor on particles.

## Acknowledgements

We thank J.A. Baross, O.E. Kawka and A. Lorre for samples, data and assistance. Funding from the Division of Ocean Sciences and Division of International

Programs of the National Science Foundation (Grants OCE-8512832, OCE-8601316 and INT-8514510) is gratefully acknowledged.

## REFERENCES

- Ageta H., K. Shiojima and Y. Arai (1968). Fern constituents: Neohopene, hopene-II, neohopadiene, and fernadiene isolated from *Adiantum* species. *Chem. Commun.*, 1105-1107.
- Ballard R. D., J. Francheteau, T. Juteau, C. Rangin and N. Normark (1981). East Pacific Rise at 21°N: The volcanic, tectonic and hydrothermal processes of the central axis. *Earth planet. Sci. Letts*, **55**, 1-10.
- Banks D. A. (1985). A fossil hydrothermal worm assemblage from the Tynagh lead-zinc deposit in Ireland. *Nature*, **313**, 128-131.
- Blumer M. (1957). Removal of elemental sulfur from hydrocarbon fractions. *Analyt. Chem.*, **29**, 1039-1041.
- Brassell S. C., P. A. Comet, G. Eglinton, P. J. Isaacson, J. McEvoy, J. R. Maxwell, I. D. Thomson, P. J. C. Tibbetts and J. K. Volkman (1980). The origin and fate of lipids in the Japan Trench. In: *Advances in Organic Geochemistry 1979*, A. G. Douglas and J. R. Maxwell, editors, Pergamon Press, Oxford, 375-392.
- Braut M., J. C. Marty and A. Saliot (1984). Fatty acids from particulate matter and sediment in hydrothermal environments from the east Pacific rise, near 13°N, in: *Advances in Organic Geochemistry 1983*, P. A. Schenck, J. W. De Leeuw and J. W. Lijmbach, editors, Pergamon Press, London, *Org. Geochem.*, **6**, 217-222.
- Braut M., B. R. T. Simoneit, J. C. Marty and A. Saliot (1988). Hydrocarbons in waters and particulate material from hydrothermal environments at the East Pacific Rise, 13°N. *Org. Geochem.*, **12**, 209-219.
- Culkin F. and R. J. Morris (1969). The fatty acids of some marine crustaceans. *Deep-Sea Res.*, **16**, 109-116.
- Curry J. R. et al. [Shipboard Scientific Party] (1982). Guaymas Basin: Sites 477, 478, and 481. In: *Initial Repts Deep Sea Drilling Proj.*, Vol. 64, Part I, J. R. Curry, D. G. Moore et al., editors, U.S. Government Printing Office, Washington, D.C., 211-415.
- De Rosa M., A. Gambacorta, L. Minale and J. D. Bu'Lock (1971). Bacterial triterpenes. *Chem. Commun.*, 619-620.
- Ensminger A. (1977). Évolution de composés polycycliques sédimentaires. *Thèse Doctorat ès Sciences, Université Louis Pasteur, Strasbourg*.
- Gagosian R. B. and J. W. Farrington (1978). Sterenes in surface sediments from the southwest African shelf and slope. *Geochim. cosmochim. Acta*, **42**, 1091-1101.
- Gagosian R. B., S. O. Smith, C. Lee, J. W. Farrington and N. M. Frew (1980). Steroid transformations in Recent marine sediments. In: *Advances in Organic Geochemistry 1979*, A. G. Douglas and J. R. Maxwell, editors, Pergamon Press, Oxford, 407-419.
- Gelpi E., H. Schneider, J. Mann and J. Oro (1970). Hydrocarbons of geochemical significance in microscopic algae. *Phytochemistry*, **9**, 603-612.
- Goodwin N. S., P. J. D. Park and A. P. Rawlinson (1983). Crude oil biodegradation under simulated and natural conditions. In: *Advances in Organic Geochemistry 1981*, M. Bjorøy et al., editors, J. Wiley and Sons, Ltd., Chichester, 650-659.
- Han J. and M. Calvin (1969). Hydrocarbon distribution of algae and bacteria and microbiological activity in sediments. *Proc. natn. Acad. Sci. USA*, **64**, 436-443.
- Haymon R. M., R. A. Koski and C. Sinclair (1984). Fossils of hydrothermal vent worms from Cretaceous sulfide ores of the Samail Ophiolite, Oman. *Science*, **223**, 1407-1409.
- Hedberg H. D. (1968). Significance of high-wax oils with respect to genesis of petroleum. *Am. Ass. Petrol. Geol. Bull.*, **52**, 736-750.
- Howard D. L., B. R. T. Simoneit and D. J. Chapman (1984). Triterpenoids from lipids of *Rhodocyclidium vannielii*. *Arch. Mikrobiol.*, **137**, 200-204.
- Kawka O. E. and B. R. T. Simoneit (1987). Survey of hydrothermally-generated petroleum from the Guaymas Basin spreading center. *Org. Geochem.*, **11**, 311-328.
- Kissin Y. V. (1987). Catagenesis and composition of petroleum: Origin of *n*-alkanes and *iso*-alkanes in petroleum crudes. *Geochim. cosmochim. Acta*, **51**, 2445-2457.
- LaFlamme R. E. and R. A. Hites (1978). The global distribution of polycyclic aromatic hydrocarbons in recent sediments. *Geochim. cosmochim. Acta*, **42**, 289-304.
- Lee R. F., J. Hirota and A. M. Barnett (1971). Distribution and importance of wax esters in marine copepods and other zooplankton. *Deep-Sea Res.*, **18**, 1147-1165.
- Mackenzie A. S., R. L. Patience and J. R. Maxwell (1980). Molecular parameters of maturation in the Toarcian shales, Paris Basin, France. I: Changes in the configurations of acyclic isoprenoid alkanes, steranes and triterpanes. *Geochim. cosmochim. Acta*, **44**, 1709-1721.
- Mackenzie A. S., S. C. Brassell, G. Eglinton and J. R. Maxwell (1982). Chemical fossils: the geological fate of steroids. *Science*, **217**, 491-504.
- Morris R. J. and F. Culkin (1977). Marine lipids: Sterols. *Oceanogr. mar. Biol. a. Rev.*, **15**, 73-102.
- Oudin E. and G. Constantinou (1984). Black smoker chimney fragments in Cyprus sulphide deposits. *Nature*, **308**, 349-352.
- Ouirsson G., P. Albrecht and M. Rohmer (1979). Palaeochemistry and biochemistry of a group of natural products. *Pure appl. Chem.*, **51**, 709-729.
- Perry G. J., J. K. Volkman, R. B. Johns and H. J. Bavor Jr. (1979). Fatty acids of bacterial origin in contemporary marine sediments. *Geochim. cosmochim. Acta*, **43**, 1715-1725.
- Prahl F. G. and G. Eglinton (1985). Faecal lipids released by fish feeding on zooplankton. *J. mar. biol. Ass. U.K.*, **65**, 547-560.
- Radke M. and D. H. Welte (1983). The methylphenanthrene index (MPI): A maturity parameter based on aromatic hydrocarbons. In: *Advances in Organic Geochemistry 1981*, M. Bjorøy et al., editors, J. Wiley and Sons, Ltd, Chichester, 504-512.
- Rona P. A. (1973). Plate tectonics and mineral resources. *Scient. Am.*, **229**, 1, 86-95.
- Rona P. A., G. Thompson, M. J. Mottl, J. A. Karson, W. J. Jenkins, D. Graham, M. Mallette, K. Von Damm and J. M. Edmond (1984). Hydrothermal activity at the Trans-Atlantic Geotraverse hydrothermal field, Mid-Atlantic Ridge Crest at 26°N. *J. Geophys. Res.*, **89**, 11365-11377.
- Saliot A. (1981). Natural hydrocarbons in sea water. In: *Marine Organic Chemistry*, E. K. Duursma and R. Dawson, editors, Elsevier, Amsterdam, 327-374.
- Scott S. D. (1985). Seafloor polymetallic sulfide deposits: Modern and ancient. *Mar. Min.*, **5**, 191-212.
- Seifert W. K. and J. M. Moldowan (1978). Applications of steranes, terpanes and monoaromatics to the maturation, migration and source of crude oils. *Geochim. cosmochim. Acta*, **42**, 77-95.
- Simoneit B. R. T. (1983). Organic Matter Maturation and Petroleum Genesis: Geothermal versus Hydrothermal. In: *The Role of Heat in the Development of Energy and Mineral Resources in the Northern Basin and Range Province*, Geotherm. Res. Council, special Report No. 13, Davis, California, 215-241.

- Simoneit B. R. T. (1984). Hydrothermal effects on organic matter - high vs. low temperature components. In: *Advances in Organic Geochemistry* 1983, P. A. Schenck, J. W. DeLeeuw and J. W. Lijmbach, editors, Pergamon Press, London, *Org. Geochem.*, 857-864.
- Simoneit B. R. T. (1985). Hydrothermal petroleum: Genesis, migration and deposition in Guaymas Basin, Gulf of California. *Can. J. Earth Sci.*, 22, 1919-1929.
- Simoneit B. R. T. (1988). Petroleum generation in submarine hydrothermal systems: an update. *Can. Mineralogist*, 26, 827-840.
- Simoneit B. R. T. and P. F. Lonsdale (1982). Hydrothermal petroleum in mineralized mounds at the seabed of Guaymas Basin. *Nature*, 295, 198-202.
- Simoneit B. R. T., R. P. Philp, P. D. Jenden and E. M. Galimov (1984). Organic geochemistry of Deep Sea Drilling Project sediments from the Gulf of California. Hydrothermal effects on unconsolidated diatom ooze. *Org. Geochem.*, 7, 173-205.
- Simoneit B. R. T., J. O. Grimalt, J. M. Hayes and H. Hartman (1987). Low temperature hydrothermal maturation of organic matter in sediments from the Atlantis II Deep, Red. Sea. *Geochim. cosmochim. Acta*, 51, 879-894.
- Spieß F. N., K. C. Macdonald, T. Atwater, R. Ballard, A. Carranza, D. Cordoba, C. Cox, V. M. Diaz Garcia, J. Francheteau, J. Guerrero, J. Hawkins, R. Haymon, R. Hessler, T. Juteau, M. Kastner, R. Larson, B. Luyendyk, J. D. Macdougall, S. Miller, W. Normark, J. Orcutt and C. Rangin (1980). East Pacific Rise: Hot springs and geophysical experiments. *Science*, 207, 1421-1433.
- Thompson G. M. J. Mottl and P. A. Rona (1985). Morphology, mineralogy and chemistry of hydrothermal deposits from the TAG area, 26°N Mid-Atlantic Ridge. *Chem. Geol.*, 49, 243-257.
- Tissot B., R. Pelet, J. Roucaché and A. Combaz (1977). Utilisation des alcanes comme fossiles géochimiques indicateurs des environnements géologiques. In: *Advances in Organic Geochemistry* 1975, R. Campos and J. Goñi, editors, ENADIMSA, Madrid, 117-154.
- Volkman J. K. (1986). A review of sterol markers for marine and terrigenous organic matter. *Org. Geochem.*, 9, 83-99.
- Volkman J. K., R. B. Johns, F. T. Gillan, G. J. Perry and H. J. Bavor Jr. (1980). Microbial lipids of an intertidal sediment. I: Fatty acids and hydrocarbons. *Geochim. cosmochim. Acta*, 44, 1133-1143.
- Wakeham S. G. (1987). Steroid geochemistry in the oxygen minimum zone of the eastern tropical North Pacific Ocean. *Geochim. cosmochim. Acta*, 51, 3051-3069.
- Wakeham S. G. and E. A. Canuel (1986). Lipid composition of the pelagic crab *Pleuroncodes planipes*, its feces, and sinking particulate organic matter in the Equatorial North Pacific Ocean. *Org. Geochem.*, 9, 331-343.