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LETTER

Evidence for gammacerane as an indicator of water column stratification

JAAP S. SINNINGHE DAMSTÉ,[1] FABIEN KENIG,[1,*] MARTIN P. KOOPMANS,[1] JÜRGEN KÖSTER,[1] STEFAN SCHOUTEN,[1] J. M. HAYES,[2] and JAN W. DE LEEUW[1]

[1] Netherlands Institute for Sea Research (NIOZ), Department of Marine Biogeochemistry, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

[2] Biogeochemical Laboratories, Departments of Geological Sciences and Chemistry, Geology Building, Indiana University, Bloomington, IN 47405, USA

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Abstract—A new route for the formation of gammacerane from tetrahymanol is proposed; in addition to dehydration and hydrogenation, sulphurisation and early C-S cleavage are shown to be important in the pathway of formation, especially in marine sediments. Evidence is twofold. First, relatively large amounts of the gammacerane skeleton are sequestered in S-rich macromolecular aggregates formed by natural sulphurisation of functionalised lipids. Selective cleavage of polysulphide linkages with MeLi/MeI led to formation of 3-methylthiogammacerane, indicating that the gammacerane skeleton is primarily bound via sulphur at position 3, consistent with the idea that tetrahymanol (or the corresponding ketone) is the precursor for gammacerane. Second, upon mild artificial maturation of two sediments using hydrous pyrolysis, gammacerane is released from S-rich macromolecular aggregates by cleavage of the relatively weak C-S bonds.

The stable carbon isotopic compositions of gammacerane and lipids derived from primary producers and green sulphur bacteria in both the Miocene Gessoso-solfifera and Upper Jurassic Allgäu Formations indicate that gammacerane is derived from bacterivorous ciliates which were partially feeding on green sulphur bacteria. This demonstrates that anaerobic ciliates living at or below the chemocline are important sources for gammacerane, consistent with the fact that ciliates only biosynthesize tetrahymanol if their diet is deprived of sterols. This leads to the conclusion that gammacerane is an indicator for water column stratification, which solves two current enigmas in gammacerane geochemistry. Firstly, it explains why gammacerane is often found in sediments deposited under hypersaline conditions but is not necessarily restricted to this type of deposits. Secondly, it explains why lacustrine deposits may contain abundant gammacerane since most lakes in the temperate climatic zones are stratified during summer.

INTRODUCTION

Gammacerane is a C_{30} triterpane first identified in the bitumen of the Green River shale (Hills et al., 1966). It is often present in samples from hypersaline marine and nonmarine depositional environments (Peters and Moldowan, 1993, and references cited therein) and, therefore, has been suggested as an indicator of hypersalinity (e.g., see de Leeuw and Sinninghe Damsté, 1990). It is thought to derive from tetrahymanol (gammacer-3β-ol), which is widely distributed in marine sediments (ten Haven et al., 1989; Venkatesan, 1989), in freshwater (Mallory et al., 1963; Holz and Conner, 1973), and in bacterivorous marine ciliates (Harvey and McManus, 1991). Tetrahymanol has also been found in photosynthetic sulphur bacteria (Kleemann et al., 1990) and in a fern (Zander et al., 1969). ten Haven et al. (1989) proposed that gammacerane is formed by dehydration and subsequent hydrogenation of tetrahymanol. This was supported by the identification of the presumed intermediate, gammacer-2-ene, in the sediments of the Nördlinger Ries (ten Haven et al., 1989).

Two major questions remain. Firstly, why should gammacerane be an indicator of hypersalinity if its precursor, tetrahymanol, is widespread in freshwater and marine ciliates? Secondly, why has the presumed intermediate in the conversion of tetrahymanol into gammacerane, gammacer-2-ene, so far only been found in one sediment? In other words, does the suggested pathway of formation provide the full story? As a partial answer to these questions, we demonstrate in this paper (1) that bacterivorous ciliates living at or below the chemocline are often the major source of tetrahymanol, (2) that tetrahymanol is often sequestered in S-rich macromolecular aggregates, and (3) that later, upon diagenesis and early catagenesis, gammacerane is released from these aggregates by selective cleavage of C-S bonds. This evidence is in full agreement with the recent suggestion of Schoell et al. (1994) that gammacerane is an indicator of water-column stratification rather than an indicator of hypersalinity per sé.

EXPERIMENTAL

The artificial maturation experiments, methods of extraction and fractionation, desulphurisation and analyses by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS), have been described in detail elsewhere (Kenig et al., 1995a; Koopmans

* *Present address:* Departments of Geology and Geophysics and of Oceanography, School of Ocean and Earth Sciences and Technology, University of Hawaii, Honolulu, HI 96822, USA.

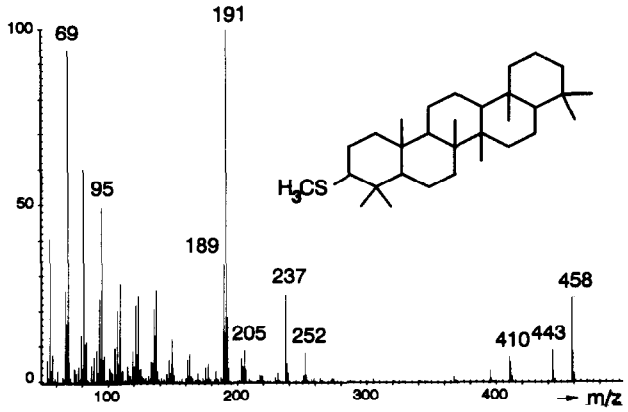

FIG. 1. Mass spectrum (subtracted for background) of 3-methylthiogammacerane.

et al., 1995; Köster et al., 1995). Gammacerane in the hydrocarbon fractions and in the desulphurised polar fractions was quantified by integration of peak areas in the FID trace or, if gammacerane partially coeluted with C_{31} 17α,21β(H)-hopanes, by integration of mass chromatograms m/z 397 (for gammacerane) and m/z 57 for the standard (a deuterated anteiso C_{22} alkane). In that case, results were corrected based on the intensities of the ions used in the mass spectra of authentic standards.

Results of compound-specific analyses of ^{13}C in the components present in the non-adduct (5 Å molecular sieve) of the desulphurised polar fraction from the Gessoso-solfifera Formation have been described elsewhere (Kenig et al., 1995b). For subfractions of the aromatic hydrocarbon fractions and non-adducts of the saturated hydrocarbon fraction of the Allgäu Formation, these analyses were performed on a HP5890 gas chromatograph coupled to a Finnigan MAT Delta C mass spectrometer. In case of the Algäu Formation, GC-MS analysis revealed that concentrations of partially co-eluting C_{31} hopanes were less than 10% of the gammacerane concentration. No coelution was apparent in the samples of the Gessoso-solfifera Formation.

RESULTS AND DISCUSSION

Desulphurisation of the polar fractions of the extracts of ten subsamples of the marl of the evaporitic cycle IV (Vai and Ricci Lucchi, 1977) of the Messinian Gessoso-solfifera Formation, Italy, released substantial amounts of gammacerane (0–200 μg/g TOC). Accordingly, large amounts of S-bound gammacerane must be bound in macromolecular aggregates present in the polar fraction. Desulphurisation of the kerogen released similar amounts of gammacerane (0–275 μg/g TOC; Schaeffer et al., 1995), indicating that the kerogen is also a pool of S-bound gammacerane. Treatment of the polar fraction of the subsample from the base of the unit with MeLi/MeI, a reagent which selectively cleaves polysulphide linkages and releases the carbon skeletons as methylthioethers (Kohnen et al., 1991), yielded large amounts of a component tentatively identified as 3-methylthiogammacerane. Its mass spectrum (Fig. 1) includes a molecular ion at m/z 458 and fragment ions at m/z 191, 237, 189, 410 (M-46), 443 (M-15), but importantly no ion at m/z 415 (M-43; isopropyl). Desulphurisation of the methylthioether fraction with deuterated nickel boride (Schouten et al., 1993a) released gammacerane bearing only one deuterium atom, indicating that only monomethylthiogammaceranes were formed upon MeLi/MeI treatment. Small amounts (20% of the gammacerane concentration) of gammacer-2-ene were also formed, probably by elimination of methylthiol. The olefin was identified by comparison of its mass spectrum with that reported by ten Haven et al. (1989). Both spectra reveal the small, but very characteristic, fragment ion at m/z 328 resulting from a retro Diels-Alder reaction. The formation of gammacer-2-ene suggests that the methylthio substituent in methylthiogammacerane was originally positioned at either C-3 or C-2. The precursor of gammacerane thus contained one functional group at C-3 or C-2 which reacted with sulphur (Kohnen et al., 1992b), consistent with the idea that tetrahymanol is the precursor for gammacerane (ten Haven et al., 1989; Venkatesan, 1989). Notably, however, saturated alcohols do not react with polysulphides under laboratory conditions thought to mimic those of natural sulphurisation (Schouten et al., 1994, and references cited therein). In contrast, ketones react readily with polysulphides (Schouten et al., 1993b). It is, therefore, more likely that gammacer-3-one, a component thought to be present in sediments (ten Haven et al., 1989) and probably formed by oxidation of tetrahymanol or present as such in organisms, is the actual precursor for S-bound gammacerane.

To study the diagenetic and catagenetic fate of S-bound gammacerane, immature samples were artificially matured by hydrous pyrolysis, which has been shown to effectively mimic oil formation (Lewan et al., 1979). Since C-S bonds are relatively weak, unusually low temperatures were applied (Koopmans et al., 1995) in the artificial maturation of a composite marl sample of Cycle IV of the Gessoso-solfifera Formation. As shown in Fig. 2, the amount of gammacerane present in the saturated hydrocarbon fractions of the samples increased significantly. In the original sample and in samples heated at temperatures up to 200°C, concentrations of gammacerane are less than 0.2 μg/g TOC. As the temperature of pyrolysis increases from 200 to 280°C, yields of gammacerane increase to 20 μg/g TOC. Yields decrease at higher temperatures, probably due to thermal destruction of products. At 239°C, 70 wt% of the gammacerane skeleton was olefinic (gammacer-2-ene) instead of saturated (gammacerane). Gammacer-2-ene was not detected at higher or lower temperatures. The increase of the free gammacerane concentration coincided with a decrease in the concentration of S-bound gammacerane in the polar fraction, suggesting strongly that gammacerane is formed by cleavage of C-S bonds in macromolecular aggregates where tetrahymanol or its corresponding ketone has been sequestered by natural sulphurisation. In a sample from the Upper Cretaceous Ghareb Formation (Jordan), hydrous pyrolysis resulted in a similar production of gammacerane (Fig. 2). The polar fraction of the Ghareb sample did not, however, release significant amounts of gammacerane upon desulphurisation. The kerogen of the related Jurf ed Darawish Oil Shale contains significant amounts of S-bound gammacerane (Hofmann et al., 1992). It is, therefore assumed that the kerogen is the major source for the gammacerane formed upon hydrous pyrolysis.

These data provide evidence for an alternative diagenetic pathway for the formation of gammacerane. Instead of dehydration and hydrogenation of tetrahymanol (ten Haven et al., 1989), sulphurisation and subsequent C-S bond cleavage may also lead to formation of gammacerane. In fact, the only

reported occurrence of the key intermediate in the proposed dehydration/hydrogenation pathway, gammacer-2-ene, may also be the product of an elimination reaction of S-bound gammacerane, since this component is an important product of hydrous pyrolysis at 239°C. Circumstantial evidence for this hypothesis is provided by results of analyses of the Nördlinger Ries sediments, in which gammacer-2-ene was identified (ten Haven et al., 1989). These sediments are also extremely rich in organic sulphur (Rullkötter et al., 1990), indicating that extensive sulphurisation of organic matter has occurred. The S-sequestration pathway is also supported by reports that gammacerane is a major product of desulphurisation of S-rich macromolecular aggregates (e.g., asphaltene and kerogen fractions) with, in cases where deuterium labelling was employed, mainly one deuterium atom incorporated (Richnow et al., 1992, 1993; Hofmann et al., 1992; Adam et al., 1993).

As shown in Fig. 3, the carbon isotopic composition of gammacerane released by desulphurisation of subsamples from marl layer IV of the Gessoso-solfifera Formation provided clues to its origin since it is significantly enriched (up to 5‰) relative to lipids derived from euphotic primary producers (i.e., algae and cyanobacteria; see Kenig et al., 1995, for details). These observations rule out photosynthetic purple sulphur bacteria as a source for tetrahymanol (Kleemann et al., 1990), since heptadecane derived from these organisms is significantly depleted relative to the primary producer lipids (Kenig et al., 1995). Therefore, it is likely that S-bound gammacerane is derived from tetrahymanol biosynthesized by ciliates. Ciliates are heterotrophic organisms which use organic material as a carbon and energy source. Heterotrophy can be associated with a carbon isotope effect, but for ciliates this effect is probably much smaller than 1.5‰ reported for vertebrates because they retain a much larger portion of assimilated carbon as biomass (Hayes et al., 1990; Hayes, 1993). Therefore, it is likely that the ciliates relied at least partially on a carbon source enriched in ^{13}C. The only possible carbon source in the Gessoso-solfifera palaeoenvironment would have been the obligately anaerobic photosynthetic green sulphur bacteria, which fix CO_2 through the reverse-TCA cycle, leading to biomass anomalously enriched in ^{13}C (Quandt et al., 1977). They comprise a well-identified component of the organic debris in the sediment through the identification of S-bound isorenieratane (Kohnen et al., 1992a; Kenig et al., 1995a,b), a diagenetic product of the diaromatic carotenoid isorenieratene, a highly characteristic pigment of brown-coloured green sulphur bacteria (Liaaen-Jensen, 1978a,b). As shown in Fig. 3, the ^{13}C content of S-bound isorenieratane varies from −11.7‰ at the base of the marl bed to ca. −14‰ in the rest of the section.

A ^{13}C enrichment of gammacerane relative to lipids derived from primary producers similar to that in the Gessoso-solfifera Formation is evident in marlstones of the Toarcian Allgäu Formation (Table 1; see Köster et al., 1995 for details). In these sediments, gammacerane is not sulphur bound but is a major component of the saturated hydrocarbon fraction (Köster et al., 1993). Again, the presence of isotopically heavy isorenieratane ($\delta = -19‰$) indicates that bacterivorous ciliates living at or below the chemocline were relying on green sulphur bacteria for at least part of their diet.

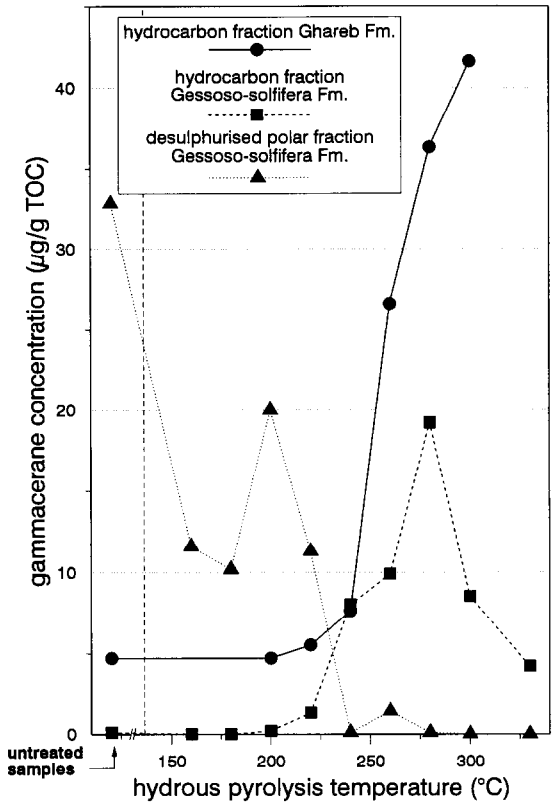

FIG. 2. Concentration of free and S-bound gammacerane in the Gessoso-solfifera and Ghareb Formations as a function of hydrous pyrolysis temperature.

If heterotrophic ciliates use green sulphur bacteria as a carbon source, they must be able to live under anaerobic conditions at least part of the time. In fact, diverse ciliates are now recognized as capable of completing their life cycles anaerobically (Fenchel and Finlay, 1991). Anaerobic ciliates have been found in sediments (e.g., Fenchel, 1969; Finlay et al., 1988; Wagener et al., 1990) and also as planktonic organisms in both marine (e.g., Fenchel et al., 1990; Zubkov et al., 1992) and lacustrine systems (e.g., Finlay et al., 1991; Guhl and Finlay, 1993; Massana and Pedrós-Alió, 1994). Moreover, ciliates living at and below the chemocline of the stratified Black Sea are thought to feed mostly on sulphide-oxidising bacteria (Zubkov et al., 1992). Similarly, the anaerobic ciliate *Caenomorpha medusula,* living in the anoxic zone of Priest Pot, a stratified freshwater lake, grazes selectively on the photosynthetic purple sulphur bacterium *Thiopedia* sp. (Guhl and Finlay, 1993). Fenchel (1969) estimated that, in the anoxic sediments of Nivå Bay, 25% of the food spectrum of the ciliates was comprised of purple sulphur bacteria, with the other 75% mainly composed of diatoms, flagellates, and other bacteria. It is thus likely that anaerobic ciliates can also use green sulphur bacteria as a food source.

Anaerobic ciliates are, like their aerobic counterparts, capable of biosynthesizing tetrahymanol (Harvey and McManus, 1991; Holler et al., 1993). Indeed, tetrahymanol can be biosynthesized under anaerobic conditions since its biosynthesis does not require squalene epoxidase (Zander et al.,

1970). However, ciliates biosynthesize tetrahymanol only if their diet contains no sterols (Conner et al., 1968, 1982; Harvey and McManus, 1991; Holler et al., 1993). Tetrahymanol is, therefore, produced only when ciliates are relying entirely on a prokaryotic food source and is a marker for bacterivory (cf. Harvey and McManus, 1991). Dominance of bacteria, and, more importantly, exclusion of sterol-bearing organisms, is expected in anaerobic environments and at aerobic-anaerobic interfaces, where chemoautotrophic bacteria can thrive. In modern stratified environments, ciliates feed on purple sulphur bacteria (e.g., Guhl and Finlay, 1993) and sulphide-oxidising bacteria (Zubkov et al., 1992), organisms found at or below the chemocline. As suggested by Schoell et al. (1994), therefore, gammacerane is a marker for water column stratification. Confirmation of this relationship solves two problems concerning the geochemistry of gammacerane. Firstly, since water columns in hypersaline depositional environments are often density stratified, it can now be understood why gammacerane is often associated with hypersalinity. Moreover, it explains why gammacerane is not absolutely restricted to sediments deposited under hypersaline conditions. Secondly, since lakes are often stratified at least seasonally, it also explains why some lacustrine deposits (e.g., Green River Shale; e.g., Collister et al., 1992) contain abundant gammacerane or its precursor.

Table 1. ^{13}C content of lipids of the Allgäu Formation.

Compound	$\delta^{13}C$ (‰ vs. PDB)
pristane	-34.3 ± 0.2
phytane	-33.7 ± 0.2
n-alkanes (average)	-33.8 ± 0.6
C_{31}-C_{33} hopanes (average)	-33.6 ± 1.3
C_{30} 4-Me triaromatic steroid	-34.9 ± 0.8
gammacerane	-29.1 ± 0.2
isorenieratane	-19.0 ± 0.3

CONCLUSIONS

1) Tetrahymanol or its corresponding keto derivative can be sequestered in S-rich macromolecular aggregates by nat-

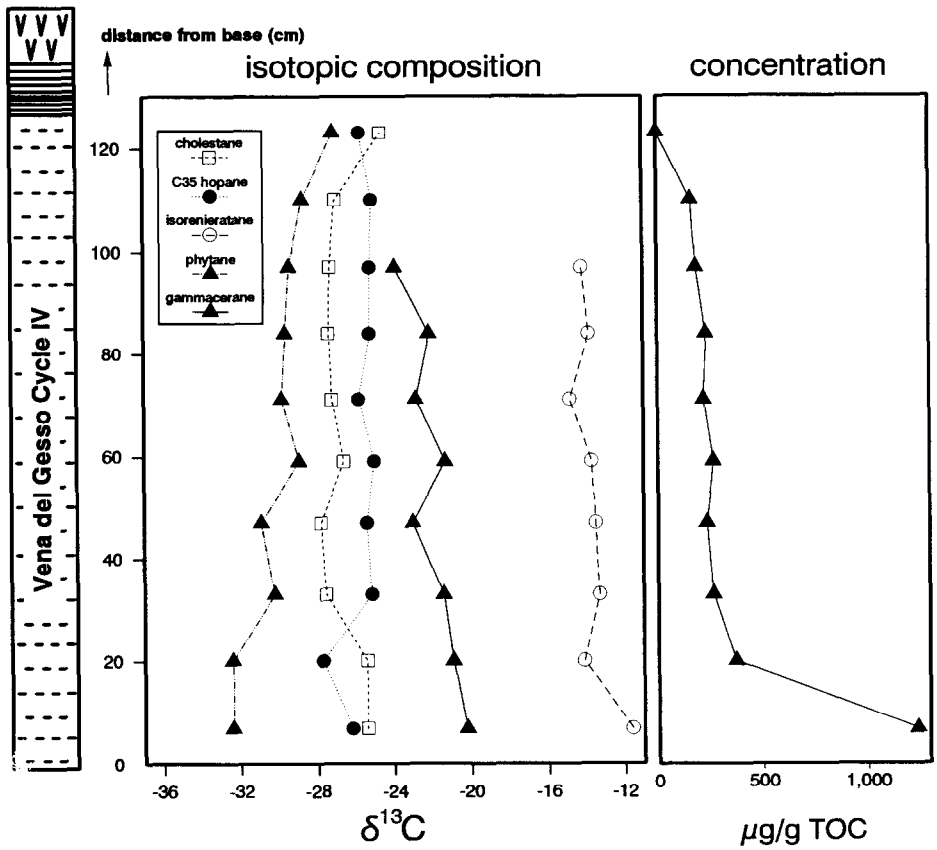

FIG. 3. Concentrations and ^{13}C contents of S-bound gammacerane in subsamples of the marl layer of Cycle IV of the Gessoso-solfifera Formation. For reference the ^{13}C contents of lipids derived of green sulphur bacteria (i.e., S-bound isorenieratane), algae (i.e., S-bound cholestane), cyanobacteria (i.e., S-bound C_{35} hopane), and S-bound phytane (i.e., primary producers and purple sulphur bacteria) are plotted (Kenig et al., 1995). In the upper two samples of the section studied the ^{13}C contents of S-bound gammacerane and S-bound isorenieratane could not be determined because they were low in concentration (gammacerane) or absent (isorenieratane) due to erosion of the chemocline (see Kenig et al., 1995 for details).

ural sulphurisation, as shown by the large amounts of 3-methylthiogammacerane released upon MeLi/MeI treatment. Artificial maturation experiments indicate that, upon further diagenesis and early catagenesis, gammacerane can be released via cleavage of relatively weak C-S bonds. This S-dependent mechanism represents an alternative pathway for the formation of gammacerane from tetrahymanol.

2) The ^{13}C contents of gammacerane released by desulphurisation of samples from the Gessoso-solfifera Formation and present as such in sediments from the Allgäu Formation indicate that it is derived from bacterivorous ciliates grazing partly on green sulphur bacteria. Recent biological literature indicates that bacterivorous ciliates also use purple sulphur bacteria and sulphide-oxidising bacteria as a food source and that they can live anaerobically. Since ciliates only biosynthesize tetrahymanol if their diet is deprived of sterols, gammacerane can be considered as an indicator for stratification of the water column.
3) These conclusions partly solve two current enigmas in gammacerane geochemistry. First, they explain why gammacerane is often found in sediments deposited under hypersaline conditions but is not necessarily restricted to such deposits. Secondly, they also explain why lacustrine deposits may contain abundant gammacerane since lakes in the temperate climatic zone tend to be stratified during summer.

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