Rapid sulfurisation of highly branched isoprenoid (HBI) alkenes in sulfidic Holocene sediments from Ellis Fjord, Antarctica.

Jaap S. Sinninghe Damsté [a,*], W. Irene C. Rijpstra [a], Marco J. L. Coolen, [a,#], Stefan Schouten [a], and John K. Volkman [b]

[a] *Royal Netherlands Institute for Sea Research, Department of Marine Biogeochemistry and Toxicology, P.O. Box 59, 1790 AB Den Burg, The Netherlands*

[b] *CSIRO Marine and Atmospheric Research, GPO Box 1538, Hobart, Tasmania 7001, Australia*

* Author to whom correspondence should be addressed (damste@nioz.nl).

[#] *currently also at:* Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, 360 Woods Hole Road, Woods Hole, MA 02543, USA

Abstract

Samples of particulate organic matter from the water column and anoxic Holocene sediment layers from the Small Meromictic Basin (SMB) in Ellis Fjord (eastern Antarctica) were analyzed to study the early incorporation of reduced inorganic sulfur species into highly branched isoprenoid (HBI) alkenes. HBIs were not detected in the water column samples from austral winter, whereas compounds containing the C_{25} HBI skeleton were abundant in all analyzed Holocene sediment layers. The structure of the $C_{25:2}$ HBI alkene together with its enriched stable carbon isotopic composition suggest that the HBI alkene is produced by a diatom or diatoms probably belonging to the *Navicula* genus present in the sea-ice which covers the area most of the year. Within just 500 years of deposition, all of the HBI alkene was sulfurised. A mixture of products was formed, including components tentatively identified as a C_{25} HBI thiane and three S-containing dimers composed of two $C_{25:1}$ HBI skeletons linked together by a sulfide bond. Most of the HBI alkene, however, was converted to polar S-containing compounds. The observed reaction rate for sulfurisation the $C_{25:2}$ HBI alkene is the highest observed so far in natural systems. Sterols and other lipids known to be prone to sulfurisation were only minimally sulfurised under these depositional conditions. The reason for this is presently unclear.

Key words: diatoms, thiane, euxinic conditions, sediment diagenesis, kinetics.

1. Introduction

The mechanisms of organic matter preservation in aquatic sediments are still far from fully explored. Bacterial mineralization of primary produced organic matter is generally highly effective leading to low storage of organic matter in the geosphere (e.g. Calvert and Pedersen, 1992). However, in environments with high primary production or where bottom waters are anoxic, a substantial part of the deposited organic carbon (perhaps up to 20%) will become sequestered in the sedimentary record, leading to the formation of organic-rich sediments that upon burial in the subsurface have the potential to generate petroleum. An important mechanism of organic matter preservation in such anoxic marine environments is natural sulfurisation: the reaction of reduced inorganic sulfur species, such as hydrogen sulfide and polysulfides, with organic matter (e.g. Schouten et al., 1994; Wakeham et al., 1995 REFS). Reaction with reduced sulfur species results in components being incorporated through cross-linking with sulfide and polysulfide bonds that are less susceptible to bacterial degradation and thus have a higher potential for preservation (Sinninghe Damsté et al., 1989a). Some compounds are more prone to sulfurisation than others. For example, laboratory sulfurisation studies (de Graaf et al., 1992, 1995; Schouten et al., 1993, 1994) have shown that ketones are much more reactive than alkenes. Of the various compound types available for sulfurisation within Holocene Antarctic Ace Lake sediments, only the C_{27}–C_{29} steroids were found to be extensively sulfurised (Kok et al., 2000). Werne et al. (2000) found that highly branched isoprenoid alkenes and malabaricatrienes in Cariaco Basin sediments were sulfurised during the first few thousands of years of diagenesis. Normally, reactive iron, present as ferric oxide and oxohydroxide coatings on clay particles (Canfield, 1989), is thought to react faster with reduced sulfur species than organic matter (Gransch and Posthuma, 1974). Organo-sulfur-rich sediments, therefore, are usually considered to be deposited in iron-depleted systems such as stratified basins where anoxic conditions can develop and the supply of iron is less than the production of sulfide. The Holocene sediments of the Small Meromictic Basin (SMB) in the Antarctic Ellis Fjord are sulfidic and organic-carbon rich. In addition, in Antarctic aquatic environments, there is little detrital iron available to complex with inorganic sulfur species in the water column and dust-derived inputs are limited by seasonal ice cover.

For these reasons, the SMB in Ellis Fjord represents a model system in which to study the early sulfurisation of organic compounds. Here, we focus on the rate of sulfurisation of a $C_{25:2}$ HBI alkene. Extensive lipid analyses of microalgae show that diatoms are most likely the biological source of HBIs in sediments (Volkman et al., 1994; Wraige et al., 1997; Sinninghe Damsté et al., 1999) and that HBI alkenes are one of the organic components most prone to sulfurisation (Sinninghe Damsté et al., 1989b; Kohnen et al., 1990; Wakeham et al., 1995; Hartgers et al., 1997; Werne et al., 2000). C_{25} HBI alkenes are produced only by species of the pennate diatom genera *Navicula*, *Haslea*, and *Pleurosigma* as well as some species of the centric genus *Rhizosolenia* (Sinninghe Damsté et al., 2004).

2. Materials and Methods

2.1. Sampling

Water samples were obtained in November 2000 from various positions in the water column at the depocentre (13 m depth; 68.59702ºS 78.22762ºE) of the Small Meromictic Basin (SMB) in Ellis Fjord, Vestfold Hills, eastern Antarctica using a 50 cm long 5 L Niskin bottle. The general features of this area are described by Trull et al. (2001). Water was obtained from: (i) the oxic mixolimnion (1.7−2.2 m; 5.2−5.7 m; 8.7−9.2 m), (ii) anoxic and sulfidic chemocline where there was a dense accumulation of obligate photolithotrophic green sulfur bacteria (9.7−10.2 m), and (iii) the anoxic and sulfidic monimolimnion (10.7−11.2 m, 12.5−13.0 m). Particulate organic matter (POM) was obtained by filtering the water samples (0.25 to 15 L, depending on particle densities), through 0.7 µm pore-size glass fiber filters (GFF; Millipore). The filtrate was then filtered through 0.2 µm pore-size polycarbonate (PC) filters (Millipore) to collect any small prokaryotic cells that were not retained during the first filtration step. The filters were stored at –40^{0}C prior to lipid extraction. The extracts from both the GFF and PC filters at each water depth were pooled.

A sediment core (113-cm-long × 5 cm diameter) was obtained from the depocentre of the SMB using a gravity corer operated from a tripod constructed on the ice. Immediately after sampling, the sediment core was kept in the dark and stored at –20ºC at Davis Station and at –40ºC at Royal NIOZ. The upper 20 cm of sediment was sliced into 1 cm horizontal sections, but deeper layers were sliced into 2 cm horizontal sections with 2 cm space intervals. The total organic carbon contents (TOC) and $\delta^{13}C$ of TOC were determined by elemental analysis (EA)/isotope-ratio-monitoring mass spectrometry (EA/irmMS). EA/irmMS analyses were performed on decalcified (by reaction with 1 M HCl for 18 h) sediments using a Carlo Erba Flash elemental analyzer coupled to a Thermo-Finnigan DeltaPLUS irmMS system. The total organic carbon content (as a percentage) was determined using external standards with known carbon content. Stable carbon isotope ratios were determined using lab standards calibrated on NBS-22 oil (IAEA) and reported in Vienna Pee Dee Belemnite (VPDB) notation.

2.2. Calibration of sediment ages

Accelerator mass spectrometry (AMS) radiocarbon (^{14}C) dating on the organic matter (after removal of inorganic carbon by acid treatment) of selected core sections (0−1 cm, 20−22 cm, 40−42 cm, 60−62 cm, 80−82 cm, 100−102 cm, 112−113 cm) was carried out at the R.J. van der Graaf laboratory, University of Utrecht, The Netherlands. The sediment ages in calendar years before present (BP) were calculated using standard techniques of calibration (Stuiver et al., 1998). The calibrated age of the top sediment was 500 years. Since the SMB has been a stratified, seasonally influenced, marine basin for some time (Gibson, 1999), we assumed that the reservoir age of the dissolved organic carbon from which the organic matter was biosynthesized was 500 years for all analyzed sediment layers and corrected the determined radiocarbon aged for this reservoir age.

2.3. Lipid extraction and fractionation

Sediment samples were freeze-dried and ultrasonically extracted with methanol, dichloromethane (DCM)/methanol (1:1, v/v) and five times with DCM. All extracts were combined and the solvent was removed by rotary evaporation under vacuum. The extracts were separated by column chromatography using Al_2O_3 as stationary phase into apolar and polar fraction using hexane/DCM (9:1, v/v) and DCM/MeOH (1:1, v/v), respectively, as eluents. Elemental sulfur was removed from the apolar fractions using activated copper powder. After adding an internal standard (6,6-d2-2-methyl-icosane), samples were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). The polar fractions were desulfurised using Raney nickel after adding a standard (2,3-dimethyl-5-(1,1-d2-hexadecyl) thiophene) (Sinninghe Damsté et al., 1988). The products were separated into apolar and polar fractions as described above and the apolars were subsequently hydrogenated and analyzed by GC and GC-MS. Compounds having HBI skeletons were quantified by integration of peaks of the HBI alkene, sulfide, sulfide dimers and the HBI alkane formed upon desulfurisation of the polar fraction and that of the internal standard in gas chromatograms. An aliquot of one of the polar fractions (18−19 cm) was hydrogenated and also separated into apolar and polar fractions; the apolars were analyzed by GC and GC-MS. Gas chromatographic analyses were performed on a Fisons MFC 800 equipped with a fused silica column (25 m x 0.32 mm i.d.) coated with CP Sil-5 (0.12 µm film thickness). He was used as the carrier gas. The samples were injected at 70ºC and the oven was programmed to 130ºC at 20ºC/min and then to 320ºC at 4ºC/min and held for 10 min at 320ºC. GC-MS analyses were performed on a Hewlett-Packard 5890 gas chromatograph interfaced with a VG Autospec Ultima mass spectrometer. The GC column and conditions were as described above.

3. Results and Discussion

3.1. Highly Branched Isoprenoid (HBI) alkene composition in SMB

Gas chromatograms showing the distribution of non-sulfurised and sulfurised hydrocarbons in sediments from Ellis Fjord are shown in Fig. 1. In the surface sediment the major constituent was a diunsaturated C_{25} HBI alkene (Fig. 1; upper panel). Other constituents include a $C_{17:1}$ *n*-alkene, squalene and a variety of sterenes and triterpenoids (e.g. fernene) present in varying amounts.

The structure of the $C_{25:2}$ HBI alkene was identified from its mass spectrum (Fig. 2) and retention index as 2,10,14-trimethyl-6-methylene-7-(3'-methylpent-1-enyl)pentadecane (**I** in Fig. 1) (Johns et al., 1999). The identification was confirmed by coinjection with alkenes found in a culture of *Navicula salinarum*: the alkene from Ellis Fjord coeluted with the first eluting of the two $C_{25:2}$ isomers found (RI 2080). This $C_{25:2}$ alkene is also found in sea-ice diatoms (Nichols et al., 1988) and in various Antarctic sediments (Venkatesan, 1988). Analysis of fossil ribosomal DNA in sediments from the same core showed that a diatom belonging in the *Navicula* genus is the likely source of HBI alkenes in this sediment (Coolen, Volkman and Sinninghe Damsté, unpublished results). Note that the predominance of the $C_{25:2}$ HBI alkene over more-unsaturated HBI alkenes seems to be a characteristic of many samples from Antarctica (Rowland and Robson, 1990).

Within a few cm depth, the distribution of hydrocarbons becomes very different such that by 8 cm depth, the HBI alkene (Table 1) and $C_{17:1}$ *n*-alkene were relatively minor constituents (Fig. 1). Acyclic isoprenoids including phytane, phytenes and squalene were major constituents together with sterenes and hopenes and the unusual triterpenoid fernene. This distribution of hydrocarbons is very similar to those found in anoxic sediments from Ace Lake (Volkman et al., 1986), with the exception that HBI alkenes were not found there (Volkman et al., 1986). In Ace Lake, phytadienes were attributed to photoautotrophs whereas phytane was attributed to an archaeal source (Schouten et al., 2001).

At deeper depths, all of the $C_{25:2}$ alkene had disappeared, to be replaced by S-containing HBIs including a cyclic HBI sulfide, a mixture of three sulfur-containing HBI-dimers (Fig. 1; Table 1) and more polar forms not amenable to GC analysis. The total abundance of these various sulfurised forms varied considerably with depth (Table 1), but the polar forms were always more abundant than the HBI sulfide or dimers. Even though no HBI alkene could be detected below 11 cm, the high abundance of S-forms in deeper sediments confirmed that HBI-containing diatoms were an important source of organic matter throughout this period of deposition, although the varying concentrations of S-HBIs suggests that their input varied over time.

3.2. Sulfur-containing HBIs

The mass spectrum of the sulfur-containing HBI monomer is shown in Fig. 2b and is identical to that of a HBI sulfide tentatively identified in the surface sediments of Lake Cisó, a monomictic lake in Spain (Hartgers et al., 1997) and Holocene sediments of Mud Lake, a shallow (<1 m) lake in Florida, USA (Filley et al., 2002). Hartgers et al. (1997) identified this S-containing HBI on the basis of the mass spectral fragmentation pattern as an HBI possessing a. thiolane ring with the sulfur atom attached at the tertiary carbon atoms C-7 and C-22 of the C_{25} HBI skeleton. An alternative, and perhaps more likely, structure is an HBI possessing a thiane ring with the sulfur atom attached at the carbon atoms C-7 and C-23 of the C_{25} HBI skeleton (see **II** in Fig. 1). The absence of an M^{+}-29 (loss of an ethyl substituent) renders this identification more likely. We postulate that this thiane can be formed by intramolecular sulfur incorporation into a $C_{25:2}$ HBI alkene that can be formed by isomerisation of the methylenic double bond from the $C_{25:2}$ HBI alkene detected in the surface sediments (Fig. 3). It has been established that the methylenic double bond of HBI alkenes isomerizes relatively easily (Belt et al., XXX??)

In the apolar fractions of the deeper sediments, three late-eluting components were present, each of which had a similar mass spectrum (e.g. Fig. 2c). The molecular ion at m/z 730 suggested that they represented a dimer composed of two $C_{25:1}$ HBI skeletons bound to each other by a sulfide linkage. The presence of one sulfur atom in these components was demonstrated previously by high resolution accurate mass MS analysis of sediments from the Deep Basin of Ellis Fjord (Robertson et al., 1995). The mass spectrum showed a dominant fragment at m/z 348, representing a $C_{25:2}$ HBI alkene, probably formed by an elimination reaction, and a mass-deficient (i.e. likely indicating the presence of a sulfur atom) fragment ion at m/z 381, representing a $C_{25:1}$ HBI alkene ion containing one sulfur atom. The remaining characteristic fragment ions (m/z 207, 235, 263, 266, M^{+}-265) also occurred in the mass spectrum of the HBI alkadiene **I** (cf. Fig. 2a). Finally, the fragment at m/z 409 (M^{+}-321) might provide a clue as to the position of the sulfide moiety, namely at C-23, which would explain the loss of a $C_{23}H_{43}$ moiety. Small amounts of HBI dimers containing two sulfur atoms were also tentatively identified (Fig. 1). They possessed a molecular ion at m/z 762 in their mass spectra and a more abundant m/z 381 fragment ion and thus probably represent dimers in which two HBI skeletons are linked by a disulfide moiety (cf. Kohnen et al., 1991; Kok et al., 2000).

It can be envisaged that the HBI dimers were formed by intermolecular sulfur incorporation linking two HBI alkadienes (Fig. 3). In this reaction, one of the two double bonds of each HBI skeleton would remain intact, whilst the other would be involved in the cross-linking reaction. It is not clear if the three detected HBI dimers (Fig. 1) represent structural isomers, reflecting the three possible isomers that can be generated from two identical $C_{25:2}$ HBIs, or stereoisomers. The almost identical mass spectra perhaps suggest the latter possibility to be the most likely one.

Desulfurisation with Raney Ni of the polar fractions followed by hydrogenation generated the C_{25} HBI alkane. Direct hydrogenation of the polar fraction of the solvent extracts did not generate the HBI alkane, providing strong support for an S-bound form of the HBI skeleton. The polar fraction of extracts of immature sediments often contain non GC-amenable macromolecular aggregates which are composed of carbon skeletons bound to each other by sulfur linkages (cf. Sinninghe Damsté et al., 1988; Kohnen et al., 1991; Adam et al., 2000; Kok et al., 2000; REFS). Additional sulfur incorporation in the HBI dimers (Fig. 3), which still possess two double bonds, can be envisaged to result in their inclusion in larger S-rich macromolecular aggregates.

3.3. HBI speciation and timing of sulfurisation

To follow the extent of sulfur incorporation, the different compound types containing the HBI skeleton (i.e. alkadiene, thiane, dimers, and polar-bound) were quantified (Table 1). These data indicate that with increasing depth the speciation of the HBI skeleton varies substantially (Fig. 4). Below 11 cm depth the alkadiene was not detected and HBIs only occur in a sulfurised form. The polar S-bound form is most abundant in this interval, representing between 50 and 95% of total S-containing forms (Fig. 4; Table 1), but with no clear trend with depth. The surface sediment (0−1 cm) already contains a relatively high amount of sulfurised HBIs compared to the horizons just below the surface (Fig. 4). This is mainly due to the higher amounts of polar S-bound HBI in the surface sediment (Table 1). This suggests that sulfurisation occurs soon after the surface sediments are deposited (or perhaps even as they are deposited).

A series of seven AMS radiocarbon dates (Table 2) corrected for the apparent reservoir effect allowed us to construct an age model for the obtained core. The ocean has a different radiocarbon reservoir than that of the majority of the radiocarbon in the biosphere. The average difference between a radiocarbon date of terrestrial and marine carbon is ca. 400 radiocarbon years (Stuiver et al., 1998). The apparent radiocarbon age of oceanic water is caused both by (i) the delay in exchange rates between atmospheric CO_2 and ocean bicarbonate, and (ii) the dilution effect caused by the mixing of surface waters with deep waters which are older. In our age model we assumed a constant reservoir effect of 500 years based upon the radiocarbon age of the 0−1 cm sediment layer (Table 2). In Fig. 5 the obtained calendar years (i.e. radiocarbon age in Table 2 corrected for the reservoir effect) are plotted versus depth. From this an average linear sedimentation rate of ca. 0.4 mm yr^{-1} was calculated. In Fig. 5 we have also plotted the degree of sulfurisation of the HBIs (defined as the ratio of the HBIs in a sulfurised form vs. all HBIs) versus depth and age, from which it is apparent that the HBI alkadiene had become completely sulfurised within ca. 500 yr. If we exclude the first data point and model the HBI sulfurisation reaction in the upper 11 cm with first order reaction kinetics (cf. Werne et al., 2000), a reaction rate constant of $1.3x10^{-2}$ yr^{-1} with an r^2 of 0.95 is obtained.

3.4. General features of sulfur incorporation

The early incorporation of sulfur into HBI alkenes in the Ellis Fjord environment was previously shown by Robertson et al. (1995) who examined a surface sediment floc from Deep Basin. A saturated C_{25} HBI-derived sulfide, an unresolved S-containing complex mixture of steroidal thiols and a suite of HBI-dimers were recognized, but only limited structural identifications were achieved at that time. Early sulfur incorporation had also been observed in the lipids of Ace Lake in the Vestfold Hills (Volkman et al., 1986; Robertson et al., 1995; Kok et al., 2000; Schouten et al., 2001), but the products are dominated by steroids. Squier et al. (2004) recently reported an S-containing chlorophyll derivative in Holocene sediments from Pup Lagoon, a freshwater lake in eastern Antarctica. Obviously, despite the low temperatures of the lake and fjord waters in Antarctica, the absence of a significant input of detrital iron renders sulfurisation of organic matter at the very early stages of diagenesis possible. However, the observed high rate of sulfurisation of the HBI alkadiene in Ellis Fjord reported here is unprecedented.

It is well known from literature data that HBI alkenes are prone to natural sulfurisation. In ancient immature sediments HBIs occur predominantly in a sulfurised form (e.g. Sinninghe Damsté et al., 2004) and they have also been encountered in Holocene sediments of the Black Sea (Kohnen et al., 1990; Wakeham et al., 1995), the Arabian Sea (Schouten et al., 2000), the Cariaco Basin (Werne et al., 2000), Walvis Bay

(Adam et al., 2000), and various lakes (Hartgers et al., 1997; Filley et al., 2002). In Black Sea sediments HBI alkenes disappear rapidly with depth (Wakeham et al., 1995); in the upper 2 cm their concentration decreases by a factor of 100 (Sun and Wakeham, 1994). However, no low- or high-molecular-weight S-containing HBIs could be detected which could explain this apparent loss (Wakeham et al., 1995). Only in Unit II (age > 3 ka) sediments were S-containing HBIs detected having two double bonds less than their precursors (Kohnen et al., 1990; Wakeham et al., 1995). In contrast, in surface sediments of Lake Cisó (age < 100 yr) only C_{25} HBI sulfides were detected and no precursor HBI alkenes were observed (Hartgers et al., 1997), suggesting rapid sulfurisation. Our data now confirm this rapid sulfurisation process of HBI alkenes and allow us to quantify the rate of this process.

At present there are only two other studies which have determined the rate of the organic matter sulfurisation process. Werne et al. (2000) determined a first order rate constant of 2×10^{-4} yr^{-1} for the sulfurisation of malabaricatriene and Kok et al. (2000) concluded that the rate of sulfurisation of steroidal ketones in Ace Lake was comparable to that observed for malabaricatriene in the Cariaco Basin (Werne et al., 2000). The rate constant of 1.3×10^{-2} yr^{-1} determined here for the sulfurisation of HBI alkenes is significantly higher than those determined previously. This agrees with the observation that HBI sulfurisation in Cariaco Basin sediments proceeds faster than malabaricatriene sulfurisation (Werne et al., 2000) and further underpins the idea that sedimentary organic matter is highly heterogeneous and contains compounds with widely varying reactivity.

The question arises why HBI alkenes are so reactive towards natural sulfurisation. Laboratory sulfurisation studies have indicated that non-conjugated double bonds are not particularly prone to sulfurisation and that internal double bonds react slower with reduced inorganic sulfur than terminal double bonds (de Graaf et al., 1992; 1995; Schouten et al., 1993; 1994). These studies also revealed that ketones react much faster than alkenes. The HBI alkadiene **I** possesses two double bonds and sulfurisation of this double bond can explain the rapid formation of the sulfurised HBI dimers (Fig. 3) and, perhaps, that of the polar S-bound HBIs but not of the HBI thiane (which requires a shift of the terminal double bond; Fig. 3). Steroid sulfurisation in Ace Lake, a comparable environmental setting to the SMB of Ellis Fjord, proceeds more slowly than HBI sulfurisation in SMB, which is remarkable since it is assumed to occur through sulfurisation of steroid ketones formed in the water column and surface sediments (Kok et al., 2000). Clearly, the chemical structure is not the only parameter determining the rate of sulfurisation of organic matter. Other factors such as the availability of the components (e.g. adsorbed on a particle vs. present in an almost intact cell) will probably exert an even stronger control on the overall reaction rate. Recently, HBI epoxides have been identified as natural products in diatoms (Belt et al., 2006) and these could perhaps react much faster than HBI alkenes. However, these epoxides were not identified in the present study and this factor would also not explain the rapid decrease in concentration of the HBI alkadiene **I**.

3.5. Contributions from sea-ice microalgal populations

HBIs were below the detection limit in the water column samples that had been taken in Austral winter. This is consistent with the proposition that the source organisms of the HBI alkenes are present in the sea-ice. After the ice cover starts to melt, benthic sea-ice diatoms are liberated into the water column and open-water blooms of diatoms (mainly non-HBI-producing) *Chaetoceros* spp. dominate in the fjords from the Vestfold Hills (McMinn and Hodgson, 1993; McMinn et al., 2000). Moreover, our parallel studies of fossil ribosomal DNA (Coolen, Volkman and Sinninghe Damsté, unpublished results) confirmed that a *Navicula* species is the likely source and such species are common in sea-ice. Nichols et al. (1988) have shown that the HBI alkadiene **I** as found in the surface sediments occurs in sea-ice diatom communities. Revill et al. (1994) and Gibson et al. (1999) showed that sea-ice diatoms have isotopically heavy organic matter presumably reflecting constraints on the availability of CO_2 in sea-ice. Revill et al. (1994) used this observation to explain why lipids in sediments from the Permian tasmanite oil shale, which were known to be deposited under ice, are isotopically heavy.

The stable carbon isotopic compositions of the HBIs were measured in several sediment layers and compared with those of sterenes (i.e. a C_{28} steratriene and C_{29} stera-3,5-diene) (Table 3). Compared to the C_{28} steratriene, the HBIs are strongly enriched in ^{13}C with $\delta^{13}C$ values around -10‰. This is indeed consistent with an origin from a *Navicula* species growing in the sea ice where CO_2 availability is limited, resulting in strongly enriched organic matter and lipids (Revill et al., 1994; Gibson et al., 1999). The similar $\delta^{13}C$ values for the HBI skeletons in the different chemical forms strongly support the proposed close diagenetic link between these compound classes (Fig. 3). Remarkably, the $\delta^{13}C$ values of the C_{29} stera-3,5-diene are also quite enriched in some samples (Table 3), suggesting that it too is derived, to varying extents, from the sea-ice diatoms. However, detailed studies of the sterols in these sediments (unpublished data, 2004) showed that most sterols have isotopic compositions between -22 and -28‰, indicating additional significant contributions from phytoplanktonic sterols from the water column.

4. Conclusions

The hydrocarbon biomarker data revealed a diversity of organic matter sources in Ellis Fjord sediments dominated in the surface layers by diatoms. At greater depth, these algal markers disappeared and the biomarker distribution became dominated by hydrocarbons from prokaryotic sources and from diagenesis of functionalized lipids. Sulfurisation clearly was a major preservation mechanism for specific unsaturated or functionalised lipids in these anoxic low-iron sediments. The rates of sulfurisation recorded are the highest yet recorded even though the depositional environment is cold and microbial activity rates are low. The primary products of sulfurisation of the diatom-derived $C_{25:2}$ HBI alkene are a sulfur-containing thiane and a suite of three S-containing dimeric HBI compounds. In contrast to Ace Lake, where sulfurisation is also a rapid process, we did not detect S-containing steroids in the surface sediments. It is at present not clear why the HBI alkadiene is so prone to sulfurisation. The presence of ^{13}C-enriched lipids, including the HBI alkene and its sulfurised forms, points to the importance of sea-ice biota, mainly diatoms, as sources of organic matter in sediments deposited under ice and demonstrates the need to sample this part of the environment and not just the water column to gain a better understanding of organic matter sources and cycling in these environments.

Acknowledgements – We greatly acknowledge Cornelia Wuchter of the Royal NIOZ and Peter Thompson and crew of the Australian Antarctic Division for assistance during sampling. We thank Drs. P. Schaeffer, K. Grice and R. Jaffé for their constructive reviews. Funding for the collection of the sediment and water samples (by MJLC and CW) was provided by ASAC grant 1166 to JKV. This work was further supported by a grant from the Netherlands Organization for Scientific Research (NWO; Netherlands Antarctic Research Proposals 851.20.006 to JSSD).

References

Adam, P., Schneckenburger, P., Schaeffer, P., Albrecht, P., 2000. Clues to early diagenetic sulfurization processes from mild chemical cleavage of labile sulfur-rich geomacromolecules. Geochimica et Cosmochimica Acta 64, 3485−3503.

Belt, S.T., Massé, G., Rowland, S.J., Rohmer, M., 2006. Highly branched isoprenoid alcohols and epoxides in the diatom *Haslea ostrearia* Simonsen. Organic Geochemistry 37, 133−145.

Calvert, S.E., Pedersen, T.F., 1992. Organic carbon accumulation and preservation in marine sediments: how important is anoxia? In: Whelan, J.K., Farrington, J.W. (Eds.) Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments. Columbia University Press, New York, pp. 231−263.

Canfield, D.E., 1989. Reactive iron in marine sediments. Geochimica et Cosmochimica Acta 53, 619−632.

Filley, T.R., Freeman, K.H., Wilkin, R.T., Hatcher, P.G., 2002. Biogeochemical controls on reaction of sedimentary organic matter and aqueous sulfides in Holocene sediments of Mud Lake, Florida. Geochimica et Cosmochimica Acta 66, 937−954.

de Graaf, W., Sinninghe Damsté, J.S., de Leeuw, J.W., 1992. Laboratory simulation of natural sulphurization. I. Formation of monomeric and oligomeric isoprenoid polysulphides by low-temperature reactions of inorganic polysulphides with phytol and phytadienes. Geochimica et Cosmochimica Acta 56, 4321−4328.

de Graaf, W., Sinninghe Damsté, J.S., de Leeuw, J.W., 1995. The low-temperature addition of hydrogen polysulphides to olefins: Formation of secondary polysulphides from 1-alkenes and heterocyclic and polymeric organic sulphur compounds from α,ω-dienes. Journal of the Chemical Society Perkin Transactions I. 635–640.

Gibson, J.A.E., 1999. The meromictic lakes and stratified marine basins of the Vestfold Hills, East Antarctica. Antarctic Science 11, 175–192.

Gibson, J.A.E., Trull, T., Nichols, P.D., Summons, R.E., McMinn, A., 1999. Sedimentation of ^{13}C rich organic matter from Antarctic sea-ice algae: A potential indicator of past sea-ice extent. Geology 27, 331–334.

Gransch, J.A., Posthuma, J., 1974. On the origin of sulphur in crudes. In Advances in Organic Geochemistry 1973. Editions Technip, Paris, pp. 727–739.

Hartgers, W.A., Lopez, J.F., Sinninghe Damsté, J.S., Reiss, C., Maxwell J.R., Grimalt, J.O., 1997. Sulfur-binding in recent environments: II. Speciation of sulfur and iron and implications for the occurrence of organo-sulfur compounds. Geochimica et Cosmochimica Acta 61, 4769–4788.

Johns, L., Wraige, E.J., Belt, S.T., Lewis, C.A., Massé, G., Robert, J.-M., Rowland, S.J., 1999. Identification of a C_{25} highly branched isoprenoid (HBI) diene in Antarctic sediments, Antarctic sea-ice diatoms and cultured diatoms. Organic Geochemistry 30, 1471–1475.

Kok, M.D., Rijpstra, W.I.C., Robertson, L., Volkman, J.K., Sinninghe Damsté, J.S., 2000. Early steroid sulfurisation in surface sediments of a permanently stratified lake (Ace Lake, Antarctica). Geochimica et Cosmochimica Acta 64, 1425–1436.

Kohnen, M.E.L., Sinninghe Damsté, J.S., Rullkötter, J., ten Haven, H.L., de Leeuw, J.W., 1990. Origin and transformations of C_{25} and C_{30} highly branched isoprenoid sulphur compounds: Further evidence for the formation of organic sulphur compounds during early diagenesis. Geochimica et Cosmochimica Acta 54, 3053–3063.

Kohnen, M.E.L., Sinninghe Damsté, J.S., Kock-van Dalen, A.C., de Leeuw, J.W., 1991. Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. Geochimica et Cosmochimica Acta 55, 1375–1394.

McMinn, A., Hodgson, D., 1993. Summer phytoplankton succession in Ellis Fjord, eastern Antarctica. Journal of Plankton Research 15, 925–938.

McMinn, A., Bleakley, N., Steinburner, K., Roberts, D., Trenerry, L., 2000. Effect of permanent sea ice cover and different nutrient regimes on the phytoplankton succession of fjords of the Vestfold Hills Oasis, eastern Antarctica. Journal of Plankton Research 22, 287–303.

Nichols, P.D., Volkman, J.K., Palmisano, A.C., Smith, G.A., White, D.C., 1988. Occurrence of an isoprenoid C_{25} diunsaturated alkene and high neutral lipid content in Antarctic sea-ice diatom communities. Journal of Phycology 24, 90–96.

Revill, A.T., Volkman, J.K., O'Leary, T., Summons, R.E., Boreham, C.J., Banks, M.R., Denwer, K., 1994. Hydrocarbon biomarkers, thermal maturity, and depositional setting of tasmanite oil shales from Tasmania, Australia. Geochimica et Cosmochimica Acta 58, 3803–3822.

Robertson, L., Revill, A.T., Volkman, J.K., Sikes E.L., 1995. Organic geochemistry of Antarctic lakes and fjords. In: Grimalt, J.O., Dorronsoro, C. (Eds.). Organic geochemistry: developments and applications to energy, climate, environment and human history. A.I.G.O.A., Donostia-San Sebastián, pp. 253–254.

Rowland, S.J., Robson, J.N., 1990. The widespread occurrence of highly branched acyclic C_{20}, C_{25} and C_{30} hydrocarbons in recent sediments and biota - a review. Marine Environmental Research 30, 191–216.

Schouten, S., van Driel, G.B., Sinninghe Damsté, J.S., de Leeuw, J.W., 1993. Natural sulphurization of ketones and aldehydes: A key reaction in the formation of organic sulphur compounds. Geochimica et Cosmochimica Acta 57, 5111–5116.

Schouten, S., de Graaf, W., Sinninghe Damsté, J.S., van Driel, G.B., de Leeuw, J.W., 1994. Laboratory simulation of natural sulphurization. II. Reaction of multi-functionalized lipids with inorganic polysulphides at low temperatures. Organic Geochemistry 22, 825–834.

Schouten, S., Hoefs, M.J.L., Sinninghe Damsté, J.S., 2000. A molecular and stable carbon isotopic study of lipids in Late Quaternary sediments from the Arabian Sea. Organic Geochemistry 31, 509–521.

Schouten, S., Rijpstra, W.I.C., Kok, M., Hopmans, E.C., Summons, R.E., Volkman, J.K., Sinninghe Damsté, J.S., 2001. Molecular organic tracers of biogeochemical processes in a saline meromictic lake (Ace Lake). Geochimica et Cosmochimica Acta 65, 1629–1640.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., de Leeuw, J.W., Schenck, P.A., 1988. Origin of organic sulphur compounds and sulphur-containing high molecular weight substances in sediments and immature crude oils. Organic Geochemistry 13, 593–606.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Kock-van Dalen, A.C., de Leeuw, J.W., Schenck, P.A., 1989a. Quenching of labile functionalised lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. Geochimica et Cosmochimica Acta 53, 1343–1355.

Sinninghe Damsté, J.S., van Koert, E., Kock-van Dalen, A.C., de Leeuw, J.W., Schenck, P.A., 1989b. Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils. Organic Geochemistry 14, 555–567.

Sinninghe Damsté, J.S., Rijpstra, W.I.C., Schouten, S., Peletier, H., van der Maarel, M.J. E.C., Gieskes, W.W.C., 1999. A C_{25} highly branched isoprenoid alkene and C_{25} and C_{27} n-polyenes in the marine diatom *Rhizosolenia setigera*. Organic Geochemistry 30, 95–100.

Sinninghe Damsté, J.S., Muyzer, G., Abbas, B., Rampen, S.W., Massé, G., Allard, W.G., Belt, S.T., Robert, J.M., Rowland, S.J., Moldowan, J.M., Barbanti, S.M., Fago, F.J., Denisevich, P., Dahl, J., Trindade, L.A.F., Schouten, S., 2004. The rise of the rhizosolenid diatoms. Science 304, 584–587.

Squier, A.H., Hodgson, D.A., Keely, B.J., 2004. Structures and profiles of novel sulfur-linked chlorophyll derivatives in an Antarctic lake sediment. Organic Geochemistry 35, 1309–1318.

Stuiver, M., Reimer, P.J., Braziunas, T.F., 1998. High-precision radiocarbon age calibration for terrestrial and marine samples. Radiocarbon 40, 1127–1151.

Sun, M.Y., Wakeham, S.G., 1994. Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea basin. Geochimica et Cosmochimica Acta

58, 3395–3406.

Trull, T.W., Gibson, J.A.E., Beaumont, K.L., Bowman, J., Burton, H.R., Davidson, A.T., Guichard, A., Kirkwood, J.M., McMinn, A., Nichols, P.D., Skerratt, J.H., Summons, R.E., Swadling, K.M., Volkman, J.K., 2001. Site holds promise for ecosystem and biogeochemical investigations. Eos 82, 306 & 311.

Venkatesan, M.I., 1988. Organic geochemistry of marine sediments in Antarctic region: Marine lipids in McMurdo Sound. Organic Geochemistry 12, 13–27.

Volkman, J.K., Allen, D.I., Stevenson, P.L., Burton, H.R., 1986. Bacterial and algal hydrocarbons in sediments from a saline Antarctic Lake, Ace Lake. Organic Geochemistry 10, 671–681

Volkman, J.K., Barrett, S.M., Dunstan, G.A., 1994. C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. Organic Geochemistry 21, 407–413.

Wakeham, S.G., Sinninghe Damsté, J.S., Kohnen, M.E.L., de Leeuw, J.W., 1995. Organic sulfur compounds formed during early diagenesis in Black Sea sediments. Geochimica et Cosmochimica Acta 59, 521–533.

Werne, J.P., Hollander, D.J., Behrens, A., Schaeffer, P., Albrecht, P., Sinninghe Damsté, J.S., 2000. Timing of early diagenetic sulfurisation of organic matter: a precursor-product relationship in Holocene sediments of the anoxic Cariaco Basin, Venezuela. Geochimica et Cosmochimica Acta 64, 1741–1751.

Wraige, E.J., Belt, S.T., Lewis, C.A., Cooke, D.A., Robert, J.-M., Massé, G., Rowland, S.J., 1997. Variations in structures and distributions of C_{25} highly branched isoprenoid (HBI) alkenes in cultures of the diatom, *Haslea ostrearia* (Simonsen). Organic Geochemistry 27, 497–505.

Figure captions

Fig. 1. Capillary gas chromatograms of hydrocarbon fractions from sediment depths 2–3 cm, 8–9 cm and 36–38 cm from a core recovered from the Small Meromictic Basin (SMB) of Ellis Fjord. The structure of the $C_{25:2}$ HBI alkene **I** is shown in the insert.

Fig. 2. Mass spectra of (**a**) the $C_{25:2}$ HBI alkene identified as 2,10,14-trimethyl-6-methylene-7-(3'-methylpent-1-enyl)pentadecane, (**b**) the C_{25} HBI thiane **II**, and (**c**) one of the sulfurised dimers of the C_{25} HBI alkene.

Fig. 3. Hypothetical reaction scheme showing how the sulfurised HBIs can be formed from the HBI alkadiene **I** biosynthesized by the sea-ice diatoms. Intramolecular sulfur incorporations results, after appropriate isomerization of one of the double bonds, into the formation of HBI thiane **II.** Intermolecular sulfur incorporation leads to formation of an HBI dimer which can subsequently be incorporated into larger macromolecular aggregates (represented by X).

Fig. 4. Speciation of the HBI skeleton amongst different compound types at various horizons of a core from the MSB of Ellis Fjord.

Fig. 5. Depth profile of age (in calendar years BP) and the degree of the sulfurisation of the C_{25} HBI skeleton (defined as the percentage of the $C_{25:2}$ HBI alkene relative to all C_{25} HBIs quantified; see Table 1). Note the complete disappearance of the $C_{25:2}$ HBI alkene

(the only non-sulfurised form of HBIs) at a depth of 12 cm corresponding to an age of about 500 calendar years.

List of Tables

Table 1: TOC, $\delta^{13}C$ of TOC and HBI concentrations (alkene and sulfurised forms) in the Ellis Fjord sediment core

Table 2: Radiocarbon ages (uncorrected for reservoir effect) for the Ellis Fjord core

Table 3: Stable carbon isotopic compositions (‰ vs. VPDB) of TOC, HBIs (alkene and sulfurised forms) and two of the major sterenes.

Table 1. TOC and HBI concentration (alkene and sulfurised forms) in the Ellis Fjord sediment core

Depth (cm)	TOC (%)	$\delta^{13}C_{TOC}$ (‰)	Concentration ($\mu g\ g^{-1}$ TOC)				
			alkene	thiane	dimers	polar S-bound	Total HBI + S-HBI
0–1	1.4	-18.2	480	30	18	320	850
2–3	3.2	-17.7	1100	9	17	94	1220
4–5	3.9	-18.4	2300	5	16	160	2480
6–7	4.9	-18.5	77	23	33	150	280
8–9	4.8	-18.4	35	71	93	250	450
10–11	8.7	-17.8	8	110	110	200	430
12–13	6.2	-18.3	nd	76	65	330	470
14–15	5.2	-17.5	nd	120	110	430	660
16–17	9.6	-17.5	nd	63	58	300	420
18–19	7.7	-18.4	nd	110	150	330	590
36–38	6.6	-17.1	nd	310	430	980	1720
56–58	7.3	-17.4	nd	180	250	570	1000
76–78	15.3	-17.5	nd	46	24	130	200
92–94	12.0	-18.3	nd	56	22	210	290
96–98	11.5	-17.7	nd	110	77	280	470
100–102	11.2	-18.2	nd	66	53	200	320
104–106	8.8	-18.0	nd	15	nd	160	175
108–110	6.4	-17.7	nd	12	nd	240	250

nd: not detected

Table 2: Radiocarbon ages (uncorrected for reservoir effect) for the Ellis Fjord core

Depth (cm)	^{14}C age (yr BP)
0-1	520
20-22	1500
40-42	1740
60-62	2200
80-82	2440
110-112	3300

Table 3: Stable carbon isotopic compositions (‰ vs. VPDB) of HBIs (alkene and sulfurised forms) and two of the major sterenes.

depth (cm)	TOC	alkene	thiane	dimer 1	dimer 2	dimer 3	$C_{29:2}\ \Delta^{3,5}$	$C_{28:3}$
2–3	-17.7	-9.1	na	na	na	na	na	na
6–7	-18.5	-9.4	-15.3	-11.0	-10.9	na	-15.1	-23.6
10–11	-17.8	nd	-12.4	-10.7	-10.4	-10.8	-14.1	-25.8
36–38	-17.1	nd	-10.4	-8.4	-8.6	-8.9	-8.9	-23.6
96–98	-17.7	nd	-11.1	-8.9	-8.9	-9.6	-8.3	-17.3

na: not analysed; nd: not detected