

Algal and archaeal polyisoprenoids in a recent marine sediment: Molecular isotopic evidence for anaerobic oxidation of methane

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[1] **Abstract:** Analyses of ^{13}C contents of individual organic molecules in a marine sediment show that crocetane, 2,6,11,15-tetramethylhexadecane, an isomer of phytane, is produced by microorganisms that use methane as their main source of carbon. The sediments lie at a water depth of 68 m in the Kattegat, the strait between Denmark and Sweden. Crocetane appears first 185 cm below the sediment-water interface, in the zone marking the transition from sulfate reduction to methanogenesis. Its $\delta^{13}\text{C}$ value is $-90 \pm 10\text{‰}$ versus Vienna Pee Dee Belemnite (VPDB). Its structure, which includes four isoprene units arranged symmetrically around a tail-to-tail linkage, suggests that it is produced by a member of the archaea. Growing at the intersection of the diffusion gradients for sulfate and methane in sedimentary pore waters, the source organism apparently functions as a methane-consuming member of the microbial consortium responsible for the anaerobic oxidation of methane [Hoehler *et al.*, 1994], in which, as first demonstrated quantitatively in these sediments [Iversen and Jørgensen, 1985], electrons are transferred from methane to sulfate. The presence of archaeal biomass throughout the sediment section is indicated by significant concentrations of 2,6,10,15,19-pentamethylcosane (PMI) and of ether-bound phytane and biphytane. The PMI reaches a minimum δ value of -47‰ well below the transition zone. Its isotopic depletion could reflect either methanogenic or methanotrophic sources. The ether-bound lipids are isotopically uniform throughout the section and are presumed to derive from archaea that utilize a carbon source unaffected by the oxidation of methane.

Keywords: Anaerobic oxidation of methane; methane; crocetane; carbon-13; methanotrophy; Kattegat.

Index terms: Low-temperature geochemistry; carbon cycling; gases; microbiology.

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1. Introduction

[2] In the 1970s, studies showed clearly that methane produced by bacteria in anaerobic, sulfate-free portions of sediment columns indeed diffused upward but was consumed at the base of the sulfate-reducing zone, well before it reached oxic waters [Barnes and Goldberg, 1976; Reeburgh, 1976; Martens and Berner, 1977; Reeburgh and Heggie, 1977]. A few years later, isotopic analyses in sediments from Skan Bay, Alaska, showed that methane dissolved in pore waters at the base of the sulfate-reducing zone is enriched in ^{13}C and that dissolved inorganic carbon (DIC) is correspondingly depleted in ^{13}C . This pattern is

indicative of (1) the consumption of CH_4 by a process with a normal kinetic isotope effect and (2) the release of the carbon to the DIC pool [Reeburgh, 1980]. Indications that methane can be oxidized by anaerobic bacteria were unexpected, or even “impossible,” because all methanotrophic bacteria ever isolated in pure culture are obligate aerobes.

[3] Anaerobic methanotrophs have still not been isolated, but evidence for their existence has become compelling. Experiments with ^{14}C -labeled CH_4 demonstrate that the rate of oxidation maximizes in a narrow band at the base of the sulfate-reduction zone and that the overall process involves a transfer of electrons from

methane to sulfate [Devol and Ahmed, 1981; Devol, 1983; Iversen and Jørgensen, 1985]. The transfer is probably mediated by a microbial consortium that includes either a methanogen (operating in reverse) or other archaeon together with a sulfate-reducer [Hoehler et al., 1994; Hinrichs et al., 1999; Boetius et al., 2000]. The overall process is clearly exergonic, but the identity of the substrate (e.g., acetate or H₂) that is transferred from the methane-consuming organism to the sulfate reducer and thereby facilitates thermodynamic control is unknown [Hoehler et al., 1994; Hoehler and Alperin, 1996; Valentine and Reeburgh, 2000].

[4] Molecular and isotopic signals related to the anaerobic oxidation of methane are now providing more information. Archaeal biomarkers found at modern [Hinrichs et al., 1999; Elvert et al., 1999; Pancost et al., 2000] and ancient methane seeps [Thiel et al., 1999; Peckmann et al., 1999] are highly depleted in ¹³C, thus indicating the utilization of methane-derived carbon and strongly suggesting that archaea are directly involved in the oxidation of methane. The structures and abundances of the archaeal biomarkers vary significantly between sites, suggesting that diverse communities can be involved in anaerobic methanotrophy. Here the search for molecular and isotopic signals related to anaerobic methanotrophy is extended to the transition zone, the depth interval in normal, nonseep sediments at which sulfate concentrations decline as those of methane rise. In terms of global area this transition zone must be the predominant setting for the anaerobic oxidation of methane in marine environments. Of the 85 Tg of CH₄ produced annually in marine sediments, all but 10 Tg are consumed before they reach the atmosphere, mostly by anaerobic oxidative processes [Reeburgh, 1996]. This consumption of 75 Tg/yr is almost twice the annual increase in the atmospheric inventory of CH₄ (40 Tg/yr).

[5] In search of specific evidence for the organisms responsible for methane oxidation, we studied the carbon isotopic compositions of biomarkers extracted from a sediment core from the Kattegat strait, between Denmark and Sweden. Methane budgets for these sediments are well documented [Laier et al., 1996]. Methane in fine-grained sediments is of microbial origin [Laier et al., 1996] and is oxidized anaerobically before reaching the seafloor [Iversen and Jørgensen, 1985]. The principal objective was to examine the molecular and isotopic characteristics of individual organic compounds in the sediments in a targeted search for components possibly produced by microorganisms that mediate the anaerobic oxidation of methane.

2. Samples and Experimental Methods

[6] Samples were collected in May 1990, during a cruise of R/V *Gunnar Thorson*. Sediments were recovered in piston cores up to 2.5 m long at station K3 (57°49.7'N, 11°13.1'E; 68-m water depth) in the Kattegat strait, between Denmark and Sweden. Closely related sites include station C (58°04.6'N, 10°04.0'E) of Iversen and Jørgensen [1985], station 10 (57°50'N, 11°16'E) of Jørgensen et al. [1990], and station PC129 (57°49.10'N, 11°10.05'E) of Laier et al. [1996]. At station 10 a sediment accumulation rate of 6.2 mm/yr was measured [Jørgensen et al., 1990]. From station K3, three cores (1, 11, and 14) have been used for biogeochemical studies. Concentrations of methane were determined for all three cores, whereas those of sulfate were measured in only cores 1 and 11. Rates of methane oxidation were measured in core 1. All analyses employed methods described by Iversen and Jørgensen [1985]. The upper 30 cm of core 14 was lost, but 5-cm segments extending from well above to well below the transition zone were frozen and preserved for

analyses of individual lipid components. Samples were received, still frozen, at Indiana University in October 1990. They were stored at -9°C for 1 year, then thawed and promptly extracted. Depths of biochemical zones (sulfate reduction, methanogenesis, and transition zone) for the three cores were aligned on the basis of concentration profiles for CH_4 . The transition zone was between 165- and 200-cm depth. To focus on this significant feature and to provide points representative of other zones, lipid extracts were prepared from depths of 40, 80, 120, 160, 170, 175, 180, 185, 190, 195, 220, and 245 cm.

2.1. Chemical Procedures

2.1.1. Concentration and Isotopic Composition of Total Organic Carbon

[7] Dried sediment samples (~ 5 mg) were treated overnight with 3 N HCl at room temperature. The samples were neutralized by repeated washing with H_2O ($\sim 5\times$) and then dried at 70°C . The decalcified sediment was then combusted in a sealed quartz tube at 850°C for >8 hours to produce CO_2 for isotopic analysis. Samples of CO_2 were analyzed using a Finnigan MAT 252 isotope-ratio mass spectrometer. Carbon isotopic compositions are expressed in terms of $\delta^{13}\text{C}$ relative to the Vienna Pee Dee Belemite (VPDB) standard. In this report, $\delta^{13}\text{C}$ is shortened to δ for simplicity in notation because only carbon isotopic abundances are considered. Concentrations of total organic carbon (TOC, wt %) were calculated from the yields of CO_2 .

2.1.2. Extraction of Soluble Organic Compounds

[8] Samples of wet sediment were extracted with dichloromethane/methanol (2:1, vol/vol; Soxhlet extractor; 48 hours). Preextracted copper particles were added to the extraction vessels to remove elemental sulfur. A 5%

solution of NaCl was used to wash the extract (2×20 mL). The organic phase was dried over anhydrous Na_2SO_4 overnight. Excess solvent was removed by rotary evaporation at $<30^{\circ}\text{C}$.

2.1.3. Preparation of Lipid Fractions

[9] The extract was separated into hexane-soluble and hexane-insoluble fractions (the latter containing the polar lipids). The hexane-soluble fraction was separated by column chromatography to yield fractions of aliphatic hydrocarbons, aromatic hydrocarbons, wax esters, triglycerides and ketones, alcohols, sterols, and polar compounds. Branched and cyclic hydrocarbons were isolated from the aliphatic hydrocarbon fraction by adduction of *n*-alkanes in a molecular sieve (5 Å, activated overnight at 310°C) using cyclohexane as the solvent (24-hour reflux). The *n*-alkanes were recovered from the sieve by refluxing with *n*-hexane for 48 hours.

[10] The wax-ester fraction was saponified using 5 mL of 0.5 N KOH in CH_3OH and 1 mL H_2O in a sealed culture tube (10 mL) at 100°C for 2 hours. Neutral and acidic components were separated by sequentially extracting the hydrolysate with hexane (4×3 mL) at pH >13 and pH <2 , using HCl and KOH to adjust the pH. The acidic lipids were methylated with 500 μL of a solution of 3% BF_3 in CH_3OH at 100°C for 2 hours. The reaction mixture was transferred to a 10-mL culture tube by rinsing with methanol (3×100 μL) and then hexane (3×100 μL). After adding 3–4 mL of 5% NaCl solution the mixture was extracted with hexane (3×1 –2 mL).

[11] Trimethylsilyl ethers of sterols were prepared using a mixture of 100 μL of BSTFA [N,O-bis(trimethylsilyl)trifluoroacetamide] and 100 μL acetonitrile heated at 70°C for 2 hours.

2.1.4. Derivatization of Polar Lipids

[12] Procedures were modified after *Panganamala et al.* [1971]. The polar-lipid fraction was hydrolyzed with 4 mL of 47% HI at 90°C for 12 hours. The reaction products were transferred to a separatory funnel and extracted with hexane (3 × 10 mL). The extract was washed successively with 0.1 M K₂CO₃ solution (20 mL) and 10% NaCl solution (2 × 20 mL). Alkyl iodides were obtained by extraction with *n*-hexane. Alkyl iodides were reacted with 100 mg of zinc powder and 3 mL of glacial acetic acid in sealed 10-mL culture tubes at 90°C for 45 min. The reaction mixture was extracted with three 10-mL portions of *n*-hexane. The combined extracts were washed with 0.1 M aqueous K₂CO₃ (2 × 10 mL) and H₂O (2 × 10 mL), then dried over Na₂SO₄.

2.2. Gas Chromatography and Gas Chromatography–Mass Spectrometry

[13] Samples were analyzed using a Hewlett-Packard 5890 gas chromatograph (GC) equipped with a 50 m × 0.32 mm ID, 0.5- μ m film thickness, Ultra-1 column, and an FID detector. Typically, the column temperature was programmed from 70° to 320°C at a rate of 5°/min, then held at 320° for 30 min. Helium was used as the carrier gas, typically at a flow rate of 1.6 mL/min. Branched and cyclic alkanes were analyzed using a Finnigan-MAT TSQ 700 coupled with a HP 5890 GC (on-column injection). A DB-5 column (30 m × 0.25 mm ID, 0.5- μ m film thickness) was used, and the temperature was programmed from 80° to 300°C at 8°/min, then held at 300°C for 30 min. All spectra were recorded at an ionization energy of 70 eV. Sterols were analyzed by GC-MS using a Finnigan-MAT Inco 50 coupled with a Varian 3400 GC (splitless injection). The temperature of the Ultra-1 column (25 m × 0.2 mm ID, 0.18- μ m film thickness) was programmed from 80° to 300°C at 5°/min, then held at 300° for 30 min.

[14] Individual biomarkers were identified by reference to published mass spectra and from GC retention times. The assignment of crocetane was confirmed by coinjection with a synthetic standard that was prepared locally [cf. *Robson and Rowland*, 1993].

2.2.1. Carbon Isotopic Analysis of Individual Compounds

[15] Carbon isotopic compositions were determined using isotope ratio monitoring GCMS techniques (irmGCMS) [*Matthews and Hayes*, 1978; *Hayes et al.*, 1990; *Merritt et al.*, 1995]. The GC (HP 5890 Series II) was equipped with an Ultra-1 column (50 m × 0.32 mm ID, 0.5- μ m film thickness) and an on-column injector. The oven was programmed from 60° to 320°C at a rate of 3°/min, followed by a final hold time of 40–50 min. Perdeuterated *n*-alkanes (C₁₆, C₂₀, C₂₄, and C₃₆) were used as internal isotopic standards. Isotopic compositions were obtained for most compounds except for those that were of low abundance and/or poorly resolved from adjacent components. Values of δ reported for sterols and for carboxylic acids have been corrected for introduction of additional carbon atoms during silylation and methylation, respectively.

3. Results and Discussion

3.1. Bulk Analyses of Cores

[16] Concentrations of sulfate in pore waters decrease only slightly, from ~25 to ~23 μ mol/mL, in the upper meter of sediment (Figure 1). They decrease sharply below 1 m, and no sulfate was detectable at 204 cm. Concentrations of methane are less than 20 nmol/mL in the upper 130 cm of sediment, then increase by more than tenfold, reaching 244 nmol/mL at 170 cm. Reliable measurements could only be made to a depth of 195 cm, where the methane concentration exceeded 1.3 μ mol/mL. At station PC129, *Laier et al.* [1996]

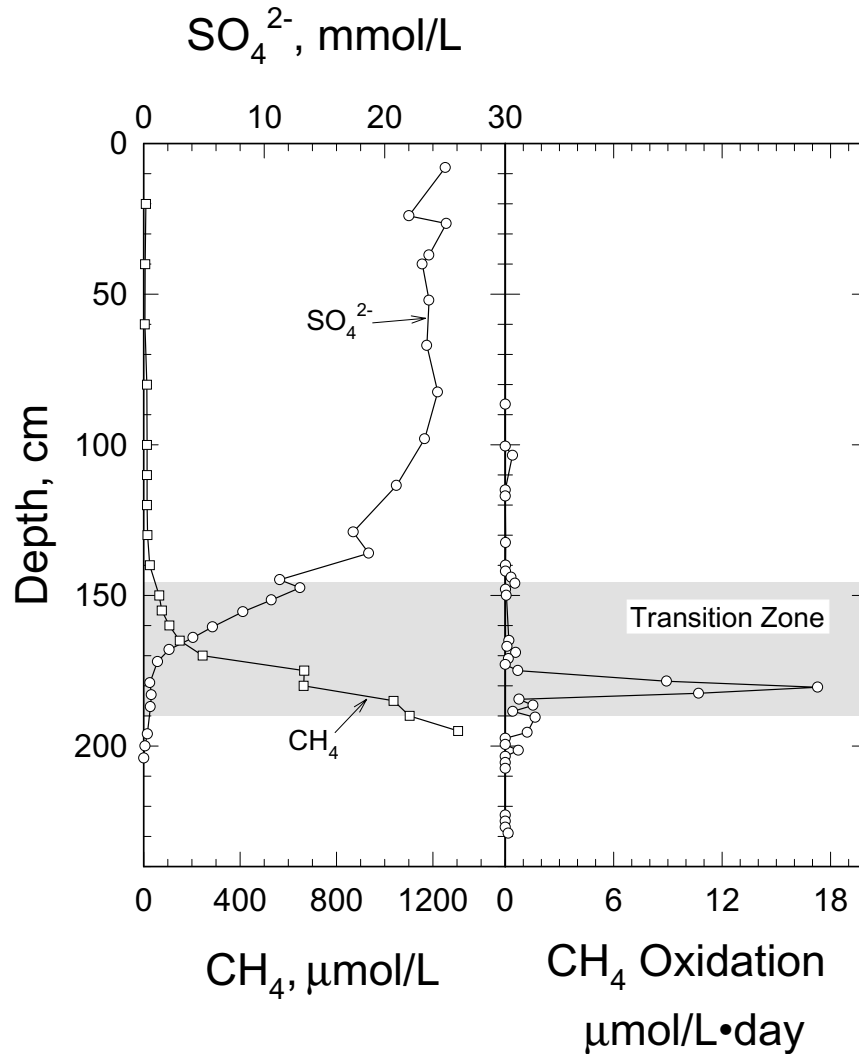


Figure 1. Concentrations of dissolved methane and sulfate in pore waters and rates of oxidation of methane per liter of sediment versus depth in sediment, station K3, Kattegat.

reported 14 $\mu\text{mol/mL}$ at 220-cm depth and calculated that saturation at ambient conditions corresponded to 9 $\mu\text{mol/mL}$. They concluded that a separate gas phase was probably present below 200 cm in the sediment and noted that its existence had been observed in acoustic profiles. As shown in Figure 1, rates of oxidation of methane were less than 0.6 $\text{nmol}/(\text{mL d})$ in sediments above 173 and below 198 cm but maximize in a 20-cm band between 175- and 195-cm depth, attaining a value of 17.3 $\text{nmol}/$

(mL d). At station C, *Iversen and Jørgensen* [1985] found a maximum rate of methane oxidation of 12.1 $\text{nmol}/(\text{mL d})$. Significant concentrations of sulfate and methane coexisted at depths between 145 and 190 cm.

[17] As shown in Figure 2, the concentration of total organic carbon (TOC) ranges between 1.1 and 1.3% in the uppermost sediment and decreases downcore to values between 0.8 and 1%. Isotopic compositions of TOC range

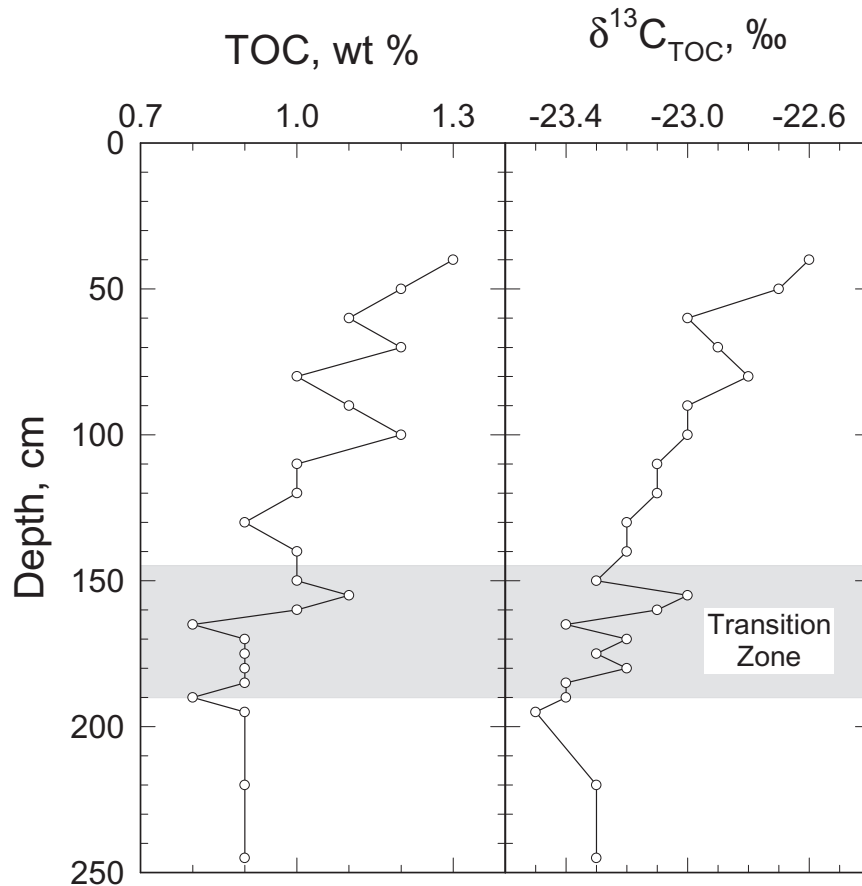


Figure 2. Concentration and isotopic composition of total organic carbon versus depth in sediment, station K3, Kattegat.

from -23.5 to -22.5‰ , consistent with the mixing of organic material from marine and terrestrial sources [e.g., Jasper and Gagosian, 1990; Prahl *et al.*, 1994].

3.2. *n*-Alkanes

[18] The distribution of *n*-alkanes varies little with depth. Odd-numbered C_{25} – C_{31} homologues, indicative of waxes produced by higher land plants [Eglinton and Hamilton, 1963], are strongly predominant. The carbon-preference index [Bray and Evans, 1961] ranges between 3.4 and 4.5 at sediment depths between 40 and 245 cm. The δ values of long-chain *n*-alkanes (C_{25} – C_{31}) range from -32.4 to -30.8‰

(Table 1), similar to those of leaf-wax alkanes from Ellesmere sediments [Rieley *et al.*, 1991] and of alkanes from plants that fix carbon using the C_3 pathway [Collister *et al.*, 1994]. They average -31.4‰ , which is 2‰ lighter than the C_{17} – C_{21} *n*-alkanes. The slight enrichment of ^{13}C in the C_{17} – C_{21} *n*-alkanes is consistent with presumed contributions from phytoplankton [Han and Calvin, 1969; Gelpi *et al.*, 1970; Schouten *et al.*, 1998].

3.3. Sterols

[19] Distributions of sterols are similar throughout the profile, indicating no marked change in sources over the depositional record. They are

Table 1. Isotopic Compositions of Individual *n*-Alkanes^a

Carbon Number	δ , ‰	Range	
		Minimum	Maximum
17	-28.8 ± 0.8	-29.3	-27.5
18	-29.4 ± 0.4	-30.1	-29.0
19	-29.2 ± 0.7	-30.0	-28.3
20	-29.7 ± 0.4	-30.2	-29.3
21	-29.6 ± 0.3	-29.9	-29.2
22	-31.1 ± 0.6	-32.1	-30.4
23	-30.2 ± 0.2	-30.5	-29.8
24	-31.1 ± 0.6	-31.8	-30.2
25	-30.8 ± 0.4	-31.2	-30.3
26	-31.1 ± 0.5	-31.6	-30.1
27	-31.5 ± 0.2	-31.8	-31.2
28	-31.0 ± 0.9	-31.4	-30.3
29	-31.8 ± 0.6	-32.2	-30.8
30	-31.2 ± 0.5	-32.0	-30.6
31	-32.0 ± 1.1	-33.2	-31.0
32	-32.2 ± 0.7	-33.1	-31.2
33	-31.1 ± 0.9	-32.2	-29.8

^aIsotopic compositions are invariant with depth. Tabulated values of δ are averages and standard deviations. Samples from depths of 40, 80, 120, 185, 220, and 245 cm.

dominated by two stenol-stanol pairs, cholest-5-en-3 β -ol with 5 α -cholestan-3 β -ol and 24-ethylcholest-5-en-3 β -ol with 24-ethyl-5 α -cholestan-3 β -ol. All of the sterols identified in the sediments (Table 2) are important components in various marine algae (Volkman *et al.*, 1998) and occur widely in contemporary marine sediments. The average δ values, determined from a set of five samples (40, 80, 120, 190, and 245 cm), range from -25.8 to -24.1 ‰ with little downcore variation. This range is consistent with reported isotopic compositions of sterols derived from marine algae from a variety of study areas [e.g., Pancost *et al.*, 1997]. On the basis of structural and isotopic evidence, therefore the sterols are attributed to planktonic sources. The sterols are enriched in ^{13}C by 3 to 4‰ relative to the C_{17n} -alkane. This difference agrees well with recent observations of isotopic fractionations associated with algal biosyntheses [Schouten *et al.*, 1998] and supports assignment of the C_{17n} -alkane to algal sources.

3.4. Branched and Cyclic Hydrocarbons

[20] The distributions of branched and cyclic hydrocarbons are typical of recent marine sediments. A representative chromatogram is shown in Figure 3. The unresolved complex mixture (UCM; i.e., the hump in the baseline) in the gas chromatogram is typical of contemporary sediments containing contributions of hydrocarbons derived from petroliferous sources [Brassell *et al.*, 1978; Brassell and Eglinton, 1980; Barrick and Hedges, 1981]. A minor UCM of comparable size occurs in sediments from the Baltic Sea [Pihlaja *et al.*, 1990], accompanied by a predominance of biogenic hydrocarbons from both autochthonous and terrigenous origins. The hopanes in sediments from the Kattegat strait also derive from a combination of natural and anthropogenic sources. Several components are typical of crude oils, including 18 α -22,29,30-trisnor-hopane (peak 7 in Figure 3; Table 2), 17 β -22,29,30-trisnor-hopane (peak 8), 17 α ,21 β -30-nor-hopane (peak 9), and 17 α ,21 β -hopane (peak 11), whereas others are biogenic, namely, 22R-17 α ,21 β -homo-hopane (peak 14) and 17 β ,21 β -hopane (peak 15). Moreover, the isotopic compositions of the hopanes help to distinguish these origins. All of the petroliferous hopanes are significantly depleted in ^{13}C (Table 2) relative to the isoprenoidal compounds of phytoplanktonic origin (i.e., the sterols), whereas the δ value of 22R-17 α ,21 β -homo-hopane (Table 2) is comparable. These triterpenoids provide no information on microbial processes within the sediment column and are not further discussed.

3.5. Acyclic Isoprenoid Alkanes

[21] 2,6,10-Trimethyl-7-(3-methylbutyl)-dodecane (peak 1 in Figure 3), which elutes just before pristane, was present in all samples. It was identified based on its mass spectrum and relative retention time [Barrick *et al.*, 1980; Yon *et al.*, 1982]. It has the highest δ values found

Table 2. Isotopic Compositions of Individual Biomarkers

Peak ^a	Compound	δ , ^b ‰	<i>n</i>	δ Range, ‰	
				Minimum	Maximum
Sterols ^c					
	cholesta-5,22-dien-3 β -ol	-24.8 ± 1.0	2	-25.5	-24.0
	5 α (H)-cholest-22-en-3 β -ol	-24.6 ± 0.6	2	-25.0	-24.2
	cholest-5-en-3 β -ol	-24.2 ± 0.8	5	-25.2	-23.3
	5 α (H)-cholestan-3 β -ol	-24.4 ± 0.6	5	-25.3	-23.8
	24-methylcholesta-5,22-dien-3 β -ol	-24.1 ± 0.8	5	-25.0	-23.0
	24-methyl-5 α (H)-cholest-22-en-3 β -ol	-25.8 ± 1.1	5	-26.8	-24.3
	24-ethylcholest-5-en-3 β -ol	-24.6 ± 0.4	5	-25.4	-24.0
	24-ethyl-5 α (H)-cholestan-3 β -ol	-24.6 ± 0.6	5	-25.0	-24.0
Acyclic isoprenoids, ^d diterpane, de-A triterpane					
1	2,6,10-trimethyl-7-(3-methylbutyl)-dodecane	-15.3 ± 0.4	4	-15.6	-14.6
2	pristane	-32.0 ± 0.4	11	-32.6	-31.5
3	C ₂₀ isoprenoid alkanes ^e	variable ^f	11	-29.8	-78.2
4	tricyclic diterpane (C ₂₀ H ₃₆)	n.d.			
5	2,6,10,15,19-pentamethylcosane	variable ^f	10	-30.0	-47.3
6	des-A-lupane	n.d.			
Hopanes ^g					
7	18 α -22,29,30- <i>trisor</i> -neohopane	-31.6 ± 0.8	7	-32.8	-30.7
8	17 β -22,29,30- <i>trisor</i> -hopane	-31.6 ± 0.7	8	-32.4	-30.4
9	17 α ,21 β -30- <i>nor</i> -hopane	-31.5 ± 0.9	8	-32.8	-30.2
10	17 β ,21 α -30- <i>nor</i> -hopane	-31.4 ± 0.7	8	-32.3	-30.4
11	17 α ,21 β -hopane	-31.3 ± 0.8	8	-32.5	-30.3
12	17 β ,21 α -hopane	-32.2 ± 0.9	8	-33.2	-30.9
13	22S-17 α ,21 β - <i>homo</i> -hopane	n.d.			
14	22R-17 α ,21 β - <i>homo</i> -hopane	-26.2 ± 0.5	8	-26.6	-25.5
15	17 β ,21 β -hopane	n.d.			
16	17 β ,21 β - <i>homo</i> -hopane	n.d.			
17	17 β ,21 β - <i>dihomo</i> -hopane	n.d.			

^aPeaks designated in Figure 3.

^bTabulated values are averages and standard deviations; n.d., not determined

^cSterols analyzed at depths of 40, 80, 120, 190, and 245 cm.

^dAcyclic isoprenoid alkanes analyzed at all depths.

^eHere 40–180 cm phytane; 185–245 cm phytane + crocetane.

^fSee Table 3.

^gHopanes analyzed at depths of 40, 80, 120, 170, 185, 190, 220, and 245 cm.

among all compounds in this study (Table 2). Its isotopic composition varies little with depth, suggesting consistent derivation from an isotopically exotic source. This highly branched isoprenoid was first found in Eocene oils [Yon *et al.*, 1982; Punanov *et al.*, 1991]. It is widely distributed in surface sediments and is a constituent, together with alkene and C₂₅ homologs, in field specimens of the green alga *Enteromorpha prolifera* [Rowland *et al.*, 1985]. Hence these algae are suggested as a

source for such highly branched compounds in sediments. The anomalous ¹³C content immediately suggests use of HCO₃⁻ as a carbon source but may also be associated with rapid growth or a small ratio of surface area to cellular volume [Popp *et al.*, 1998].

[22] At sediment depths of 180 cm or less the δ values of pristane (2,6,10,14-tetramethylpentadecane) and phytane (2,6,10,14-tetramethylhexadecane) are uniform (Tables 2 and 3, Figure 4).

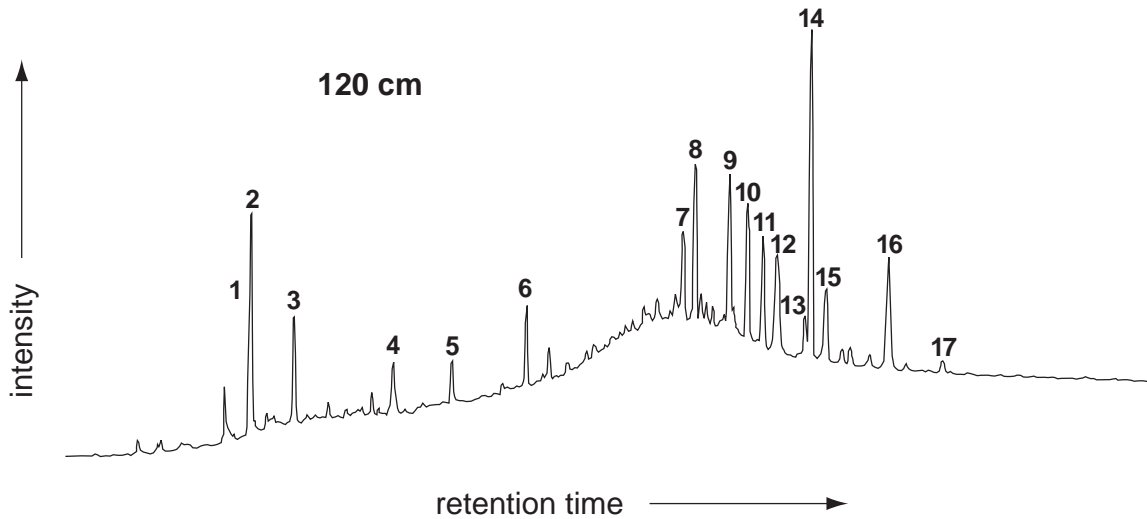


Figure 3. Gas chromatogram of branched and cyclic hydrocarbons from 120-cm depth in sediment column. The numbered peaks are identified in Table 2.

Those of phytane range from -31.8 to -29.8‰ and average $-30.8 \pm 0.4\text{‰}$ ($n = 6$), $\sim 1\text{‰}$ more positive than those of pristane in the same set of samples, which average $-32.0 \pm 0.2\text{‰}$. Both of these compounds can be generated diagenetically from the phytol side chain of chlorophyll [Brooks *et al.*, 1969; Didyk *et al.*, 1978]; pristane also derives from the side chain of tocopherols [Goossens *et al.*, 1984; ten Haven *et al.*, 1987]. Archaea are an important additional source [Holzer *et al.*, 1979; Tornabene *et al.*, 1979] of sedimentary phytane [Brassell *et al.*, 1981; Rowland, 1990].

[23] The carbon isotopic relationship between (1) pristane and phytane and (2) the sterols indicates that the acyclic isoprenoids must have multiple sources. In phytoplankton, phytol is commonly enriched in ^{13}C by $2\text{--}4\text{‰}$ relative to sterols [Bidigare *et al.*, 1997; Kuypers *et al.*, 1999] (but see also Schouten *et al.* [1998]). In these sediments, pristane and phytane are instead depleted in ^{13}C relative to sterols by 6‰ . Accordingly, only a small portion of pristane and phytane can derive from autochthonous planktonic biomass. The remainder

must come from mature organic material and/or terrestrial higher plants. The isotopic compositions of the pristane and phytane are consistent with either of these possibilities.

[24] The depletion of ^{13}C in pristane relative to phytane suggests that a small portion of the phytane was contributed by ^{13}C -enriched sources in the water column or sediment, such as algae or archaea. Notably, the phytane derived from cleavage of archaeal ether lipids (discussed below) is significantly enriched in ^{13}C .

3.6. C_{20} Isoprenoid Alkanes

[25] A subtle change in the shape of the GC peak for the C_{20} isoprenoid alkane at sediments depths below 185 cm is accompanied by sharply lower δ values (Table 3). At the same point, a double inflection appears in the continuously recorded 45/44 ion-current ratio, indicating the presence of multiple components. Mass spectra recorded continuously across the width of the C_{20} -isoprenoid peak indicate the presence of two compounds. Subtraction of summed spectra representative of the tail of the peak from spectra

Table 3. Biomarkers for Which Isotopic Compositions Vary With Depth^a

Depth, cm	$\Sigma C_{20} \text{isop}^b$ $\delta, \text{‰}$	Crocetane ^c		PMI ^d $\delta, \text{‰}$	Ether-Bound Carbon Skeletons	
		$\delta, \text{‰}$	f_{Cr}		Phytane $\delta, \text{‰}$	Biphtane $\delta, \text{‰}$
40	-29.8 ± 0.3	absent	0	abs.	-26.8 ± 0.5	abs.
80	-30.1 ± 0.1	absent	0	-30.4 ± 0.3	-26.1 ± 0.1	-26.4 ± 0.2
120	-30.0 ± 0.6	absent	0	-31.2 ± 0.3	-25.6 ± 0.3	-26.0 ± 0.3
170	-31.5 ± 0.1	absent	0	-30.0 ± 0.3	-27.3 ± 0.4	-26.4
175	-31.8 ± 0.5	absent	0	-30.8 ± 1.4	n.d.	n.d.
180	-31.9 ± 1.1	absent	0	-31.9 ± 0.8	n.d.	n.d.
185	-40.0 ± 0.5	-67.0 ± 4.7	0.25	-32.3 ± 0.6	n.d.	n.d.
190	-47.6 ± 0.5	-90.3 ± 5.5	0.28	-32.7 ± 0.5	-26.0 ± 0.4	-25.9 ± 1.4
195	-78.2 ± 0.6	-100.4 ± 3.0	0.68	-35.0 ± 0.9	-26.7 ± 0.5	-27.1
220	-40.1 ± 0.3	-84.5 ± 8.3	0.17	-36.0 ± 0.6	-28.6 ± 0.1	-27.4 ± 1.1
245	-52.6 ± 0.5	-91.0 ± 4.4	0.36	-47.3 ± 0.1	-28.7 ± 0.3	-27.1

^aHere n.d., not determined.

^bSum of C_{20} acyclic isoprenoid alkanes: 40–180 cm, phytane; 185–245 cm, phytane + crocetane.

^cIsotopic compositions of crocetane calculated from those of the C_{20} isoprenoid peaks and f_{Cr} , assuming $\delta_{Ph} = -31.0\text{‰}$. Values of f_{Cr} determined from mass spectra of C_{20} isoprenoid peaks, as described in text. Uncertainties in δ_{Cr} calculated by propagation of errors, assigning $\sigma_{\delta_{Ph}} = 1.0\text{‰}$ and estimating that the mass spectral peak intensities of which f_{Cr} is based were measured with a constant standard deviation equal to 2% of full scale.

^d2,6,10,15,19-pentamethylcosane

corresponding to the front yielded a spectrum identical with that of crocetane (2,6,11,15-tetramethylhexadecane [Robson and Rowland, 1993]). This assignment is consistent with the retention index of crocetane, which is slightly less than that of phytane [Robson and Rowland, 1993]. When authentic crocetane was coinjected with the sample, the leading edge of the C_{20} -isoprenoid peak was enhanced, but its mass spectrum was not changed, confirming the identification of the second component.

[26] The mixing of phytane and crocetane can be treated quantitatively, and, as a result, values of δ for the individual compounds can be obtained. If we let $f_{Cr} \equiv (\text{moles crocetane})/(\text{moles crocetane} + \text{moles phytane})$, the reasonable assumption that these closely related isomers possess equal ionization cross sections leads to

$$f_{Cr} = I_{Cr}/I = I_{Cr}/(I_{Cr} + I_{Ph}), \quad (1)$$

where I is used to designate reconstructed total ion currents due to crocetane (I_{Cr}) and to phytane (I_{Ph}) and the subscript Σ designates

reconstructed total ion current for the full width of the peak. Separate consideration of ion currents at mass 169, which occur in the spectra of both compounds but which are particularly intense in crocetane (corresponding to the fragment at m/z 183 in phytane [Robson and Rowland, 1993], leads to

$$^{169}i = ^{169}i_{Cr} + ^{169}i_{Ph}, \quad (2)$$

where I is used to designate specific ion currents that can be related to the total ion currents associated with each compound:

$$^{169}i_{Cr} = k_{Cr}I_{Cr} \text{ and } ^{169}i_{Ph} = k_{Ph}I_{Ph}, \quad (3a, b)$$

where k_{Cr} and k_{Ph} are constants characteristic of the individual mass spectra, with $k_{Cr} > k_{Ph}$. Substitution and division by I_{Σ} then yields

$$\begin{aligned} ^{169}i/I &= (k_{Cr}I_{Cr}/I) + (k_{Ph}I_{Ph}/I) \\ &= k_{Cr}f_{Cr} + k_{Ph}(1 - f_{Cr}). \end{aligned} \quad (4)$$

Rearrangement of (4) yields

$$f_{Cr} = [(^{169}i/I) - k_{Ph}]/(k_{Cr} - k_{Ph}) \quad (5)$$

and shows that f_{Cr} can be calculated from observed values of $^{169}i_{\Sigma}/I_{\Sigma}$, k_{Cr} , and k_{Ph} .

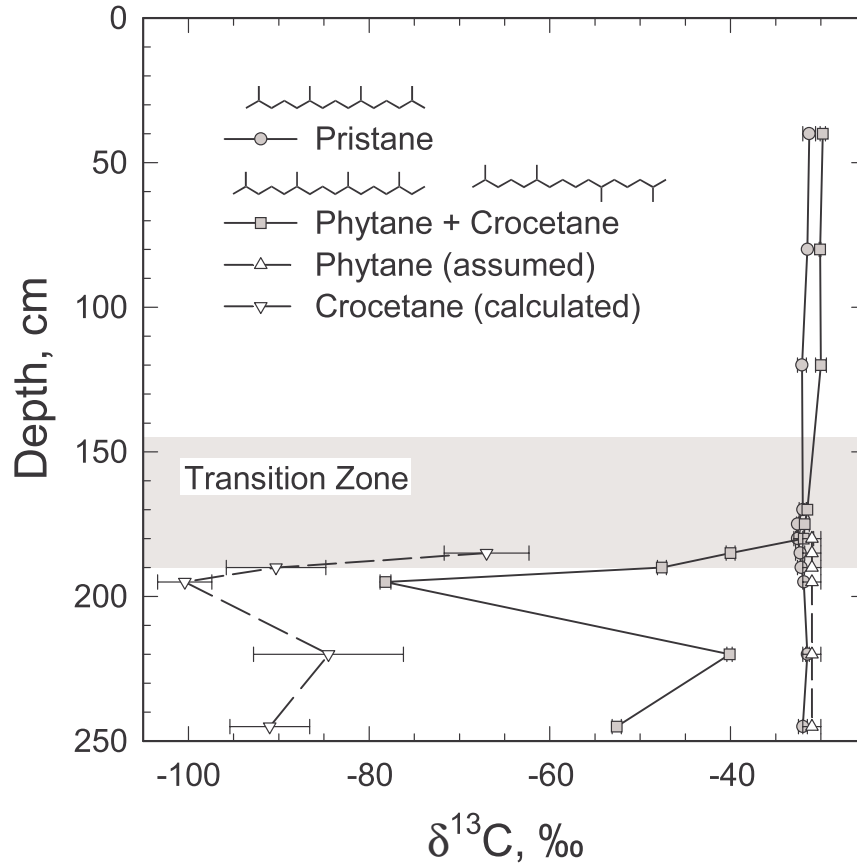


Figure 4. Carbon isotopic compositions of pristane and of the gas chromatographic peak containing both phytane and crocetane versus depth in sediment. The isotopic composition of the crocetane component was calculated as described in the text (equation (6) and accompanying discussion).

Results are summarized in the fourth column of Table 3.

[27] The correlation between δ values and composition of the C_{20} -isoprenoid peak is unequivocal. Mass spectra across the full width of the C_{20} -isoprenoid peak from sediment depths of 180 cm or less indicate that only phytane is present and yield δ values of about -31‰ . At 185 cm and below the mass spectra indicate the additional presence of crocetane ($f_{Cr} > 0$). For sediments in this zone the extent to which the δ value of the C_{20} -isoprenoid peak is decreased from -31‰ is roughly proportional to f_{Cr} .

[28] Crocetane consists of four isoprene units arranged symmetrically around a tail-to-tail linkage (cf. phytane: four isoprene units all joined head-to-tail). It was initially synthesized and named by *Karrer and Golde* [1930]. In an early systematic study of isoprenoid hydrocarbons, *McCarthy* [1967] recognized that the broad asymmetric GC peak corresponding to “phytane” in some Precambrian shale extracts actually contained at least two components. He even suggested that the extra component was probably crocetane based on coelution with a synthetic standard. However, this insight is recorded only in his thesis and appears never

to have reached the published literature. *Robson and Rowland* [1993] reported that crocetane was formed as a by-product during synthesis of 2,6,9,13-tetramethyltetradecane but identified no natural source. According to their study, crocetane and phytane can be separated gas chromatographically by use of a dimethyl silicone stationary phase (specifically OV-1, which yielded Kovats retention indices of 1792 and 1809 for crocetane and phytane, respectively). Neither the present investigation nor that of *Thiel et al.* [1999] was able to reproduce this separation on an equivalent column (HP-1). Well after completion of this work, it was reported to us that crocetane and phytane standards that we had supplied could be fully resolved by isothermal chromatography at 150°C on a DB-17 column (50% methyl, 50% phenyl silicone), 30 m × 0.32 mm ID, 0.5-μm film thickness (H. Alwan, J&W Scientific, private communication, 1994).

[29] The isotopic composition of the crocetane can be estimated even though the components of the C₂₀-isoprenoid peak have not been separated chromatographically. For each sample we can write

$$\delta_{\Sigma} = f_{Cr}\delta_{Cr} + (1 - f_{Cr})\delta_{Ph}, \quad (6)$$

where the δ values refer to the overall crocetane + phytane peak (δ_{Σ}) and to its crocetane (δ_{Cr}) and phytane (δ_{Ph}) components. The values of δ_{Cr} reported in Table 3 are based on the assumption that $\delta_{Ph} = -31.0 \pm 1.0\text{‰}$ at all depths below 180 cm (i.e., that, like other algal and terrestrial products in this core, its δ value does not vary by more than a few permil).

[30] The extreme depletion of ¹³C in crocetane indicates that this compound was produced by microorganisms that utilized methane as their carbon source. Crocetane has no known microbiological source. However, the prevalence of tail-to-tail-linked isoprenoids in archaea [e.g., *Holzer et al.*, 1979] points strongly to deriva-

tion of crocetane from methane-consuming archaea. The observed depletions of ¹³C are in good agreement with those in other biosynthetic products of methanotrophic archaea [*Hinrichs et al.*, 1999]. Recently, ¹³C-depleted crocetane was observed in other studies of environments in which it is likely that methane is being oxidized anaerobically. *Elvert et al.* [1999] found crocetane together with C₂₅ acyclic isoprenoids in surface sediments from the Cascadia margin. These sediments are saturated with methane from decomposition of underlying clathrates. *Pancost et al.* [2000] and *Hinrichs et al.* [2000] reported the occurrence of crocetane in methane-rich sediments from the Mediterranean Sea and from the Santa Barbara Basin, respectively. *Thiel et al.* [1999] and *Peckmann et al.* [1999] found crocetane and a few other compounds extremely depleted in ¹³C in a Miocene limestone and a Jurassic carbonate. The latter deposits apparently formed at ancient methane vent systems. Notably, they provide evidence for the long-term preservation of crocetane.

3.7. Pentamethylicosane

[31] 2,6,10,15,19- Pentamethylicosane differs from crocetane only by the addition of a single isoprene unit, joined head to tail, at one end of the molecule. It was identified in the sediments by comparison of its mass spectrum and GC retention time with published data [*Holzer et al.*, 1979; *Brassell et al.*, 1981]. In contrast to the C₂₀-isoprenoid-alkane peak, irmGCMS and GCMS analyses of the C₂₅-isoprenoid-alkane peak indicate that it contains only a single component. The δ values of 2,6,10,15,19-PMI in the samples above 180 cm range from -31.9 to -30.0‰ and then decrease steadily from -32.3 to -47.3‰ below 185 cm (Table 3, Figure 5). They are not correlated with those of the C₂₀-isoprenoid peak and thus indicate that 2,6,10,15,19-PMI and crocetane have independent sources within the sediments. This con-

trasts with the other recent observations of crocetane, in which its isotopic composition has been found to be similar to that of coexisting 2,6,10,15,19-PMI [Elvert *et al.*, 1999; Thiel *et al.*, 1999; Hinrichs *et al.*, 2000].

[32] 2,6,10,15,19-PMI is already present above the zone of methane consumption and its maximum isotopic depletion occurs well below the transition zone. Taken together with the previous observations Elvert *et al.*, 1999; Thiel *et al.*, 1999; Hinrichs *et al.*, 2000], this indicates that 2,6,10,15,19-PMI may be produced by anaerobic methanotrophs but that its biosynthesis is not restricted to these microbes. The source organisms of 2,6,10,15,19-PMI could certainly include methanogens, because the alkane occurs widely in methanogenic archaea [Tornabene *et al.*, 1979, 1980; Rowland *et al.*, 1982; Risatti *et al.*, 1984; Rowland, 1990]. Some of these species, called methylotrophic methanogens, can coexist with sulfate-reducing bacteria because they yield methane, biomass, and CO₂ from fermentation of methyl-bearing substrates for which the sulfate reducers do not compete [Oremland and Polcin, 1982; King, 1984; Ward and Winfrey, 1985]. Iversen and Jørgensen [1985] have provided evidence for the presence of methylotrophic methanogens at station C. Methane was present “in most of the sulfate zone,” where it was “consumed by anaerobic oxidation in the same layer of sediment and at a rate similar to that at which it is produced” [Iversen and Jørgensen, 1985, p. 953]. The 2,6,10,15,19-PMI within the sulfate-reducing zone might therefore derive from methylotrophic methanogenesis.

[33] The isotopic compositions of the 2,6,10,15,19-PMI are consistent with this assignment but require careful interpretation. Summons *et al.* [1998] found that PMI produced by *Methanosarcina barkeri* growing on trimethy-

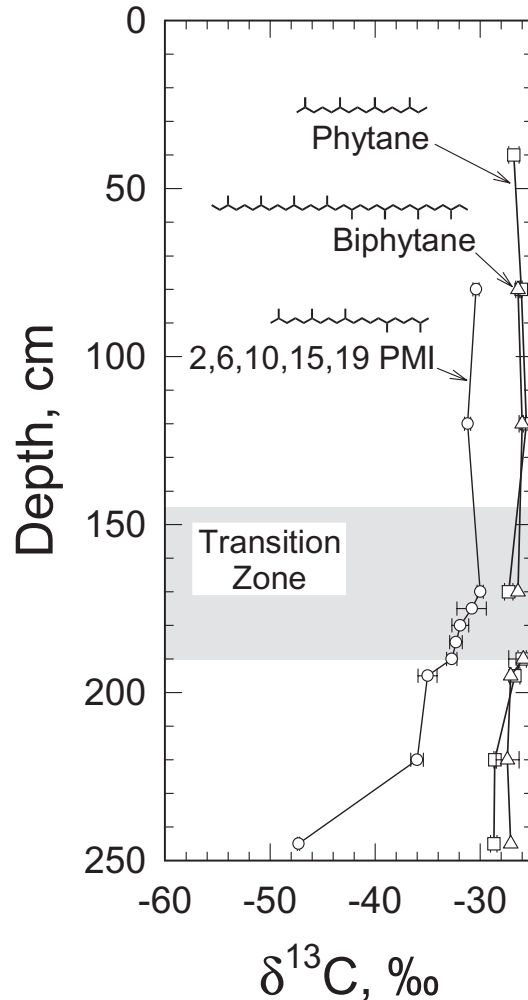


Figure 5. Isotopic compositions of 2,6,10,15,19-pentamethylicosane and of the ether-bound phytanyl and biphytanyl moieties versus depth in the sediment. Samples of ether-bound lipids were not prepared for depths between 170 and 190 cm.

lamine is depleted in ¹³C relative to biomass by $\Delta_{\text{biosynth}} \approx 14\text{‰}$. If the -31‰ PMI in the sulfate-reducing zone were attributed to such methanogens, then the δ value of the biomass of the putative methylotrophic methanogens, δ_{mm} , would be $\delta_{\text{PMI}} + \Delta_{\text{biosynth}} = -31 + 14 = -17\text{‰}$. This value appears high but could very plausibly result from mass balances associated with the fermentation of methyl-bearing substrates.

[34] The ^{13}C content of 2,6,10,15,19-PMI begins to decline within the transition zone and reaches values (-47‰), suggesting contributions from methanotrophy near the bottom of the core. Inorganic electron acceptors are, however, absent at that depth. If the depletion in ^{13}C reflects methanotrophy, it must have occurred while that layer of sediment passed through the transition zone, roughly 200 years prior to sampling (given reported rates of sedimentation) [Jørgensen *et al.*, 1990]. Methanogens, apparently using substrates that are depleted in ^{13}C , are an alternative source.

3.8. Ether-Bound Isoprenoids

[35] The polar-lipid fraction was treated with HI to cleave ether linkages. The resulting alkyl iodides were reductively dehalogenated to yield hydrocarbons that were identified from their mass spectra. The products included, in order of decreasing abundance, phytane, biphytane, and 2,6,10,14,18-PMI. The last of these, the only C_{25} isoprenoid among the polar-lipid-cleavage products, contrasts structurally with the tail-to-tail-linked 2,6,10,15,19-PMI present in the free-lipid fraction. The phytane and biphytane carbon skeletons occur in archaea as components of ether-linked polar lipids [e.g., DeRosa *et al.*, 1986; Hoefs *et al.*, 1997; DeLong *et al.*, 1998].

[36] Isotopic compositions of ether-linked phytane and biphytane moieties were determined (Table 3) but yields of the C_{25} regular isoprenoid hydrocarbon were too low for isotopic analysis. Significant yields of phytane and biphytane were obtained from all samples analyzed, even in the presence of concentrations of sulfate as high as 25 mmol/L. Phytane and biphytane are enriched in ^{13}C relative to most other hydrocarbon biomarkers except the sterols. As shown in Figure 5, the enrichment relative to 2,6,10,15,19-PMI is large enough to suggest strongly that there are two distinct

sources for archaeal biomarkers in the sulfate-reducing zone.

[37] The isotopic compositions of ether-linked phytane and biphytane shift only marginally at the transition zone (Figure 5, Table 3), suggesting either that their producers are not participating in methanotrophic processes or that contributions from the methanotrophs are swamped by the archaeal lipids already present. Methanogens are expected below the transition zone and would be reasonable sources for these compounds. However, the absence of a shift in the δ values of ether-linked lipids across the transition zone requires that if methanogens are important sources for these compounds, the isotopic compositions of their lipids must resemble those of the ether-linked lipids at shallower depths. We do not know whether this is likely. Two questions follow. First, are there alternative sources for the ether-linked lipids? Second, can consideration of the methane budget constrain the possibilities?

[38] In fact, the presence of ether-bound phytane and biphytane moieties throughout the sediment column may indicate derivation from planktonic archaea, which can contribute significantly to microbial biomass in certain environments [DeLong *et al.*, 1994], and which are also producers of ether-bound phytane and biphytane [DeLong *et al.*, 1998]. However, the absence of any biphytanes with cyclopentyl structural units, a general feature of ether lipids from planktonic archaea [DeLong *et al.*, 1998; Hoefs *et al.*, 1997; Schouten *et al.*, 1998] strongly suggests a sedimentary organism as source of the archaeal ether lipids. Methylo-trophic methanogens remain as a second alternative.

[39] Results of other studies provide information about the methane budget. The isotopic compositions ($\delta^{13}\text{C} = -72.3 \pm 0.7\text{‰}$, $\delta\text{D} = -183 \pm 5\text{‰}$, $n = 8$) and age (2970 ± 140

radiocarbon years, uncorrected for reservoir effects) of the methane found at site PC129 nearby [Laier *et al.*, 1996] indicate decisively that the methane in Kattegat sediments has been produced by CO₂-reducing methanogenic bacteria. The content of radiocarbon is so high that no more than 20% of the methane could derive from pre-Holocene organic matter, even if the remainder were of modern origin. It is most likely that the radiocarbon age accurately reflects the age of the organic material from which the methane was produced: either oceanic debris with an average age of ~2600 years or some mixture of more recent oceanic debris with slightly older material eroded from continental soil profiles.

[40] The present analyses (Figure 1) did not provide for retention and quantification of pressurized gases. Thus they cannot reveal if the highest recorded concentration of methane (1.3 mmol/L at 195 cm; Figure 1) represents the maximum in the core or whether concentrations continue to increase with depth. At site PC129, nearby, concentrations of methane in fine-grained sediments deeper than the base of the present core are tenfold higher than those reported here [Laier *et al.*, 1996]. Similarly, assessment of carbon budgets requires additional, deeper sources of methane. Integration of the methane-oxidation rate (Figure 1b) indicates a flux of ~175 nmol/(cm² d) or 64 μmol/(cm² yr). That methane is certainly ascending from depths below 190 cm.

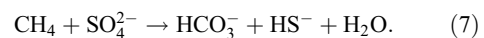
[41] The concentration of TOC between 190 and 245 cm is roughly constant (Figure 2). Rates of burial, sediment properties, and concentrations of organic carbon reported by Iversen and Jørgensen [1985], Jørgensen [1989], Jørgensen and Revsbech [1989], and Jørgensen *et al.* [1990] show that generation of 64 μmol CH₄/(cm² yr) within this interval would lead concentrations of TOC to decrease by ~0.25%. The initial concentrations of organic carbon in

these sediments are not known. It is therefore possible that the 0.9% now found at 245 cm is the residue of 1.15% that passed through the 190-cm horizon. If so, the methane being consumed within the transition zone would be coming from this interval. If not, some or all of the methane is from greater depths.

[42] From these considerations an answer to the isotopic mystery (why do the isotopic compositions of the ether-linked lipids not change with depth, even though a progression of archaeal activities is expected?) can be suggested. First, there are microbial sources that could account for the isotopic constancy. Second, there is little or no evidence for important methanogenesis (and thus for concomitant production of isotopically distinctive ether-linked lipids) in the depth range studied here. The alternative sources, most probably the methylotrophic methanogens, are thus favored.

3.9. Bioenergetics and Mechanisms

[43] The net chemical reaction occurring in the transition zone is [Iversen and Jørgensen, 1985; Hoehler *et al.*, 1994]



From standard free energies of formation tabulated by Thauer *et al.* [1977] we obtain $\Delta G^\circ = -16.6$ kJ. Because the concentrations of all reactants and products are known, the in situ ΔG can be estimated. For CH₄, Figure 1 indicates a concentration of 0.4 mmol/L at the methane-oxidation maximum. The concentration in equilibrium with $P_{\text{CH}_4} = 1$ atm at 5°C (sediment temperature reported by Laier *et al.* [1996]) would be 1.8 mmol/L [Duan *et al.*, 1992]. The activity relative to that at the standard state is thus 0.22. For SO₄²⁻, Figure 1 indicates a concentration of 2 mmol/L. For HCO₃⁻, Jørgensen *et al.* [1990] report a concentration of 25 mmol/L in the transition

zone at their station 10. At the same point, the concentration of total H_2S is 0.75 mmol/L, and we thus estimate a concentration of 0.4 mmol/L for HS^- . Employing the seawater activity coefficients for the ionic species [Miller and Pierrot, 1998], we obtain $\Delta G = -22.6$ kJ.

[44] Mechanisms for the biological catalysis of the anaerobic oxidation of methane have been reviewed by Hoehler *et al.* [1994]. Diverse lines of evidence point to the existence of a microbial consortium comprised of methane-consuming and sulfate-reducing organisms. Hoehler *et al.* [1994] conclude that the methane consumer is probably a methanogen operating in reverse and producing $\text{CO}_2 + \text{H}_2$. Prompt consumption of the H_2 by the sulfate-reducing partners would hold the concentration of either intermediate to levels low enough that the methane consumption itself would yield enough energy to sustain the “reversed methanogens.” The overall process would be driven by the (barely) favorable ΔG borne out by the preceding calculations.

[45] Because the molecular structures of the lipids are distinctive, the present results and other recent reports [Elvert *et al.*, 1999; Thiel *et al.*, 1999; Hinrichs *et al.*, 1999, 2000; Pancost *et al.*, 2000] provide strong evidence for consumption of methane by archaea. This confirms the key point of the consortium hypothesis. Hinrichs *et al.* [1999] also combined molecular isotopic analyses with a survey of 16S rRNA genes. The samples that contained ^{13}C -depleted archaeal lipids yielded six archaeal phylotypes, all previously unknown. Of these, one was closely related to methanogens of the order Methanosarcinales, but the other five defined a new cluster that is phylogenetically distinct from the known orders of methanogens. Accordingly, Hinrichs *et al.* [1999] noted that the “reversed methanogens” might in fact be archaea that were specialized as methane consumers rather than methane producers.

[46] The energy available to the methane-consuming organisms must place them at the margin of viability. Energy released by metabolic reactions is conserved within cells through a sequence of processes leading to the synthesis of ATP. With reactants and products at physiological concentrations that reaction requires circa 50 kJ/mole and, with allowance for energy lost as heat in irreversible reaction steps, Schink [1997, p. 263] concludes that a realistic requirement is “about 60 kJ per mole.” The synthesis of ATP is coupled to proton transport. Schäfer *et al.* [1999, p. 600] review recent evidence indicating that “For every four protons translocated... one molecule of ATP is synthesized.” Accordingly, 15 kJ (= 60/4) is a kind of biochemical energy quantum. If there is a stoichiometric relationship in which one proton is transported for each mole of substrate consumed, then a minimally adequate metabolic reaction must yield at least 15 kJ per mole of substrate. Here the energy available, 22.6 kJ per mole of substrate, appears inadequate for a consortium in which energy must be shared between two organisms (but see concepts introduced by Valentine and Reeburgh [2000]).

[47] Recycling of carbon from methane to biomass within the sediment has the potential to affect the isotopic composition of sedimentary TOC since it will lead selectively to the retention of carbon depleted in ^{13}C . Although production of biomass by aerobic microorganisms growing optimally on methane can be high [Harder and van Dijken, 1976], production under anaerobic conditions may be much lower. Heijnen and van Dijken [1992] review biomass production in terms of energy requirements and the redox levels of available carbon sources. For anaerobic growth on methane they estimate an energy requirement of 1011 kJ/(mole of biomass carbon). From the integrated rate of methane oxidation (0.64 mol/(m² yr)) we calculate that the energy available to the methane-oxidizing

community is $14 \text{ kJ}/(\text{m}^2 \text{ yr})$. Production of $14 \text{ (mmol biomass C)}/(\text{m}^2 \text{ yr})$ is thus expected.

[48] The rate of burial of organic carbon in these sediments, which must be compared to the methanotrophic production in order to determine the net shift in the δ value of TOC, can similarly be estimated. On the basis of analyses of ^{210}Pb , *Jørgensen et al.* [1990, p. 41] report that at their station 10, which is very near the present site, sediments are accumulating below the mixed zone (“at 10 to 20 cm depth”) at 6.2 mm/yr or $430 \text{ mg dry sediment/yr}$. If the density of the dry mineral matter is 2.8 g/cm^3 , this corresponds to a porosity of 75%. *Iversen and Jørgensen* [1985] report a porosity of 60% for sediments in the transition zone. With allowance for dewatering and compaction, the rate of sediment accumulation at 190 cm depth is $\sim 3.9 \text{ mm/yr}$. Since the concentration of TOC is $0.9 \text{ g}/100 \text{ g dry}$, carbonate-free sediment, this corresponds to an organic-carbon burial rate of $3.2 \text{ mol}/(\text{m}^2 \text{ yr})$.

[49] If 14 mmol C with $\delta \approx -90\text{‰}$ (a reasonable estimate for the products of the methanotrophic community, since the biomass of the bacterial, syntrophic partner is also expected to be strongly depleted in ^{13}C) are mixed with 3.2 mol C with $\delta = -23.3\text{‰}$, the δ of TOC is expected to shift by 0.3‰ . No stepwise change is evident in the record shown in Figure 2, but the trend in δ values is not inconsistent with this expectation. The signal could also be blurred and attenuated by partial remineralization of the methanotrophic products.

[50] To search for direct evidence of the involvement of a second organism, expected to be a sulfate-reducer, we examined the isotopic compositions of $\text{C}_{14}\text{--C}_{19}$ carboxylic acids in samples from the Kattegat sediments. The δ values of some acids shift by as much as 2‰ between depths of 185 and 190 cm in the core, but there is no pattern that can be clearly associated with

propagation of the methane-oxidation signal from the methane consumer to its putative partner. Sediments related to the anaerobic oxidation of methane in the Eel River and Santa Barbara Basins present a very different picture [*Hinrichs et al.*, 2000]. The samples in those locations were collected near cold seeps at which substantial quantities of methane are bubbling into the ocean. The products of anaerobic methanotrophy are abundant enough to depress the ^{13}C content of the total extractable lipid fractions. The ^{13}C -depleted archaeal biomarkers ($\delta < -100\text{‰}$) are accompanied by carboxylic acids with isotopic compositions bridging the gap between pure methanotrophic and typical marine products. Those most strongly depleted are the odd-carbon and branched-chain acids characteristic of eubacteria. The isotopic signals indicate that some eubacteria are associated with the methane-consuming archaea closely enough that the isotopic signal characteristic of the methane, released as CO_2 or acetate in pore waters, is not strongly diluted. It supports, but does not prove, the hypothesis that a consortium including a eubacterial sulfate reducer is responsible for the anaerobic oxidation of methane.

[51] The archaeal ether lipids found in sediments from the Eel River Basin by *Hinrichs et al.* [1999] were not found in Kattegat sediments. Conversely, crocetane was not found in the sediments from the Eel River seeps. Considering these findings in combination with those of *Elvert et al.* [1999] and *Thiel et al.* [1999], it would appear that there are at least two different archaeal methane consumers.

4. Conclusion

[52] The present results provide compelling evidence that crocetane is produced by an organism capable of consuming methane anaerobically. The depletion of ^{13}C relative to that in the methane ($20\text{--}25\text{‰}$, given a methane

source at -72‰) is so great that methane must be the only carbon source used by this organism for the biosynthesis of lipids. The molecular structure of crocetane, which includes a tail-to-tail junction, suggests strongly that the organism is archaeal. The isotopic differences between crocetane and 2,6,10,15,19-pentamethylcosane indicate that these molecules are not produced by the same organism and, together with other observations, indicate the existence of several different organisms capable of oxidizing methane anaerobically.

Acknowledgments

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