

Volume 72, Number 14		July 15, 2008		
 <b>Geochimica et Cosmochimica Acta</b> JOURNAL OF THE GEOCHEMICAL SOCIETY AND THE METEORITICAL SOCIETY				
Executive Editor: FRANK A. PODOSKA		Editorial Manager: LINDA TROWER Editorial Assistants: KAREN KELLY KATHY SMITH		
Webmaster: ROBERT H. NICKEL, Jr. Production Manager: CHRIS ADAMS				
<b>ASSOCIATE EDITORS:</b> ROBERT C. ALLEN ROBERT C. ALP YUL ANDEL CAROL ANNETT MERVIN RAY MATHIAS LINDA G. BRONKHORST THOMAS S. BRONKHORST JAY A. BRONKHORST ALAN D. BRONKHORST DAVID J. BRONKHORST ROBERT C. BRONKHORST WILLIAM H. CANN THOMAS CHAKRABORTY ANNE CLARKE DAVID R. COLE	<b>EDITORIAL BOARD:</b> JOHN CHERRY CHRISTOPHER DAVIDSON ZAFARUDDIN DAVAN JAMES FARQUHAR FREDERICK A. FERRY SUSAN GLAZIER JEROME R. HALL H. ROBERT HAPPEL GORDON R. HILL GREGORY F. HUBERT JAMES HUBERT JIN-YOUNG LEE KAREN JONSSON CLARE JONSSON NARUKI KITA CHRISTOPHER KRETZ	<b>EDITORIAL BOARD:</b> RUSSELL KORTZ STEPHAN M. KRAUSE S. KRUMHOLTZ ALEXANDER N. KURTZ JAMES KURTZ TYRONNE LEE GREGORY A. LUGAN TIMOTHY J. LYONS MICHAEL L. MANNING BERNARD MARET JAMES MATHIAS JAMES McMANUS ANTHONY MURPHY MARTIN A. MURPHY JACK J. MURPHY DAVID W. MURPHY	<b>EDITORIAL BOARD:</b> ALONSO MUCCI BOB MURPHY HIDEO NAGAIWA MARTIN NICKEL PETER A. OGDEN ERIC H. OHLBERG NANCY PITZARULO MARK REAGAN W. URS REAGAN EDWARD M. REAGAN J. KEVIN RUSSELL SAM S. RUSSELL F. J. RYAN JACQUES SCHOTT JUDITH SHERWOOD THOMAS J. SIMON	<b>EDITORIAL BOARD:</b> J. S. SINGER DONALD L. STARK GABRIELE STROTH DIMITRI A. SYRINAKIS MICHAEL J. TOLAN PETER TRUESDELL DENIS T. VAUGHAN RICHARD J. WALKER JAMES A. WARDEN RUSSELL WALKER RAY A. WALKER RAY A. WALKER CHEN ZHU
Volume 72, Number 14		July 15, 2008		
<b>Articles</b>				
K. H. LEMKE, T. M. SEWARD: Solvation processes in steam: <i>Ab initio</i> calculations of ion-solvent structures and clustering equilibria	3293			
K. S. TANWAR, S. C. PETITO, S. K. GHOSH, P. J. ENO, T. P. TRAINER: Structural study of Fe(II) adsorption on hematite(1102) ...	3311			
J. R. HOUSTON, J. L. HERRERO, R. S. MAXWELL, S. A. CARROLL: Association of dissolved aluminum with silica: Connecting molecular structure to surface reactivity using NMR	3326			
J.-F. BOUY, A. R. FELMY: On the protonation of oxo- and hydroxo-groups of the goethite ( $\alpha$ -FeOOH) surface: A FTIR spectroscopic investigation of surface O-H stretching vibrations	3338			
G. K. DEUSCHEL, D. ENERSON, R. SITKA, P. SUCHOCKI, G. W. LITTELL III: Low-oxygen and chemical kinetic constraints on the geochemical niche of neutrophilic iron(II) oxidizing microorganisms	3358			
O. W. DICKWORTH, J. R. BARBAR, G. SIVITER: Sorption of ferric iron from ferrioxamine B to synthetic and biogenic layer type manganese oxides	3371			
E. A. HAACK, C. T. JOHNSTON, P. A. MAHER: Mechanisms of siderophore sorption to smectite and siderophore-enhanced release of structural Fe <sup>3+</sup>	3381			
C. SOTER, C. P. SLOMP, M. A. CHARETTE, K. TUNICAY, C. MIELLE: Flow and nutrient dynamics in a subterranean estuary (Waquoit Bay, MA, USA): Field data and reactive transport modeling	3398			
O. ROHNEL, E. SHOLKOVITZ, M. CHARETTE, K. J. EDWARDS: Iron isotope fractionation in subterranean estuaries	3413			
C. SOTER, P. V. CAPPELLAS, P. REONIER: Surface complexation effects on phosphate adsorption to ferric iron oxyhydroxides along pH and salinity gradients in estuarine and coastal aquifers	3431			
B. LAZAR, Y. WEINSTEIN, A. PANTAN, E. MAGAL, D. BROU, Y. KOLODNY: Ra and Th adsorption coefficients in lakes - Lake Kinneret (Sea of Galilee) "natural experiment"	3446			

Continued on outside back cover

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



## Investigating pathways of diagenetic organic matter sulfurization using compound-specific sulfur isotope analysis

Josef P. Werne<sup>a,b,\*</sup>, Timothy W. Lyons<sup>c</sup>, David J. Hollander<sup>d</sup>, Stefan Schouten<sup>a</sup>,  
Ellen C. Hopmans<sup>a</sup>, Jaap S. Sinninghe Damsté<sup>a</sup>

<sup>a</sup> *Department of Marine Organic Geochemistry, Royal Netherlands Institute for Sea Research (NIOZ),  
P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands*

<sup>b</sup> *Large Lakes Observatory and Department of Chemistry & Biochemistry, University of Minnesota Duluth, Duluth, MN 55812, USA*

<sup>c</sup> *Department of Earth Sciences, University of California, Riverside, CA 92521, USA*

<sup>d</sup> *College of Marine Science, University of South Florida, St. Petersburg, FL 33701, USA*

Received 28 November 2007; accepted in revised form 30 April 2008; available online 15 May 2008

---

### Abstract

We present the results of compound-specific sulfur isotope analyses performed on organic sulfur compounds (OSCs) isolated from sediments deposited in the euxinic Cariaco Basin, Venezuela. Individual OSCs (sulfurized highly branched isoprenoids and malabaricatriene) have sulfur isotope compositions of ca.  $-15\text{‰}$ , which is  $^{34}\text{S}$  enriched by 5–15‰ relative to coeval bulk organic and inorganic sulfur pools. These observed differences in the sulfur isotope composition of bulk organic sulfur in the kerogen and bitumen pools and individual OSCs demonstrate that there are multiple pathways of organic sulfur formation operating simultaneously in marine sediments. Comparison of our measured compound-specific sulfur isotope data with values predicted using simple isotopic mass balance assumptions suggests that the sulfurization process likely involves multiple sources of inorganic sulfur. Further, the isotopic composition of these various precursor inorganic sulfur species and the specific pathway of sulfur incorporation into organic matter (OM) impart distinct isotopic compositions to the resulting organic sulfur compounds. These data represent the first compound-specific sulfur isotope measurements made in marine sediments, and demonstrate the utility of compound-specific sulfur isotope analysis in identification of inorganic sulfur sources for OM sulfurization and tracking pathways of sulfur incorporation, which will lead to a more complete understanding of diagenetic sulfurization of OM.

© 2008 Elsevier Ltd. All rights reserved.

---

### 1. INTRODUCTION

Sulfurization of organic matter (OM) is a globally significant biogeochemical process. Organic sulfur (OS) is second only to pyrite in its quantitative significance as a reduced sulfur pool in the sedimentary environment (Berner and Raiswell, 1983; Garrels and Lerman, 1984; Anderson and Pratt, 1995; Vairavamurthy et al., 1995), frequently reach-

ing values of 35%, and occasionally as high as 80% (Zaback and Pratt, 1992; Anderson and Pratt, 1995; Werne et al., 2004). Diagenetic sulfurization can also affect the preservation of OM, enhancing the preservation potential of specific biomarker lipids (see Sinninghe Damsté and deLeeuw, 1990, for a review) and carbohydrates (van Kaam-Peters et al., 1998; Kok et al., 2000a; van Dongen et al., 2003a,b). Sulfurization may therefore induce a bias in biomarker distributions (e.g., Kohnen et al., 1991a) since OSC are also less amenable to traditional geochemical analyses (Kohnen et al., 1991b,c).

OS in sediments has been identified in reduced forms as thiols (Vairavamurthy and Mopper, 1987) as well as sulfide and polysulfide linkages within and between compounds in

---

\* Corresponding author. Address: Large Lakes Observatory and Department of Chemistry & Biochemistry, University of Minnesota Duluth, Duluth, MN 55812, USA. Fax: +1 218 728 6979.

E-mail address: [jwerne@d.umn.edu](mailto:jwerne@d.umn.edu) (J.P. Werne).

the macromolecular matrix of organic-rich deposits (Aizenshtat et al., 1983; Sinninghe Damsté et al., 1988; Adam et al., 1991; Kohlen et al., 1991b; Schouten et al., 1995b). S–S bonds are known to be cleaved more easily than C–S or C–C bonds (Orr, 1986; Eglinton et al., 1990; Baskin and Peters, 1992; Aizenshtat et al., 1995; Koopmans et al., 1998), thus the form and content of the sulfur present in the original organic-rich sedimentary deposit has a significant impact on the kinetics of petroleum formation (Lewan, 1993, 1998; Krein and Aizenshtat, 1995; Putschew et al., 1998). Oxidized forms of OS include ether-bonded sulfates, sulfonates and sulfoxides (Vairavamurthy et al., 1994, 1997; Schouten et al., 1995a). Although it is generally agreed that diagenetic OM sulfurization is primarily abiotic (Dinur et al., 1980; Sinninghe Damsté and deLeeuw, 1990; Amrani and Aizenshtat, 2004a), biological mechanisms cannot be ruled out, especially for compounds such as sulfonates (Vairavamurthy et al., 1994). Furthermore, the species of inorganic sulfur incorporated into OM are either products or byproducts of various microbial and/or microbially mediated reactions. In all likelihood, many different reduced and oxidized sulfur species play a role in OM sulfurization depending on specific environmental conditions. Unfortunately, the extremely reactive nature of key intermediate inorganic sulfur species such as polysulfides and thiosulfate makes sampling and analysis difficult, and thus their contributions to OM sulfurization are difficult to quantify.

There are two basic pathways by which OS is formed. The first is assimilatory sulfate reduction, which is the active uptake of sulfate into the cell followed by its reduction to sulfide to produce amino acids and other sulfur-requiring cellular components. These components, primarily the amino acids cysteine and methionine, are formed primarily by the reduction of sulfate from the environment to sulfide; however, other forms of sulfur can be incorporated (Le Faou et al., 1990; Thauer and Kunow, 1995). This 'primary biogenic' sulfur typically represents about 0.5–1% of the dry weight of the living biomass (Zehnder and Zinder, 1980). Based primarily on S-isotope mass balance estimates, ~10–25% of sedimentary organic sulfur is thought to be primary biogenic sulfur, with the proportion of biogenic sulfur decreasing during ongoing diagenesis due to the greater lability of amino acids compared to other forms of OM (Anderson and Pratt, 1995; Wakeham et al., 1997; Werne et al., 2003).

The second, and more significant, pathway is the incorporation of reduced sulfur into OM during diagenesis. The mechanisms of diagenetic sulfur incorporation into OM are still debated but fundamentally require that the sulfur is derived from pore water sulfide produced via (bacterial) sulfate reduction, either directly or via reactive intermediates (Aizenshtat et al., 1983; Brassell et al., 1986; LaLonde et al., 1987; Kohlen et al., 1989, 1991b,c; Vairavamurthy and Mopper, 1989; Vairavamurthy et al., 1992; Anderson and Pratt, 1995; Adam et al., 1998; Filley et al., 2002; Amrani and Aizenshtat, 2004a,b). All of the possible inorganic sulfur sources, including sulfate ( $\text{SO}_4^{2-}$ ), sulfide ( $\Sigma\text{H}_2\text{S}$ , including  $\text{HS}^-$  and  $\text{S}^{2-}$ ), and reactive intermediates such as elemental sulfur ( $\text{S}^0$ ), polysulfides ( $\text{S}_x^{2-}$ ,  $x > 1$ ) and

thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ) can be produced in the natural environment by microbial processes. Intermediates can also be produced by abiotic processes, such as the formation of polysulfides via reactions between iron oxides and bisulfide or between dissolved sulfide and elemental sulfur (Pyzik and Sommer, 1981). Thus the fundamental control on the biogeochemistry of sulfur isotopes, and specifically on the sulfur isotope composition of OM, is the oxidative and reductive cycling of different forms of sulfur by microorganisms. Microbial sulfur isotope fractionation has been thoroughly reviewed (Canfield, 2001; Brückert, 2004; Brunner and Bernasconi, 2005). We will therefore present only a brief summary here as required for our discussion of organic sulfur isotopes below and refer the interested reader to these other papers for a more detailed treatment of the subject.

The sulfur isotope composition of sedimentary OS is dependent on (1) the sulfur isotope composition of the source sulfur, specifically reactive inorganic sulfur species, and (2) any isotopic fractionations associated with OM sulfurization. There is generally little sulfur isotope fractionation associated with assimilatory sulfate reduction (~1–3‰, Kaplan and Rittenberg, 1964; Trust and Fry, 1992), so this primary biogenic sulfur typically has an isotope composition similar to the ambient dissolved sulfate, which is ~+21‰ for modern seawater (Kaplan and Rittenberg, 1964; Rees et al., 1978; Böttcher et al., 2000). In contrast, the sulfur isotope composition of diagenetically formed OS is typically  $^{34}\text{S}$  depleted relative to primary biogenic sulfur by 20–60‰ because it is derived ultimately from pore water sulfide (Anderson and Pratt, 1995; Werne et al., 2004), which is  $^{34}\text{S}$  depleted relative to sea water sulfate due to fractionation associated with dissimilatory sulfate reduction (Chambers and Trudinger, 1979; Habicht and Canfield, 1997, 2001; Brückert et al., 2001; Detmers et al., 2001). Because isotope fractionations associated with incorporation of inorganic sulfur species into OM during diagenesis are generally believed to be small (Price and Shieh, 1979; Fry et al., 1984, 1986, 1988) it is thought that diagenetic OS will be isotopically similar to the inorganic sulfur species from which it is derived. It should be noted, however, that the common assumption of negligible fractionation during incorporation of inorganic S into OM is being challenged (cf. Amrani and Aizenshtat, 2004b).

One avenue of investigation that can provide insight into sedimentary sulfur cycling is the use of stable sulfur isotope analysis to track pathways of sulfur transformations between various inorganic and organic forms (Mossman et al., 1991; Zaback and Pratt, 1992; Henneke et al., 1997; Passier et al., 1997; Canfield et al., 1998; Werne et al., 2003). Attempting to understand the relationships between organic and inorganic sulfur species, particularly from an isotopic perspective, has been difficult because bulk OS represents a mixture of compounds derived from many sources by different pathways. Recent analytical advances in stable sulfur isotope geochemistry have provided new avenues of investigation previously unavailable. Isotopic measurement of various forms of OS in conjunction with measurement of inorganic sulfur species has enhanced our understanding of the complex role of OS in

sedimentary sulfur cycling and the pathways of its formation (Werne et al., 2003, 2004). For example, molecular sulfur isotope analysis suggests that alkylthioadamantanes are products of thermochemical sulfate reduction (Hanin et al., 2002). In this paper we use compound-specific sulfur isotope analysis to explore pathways of OM sulfurization in marine sediments through a study of OS compounds isolated from Holocene and latest Pleistocene sediments of the Cariaco Basin, Venezuela.

## 2. MATERIALS AND METHODS

### 2.1. Site description, sampling, age control

The Cariaco Basin is a pull-apart structure located on the continental shelf immediately north of Venezuela (Fig. 1). It consists of two sub-basins, with a maximum depth of about 1400 m, separated by a saddle at 900 m. The basin is currently anoxic below ~300 m water depth. Samples were collected at Ocean Drilling Program (ODP) Site 165 (Core 1002B) on the western side of the saddle at about 900 m water depth (Shipboard Scientific Party, 1997; Lyons et al., 2003). Sediments were frozen immediately after collection, stored frozen until analysis, and freeze-dried. Dried samples were homogenized, and splits were taken for the various analyses. Bulk analyses are described in Werne et al. (2003). Age control for this study was provided by a suite of accelerator mass spectrometer (AMS)  $^{14}\text{C}$  dates on individual planktonic foraminifera from core PL07-39PC (Lin et al., 1997), which were correlated to 1002B using magnetic susceptibility (Shipboard Scientific Party, 1997; Werne et al., 2000a). The study interval spans the last ~12  $^{14}\text{C}$  kyr (14.5–15 calendar kyr; Peterson et al., 1991; Hughen et al., 1996a,b, 1998; Lin et al., 1997; Lyons et al., 2003).

One of the inherent difficulties in measuring the sulfur isotope composition of specific organic compounds is that they typically have only 1 or 2 sulfur atoms, compared to ca. 20–30 carbon atoms. Thus, in order to obtain sufficient

quantities of S for isotopic analysis, we must use large amounts of sediment (i.e., 60–300 g). Because CSSIA requires such large samples, a pilot study was undertaken using easily available samples before we attempted to apply our new CSSIA methodology to the more-limited Cariaco Basin core samples obtained from the Ocean Drilling Program. For this pilot study, we used fresh outcrop material from the Miocene Monterey Formation in which OS compounds had previously been identified (Shell Beach sampling site, see Schouten et al., 1995b for details). These samples contained multiple OSC that would have to be separated prior to isotopic analysis, which made them appropriate “test” samples to prepare for the Cariaco materials. Furthermore, the Monterey samples contained OSC with both 1 and 2 S atoms, so we could explore the limits of the method by analysis of both compounds.

### 2.2. Extraction, separation, and preliminary analysis of OSCs

Dried sediments (ca. 200–250 g dry weight for the Cariaco and 450 g for the Monterey) were Soxhlet-extracted with a 2:1 methylene chloride:methanol solution for 48 h to obtain the total lipid extract (TLE). The TLE was then fractionated on an alumina column (2 × 25 cm, alumina activated for 2 h at 150 °C) by sequential elution as follows: the apolar fraction in 150 ml hexane:CH<sub>2</sub>Cl<sub>2</sub> (9:1 v/v), the polar fraction in CH<sub>2</sub>Cl<sub>2</sub>:MeOH (1:1), and any remaining polar organic compounds were eluted using methanol. Column dimensions and solvent volumes were proportionally greater for the larger Monterey sample. For this study, only the apolar fraction was analyzed further. OSCs were identified and quantified via gas chromatography (GC) using flame ionization (FID), sulfur-specific flame photometric (FPD), and mass spectrometric (MS) detectors. GC analyses were performed using a Hewlett-Packard 5890 with an on-column injector fitted with a CP Sil-5 fused silica capillary column (25 m × 0.32 mm, film thickness 0.25 μm).

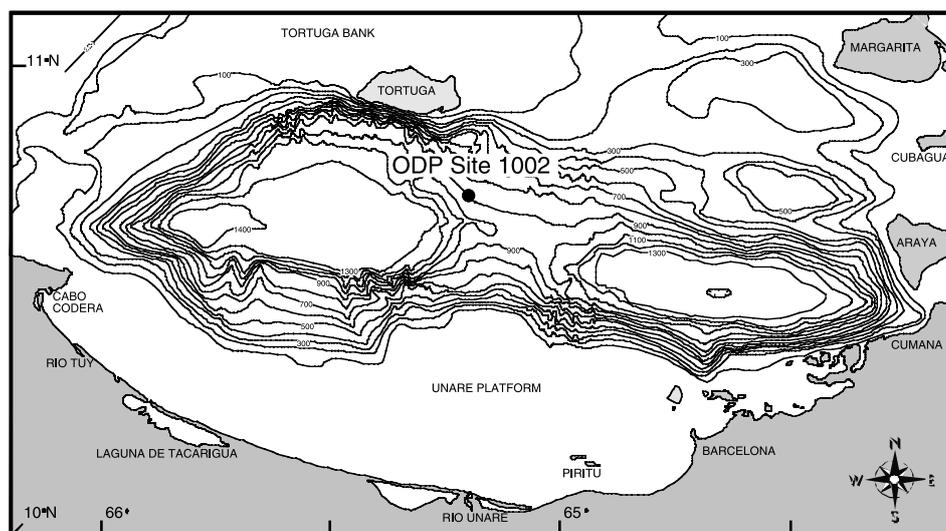


Fig. 1. Map showing location of ODP core site 1002B in the Cariaco Basin.

Samples were injected at 70 °C, with the oven temperature programmed to increase at a rate of 10 °C/min to 130 °C, then at 4 °C/min to 310 °C, maintaining the final temperature of 310 °C for 20 min. Helium was used as the carrier gas. An HP 5890 GC interfaced with a VG Autospec-UltimaQ mass spectrometer was used for GC–MS analysis. Operating conditions were as follows: mass range 50–800, cycle time 1.8 s, 70 eV ionizing energy. Temperature programming was the same as for GC analysis, and we used a CP Sil-5 fused silica capillary column (25 m × 0.32 mm, film thickness 0.25 µm) with helium as the carrier gas.

### 2.3. Isolation and isotopic analysis of OSCs

At present, there is no method available for simultaneous analysis of the sulfur isotope composition of multiple OS compounds in the same organic fraction (e.g., via isotope ratio monitoring GC–MS; irmGC–MS) due to the low relative abundance of S in OSC (typically 1 or 2 S atoms per 30 atoms of C). Thus, we had to isolate the individual OSC prior to isotopic analysis by semi-preparative high performance liquid chromatography (prep-HPLC). Prep-HPLC was performed using an HP (Palo-Alto, CA, USA) 1100 series LC equipped with an auto-injector. Separation was achieved on a SymmetryPrep C<sub>18</sub> HPLC column (7.8 × 150 mm, 7 µm; Waters Corporation, Milford, MA, USA). Injection volumes varied from 25 to 100 µl. OSC were eluted with 2 ml/min of MeOH:acetone (1:1, v/v). Total run time was 60 min. The eluent was collected, and the resulting fractions were analyzed by flow injection analysis-atmospheric pressure chemical ionization mass spectrometry (FIA-APCI-MS, method modified from Smitenberg et al., 2002) and GC-FPD to confirm adequate separation of OSC. Initial tests of prep-HPLC on a suite of standards containing thiophenes and thiolanes demonstrated quantitative recovery, thus no S-isotopic fractionation is expected.

The isotopic composition of individual OSC were analyzed by combustion in tin boats with a V<sub>2</sub>O<sub>5</sub> catalyst in a continuous flow elemental analyzer connected to a Finnigan 252 stable isotope ratio monitoring mass spectrometer (EA-irmMS) at the Indiana University Bloomington-Stable Isotope Research Facility (IUB-SIRF). Samples were transferred from LC vials to tin boats dissolved in hexane. Excess hexane was then evaporated, leaving the OSC adsorbed to the V<sub>2</sub>O<sub>5</sub> catalyst in the tin boats. Sulfur isotope compositions are expressed as per mil (‰) deviations from V-CDT using the conventional delta notation. Measurements were calibrated directly against multiple laboratory standards, including NBS-127 and EMR-CP (+20.00‰ and –1.07‰ V-CDT, respectively). The standard deviation for replicate standard analysis was better than ±0.1‰. Due to the low abundance of S in our compound-specific samples, we were forced to use the entire sample available for each isotopic measurement, preventing rigorous error analysis of the actual sample sulfur isotope values. We therefore conservatively estimate the errors to be ±2.5‰.

## 3. RESULTS

### 3.1. Isolation and isotopic analysis of Monterey Formation OSC

OSC sufficient for sulfur isotopic analysis were isolated from a large sediment sample from the Miocene Monterey Formation. The apolar fraction of the total lipid extract from this sediment contained two distinct OSC, a disulfide with a bicyclic carbon skeleton (Schouten et al., 1995b, compound **III** Fig. 2, though it has been suggested that the structure may actually be a “spiro-type” structure such as compound **IIIa**, Gug et al., 2007) and a hopanoid thiophene (Valisolalao et al., 1984; compound **IV**; Fig. 2). Because our method of S-isotope analysis required that only

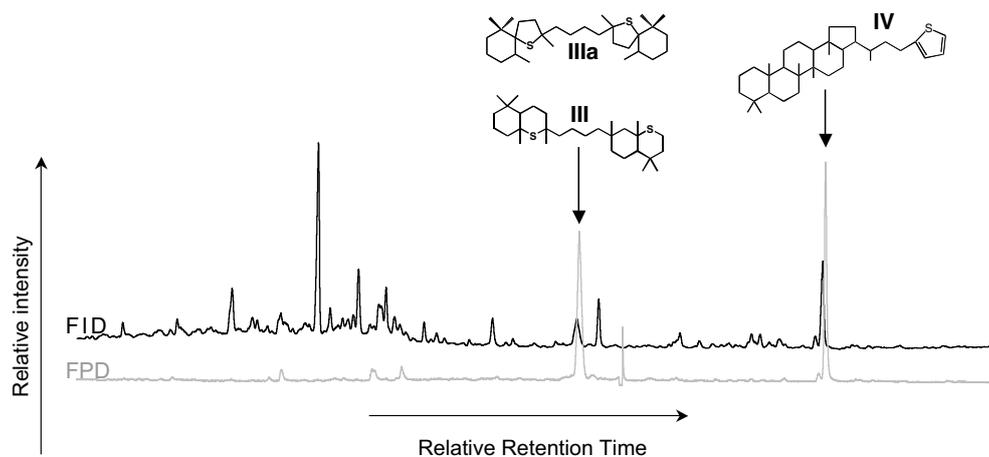


Fig. 2. Gas chromatograms of the apolar fraction of the total lipid extract from the Monterey Formation sample. FID and S-specific FPD traces before separation show multiple OSC in the same fraction, a C<sub>30</sub> triterpenoid dithiane (**III**) and a hopanoid thiophene (**IV**). Structure of compound **III** was first tentatively identified as shown by Schouten et al. (1995) based on mass spectral data, however, this structure has recently been demonstrated to be incorrect by coinjection with a synthetic standard, and a new “spiro-type” structure (compound **IIIa**) proposed (Gug et al., 2007).

one OSC is present in a given fraction, we first separated the different OSC by prep-HPLC. Base peak chromatograms for the Monterey sample shows separation between the various OSC that was sufficient to isolate the relevant OSC for isotopic analysis (Fig. 3). The fraction containing the disulfide was recognized by its protonated molecule ( $[M+H]^+$ ) at  $m/z$  479 and that containing the hopanoid thiophene by its protonated molecule at  $m/z$  509 (Fig. 3). Analysis by GC (with both FID and FPD detection) of the fractions collected after semi-preparative HPLC separation demonstrate clearly that the hopanoid thiophene and disulfide were separated from each other, as well as any other S-containing compounds that may have initially been present in small

amounts (Fig. 4). Furthermore, analysis of all other fractions collected demonstrated that OSC were only present in the two fractions shown in Fig. 4, indicating that our separation was quantitative.

Once the two OSC were purified, they were analyzed for their sulfur isotope compositions. The sulfur isotope compositions determined for individual OSC isolated from the Miocene Monterey Formation were  $+5\text{‰}$  and  $+6\text{‰}$  for the hopanoid thiophene and disulfide, respectively. The measured bulk  $\delta^{34}\text{S}_{\text{OS}}$  value for this sample is  $+8\text{‰}$ , slightly  $^{34}\text{S}$ -enriched relative to the individual OSC. Thus, these values are quite reasonable, suggesting that our analytical methods were appropriate.

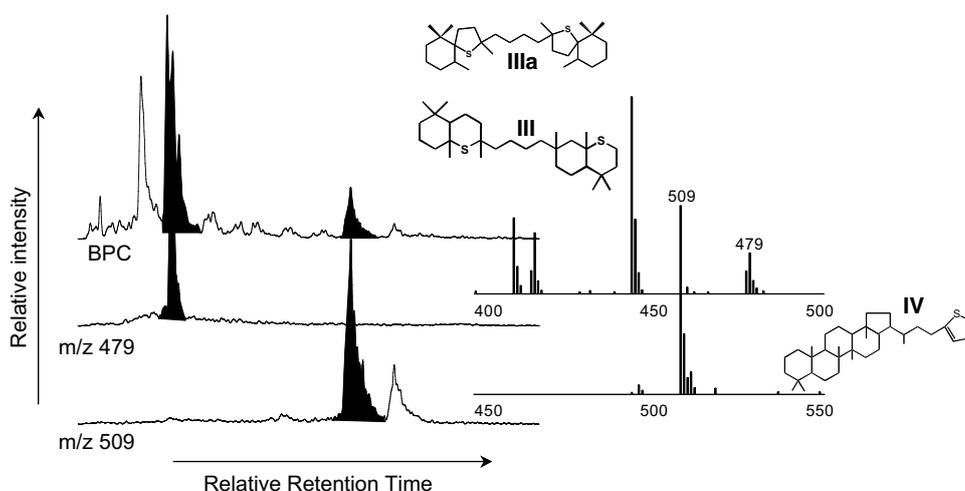


Fig. 3. Mass chromatograms and mass spectra of selected compounds from the apolar fraction of the total lipid extract from the Monterey Formation sample. Basepeak chromatogram shows both OSC, and the specific ion monitoring chromatograms show only the triterpenoid dithiane ( $m/z$  479, upper mass spectrum) and hopanoid thiophene ( $m/z$  509, lower mass spectrum), respectively. Mass spectra both show the  $[M+H]^+$  molecular ions. The two compounds are clearly separable by semi-preparative HPLC.

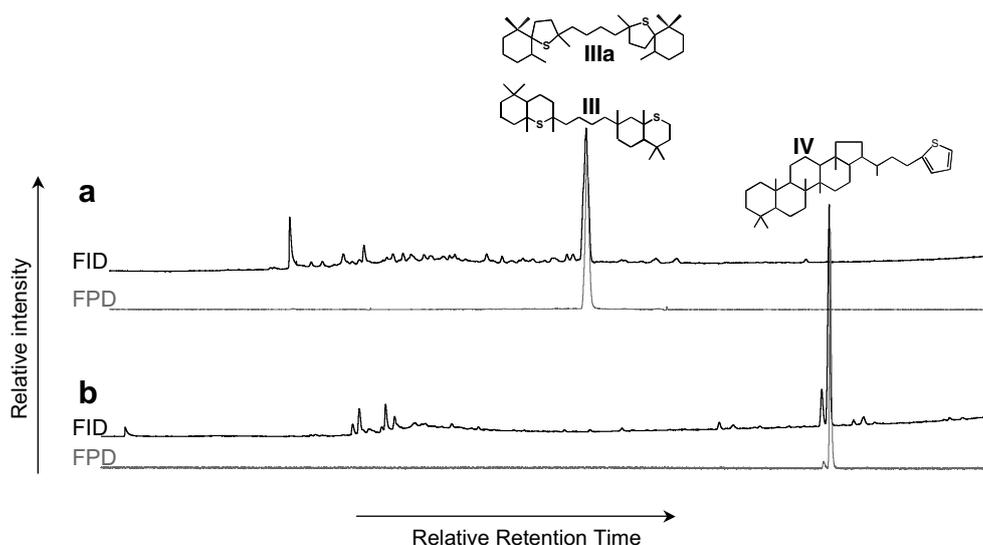


Fig. 4. Gas chromatograms of the apolar fraction of the total lipid extract from the Monterey Formation sample after separation by semi-preparative HPLC. FID (black) and FPD (gray) traces demonstrate that each fraction only contains one OSC. (a) FID and FPD traces of the separated disulfide fraction. (b) FID and FPD traces of the separated hopanoid thiophene fraction.

### 3.2. Cariaco Basin

The apolar fractions of the extracts of the sediments from the Cariaco Basin contained a mixture of OSC, namely the triterpenoid thiane identified by Werne et al. (2000b) and a series of C<sub>25</sub> highly branched isoprenoid thiophenes and thiolanes (HBIs, e.g., compound **V** (Fig. 5), a representative HBI thiophene) identified by Kohnen et al. (1991c). The latter OSC were in very low abundance and had similar polarities and were therefore collected in the same fraction for subsequent analysis as a class. The Cariaco OSC were separated into two fractions using prep-HPLC and FIA-APCI-MS analysis. In these samples, the triterpenoid thiane was identified by its protonated molecule at *m/z* 445 and the HBIs by their protonated molecule at *m/z* 381 (Fig. 5). The separation achieved for Cariaco Basin samples was also complete as demonstrated by GC-FPD analysis (data not shown).

The sulfur isotope composition of the triterpenoid thiane (**II**) identified in Cariaco sediments ranges from  $-12.5\text{‰}$  to  $-17.3\text{‰}$ , and the HBIs (**V**) have values ranging from  $-4.7\text{‰}$  to  $-21.4\text{‰}$  in the depth interval from 230 cm to 600 cm, where there was sufficient abundance of OSC for CSSIA (Fig. 6). It should be noted that the  $\delta^{34}\text{S}$  values of these OSC are much narrower below 300 cm depth, ranging from approximately  $-11\text{‰}$  to  $-17\text{‰}$ , barely above the estimated error of the analysis. These compounds are sulfurized in the upper 300 cm of sediments (Werne et al., 2000b), so these values represent the  $\delta^{34}\text{S}$  of the triterpenoid thiane *after* the sulfurization reaction is almost completed. The measured  $\delta^{34}\text{S}$  values therefore represent the final signal, after the compounds have passed through the complete interval of sulfur incorporation during which it would be expected that  $\delta^{34}\text{S}$  values would vary as a function

of ongoing sulfurization reactions. Unfortunately, due to low compound abundance in the upper 2 m of sediment, we were unable to perform reliable CSSIA in the zone of active sulfurization. Despite this gap, the available data provide insight into the timing and mechanisms of OM sulfurization and confirm the feasibility of performing CSSIA in natural marine sediments by providing a methodological template for future complementary studies.

### 4. DISCUSSION

Previous research in the Cariaco Basin demonstrated that OS in Cariaco Basin sediments is comprised of a mixture of biosynthetic and sulfur added during diagenesis (Werne et al., 2003). Of particular note, a direct precursor–product relationship for a diagenetic sulfurization reaction was identified, in this case involving conversion of a malabaricatriene precursor to a triterpenoid thiane product (compounds **I** and **II**, respectively, Fig. 7A) via inorganic sulfur incorporation at sites of unsaturation (Werne et al., 2000b). The identification of an unambiguous precursor–product relationship for a diagenetic sulfurization reaction in the well-dated Cariaco Basin sedimentary system provided an opportunity to assess OM sulfurization more quantitatively than was previously possible. Because the  $\delta^{34}\text{S}$  values of many inorganic sulfur species were measured and the rate of incorporation of sulfur into OM was known, Werne et al. (2003) were able to use a simple isotopic mass balance model to predict the sulfur isotope composition of diagenetically formed OS compounds (OSC) in Cariaco Basin sediments. Critical assumptions of that model were: (1) that dissolved pore water sulfide was the source of S to the OSC, (2) HS<sup>−</sup> was incorporated directly into OM (i.e., not first converted to other [intermediate] forms and

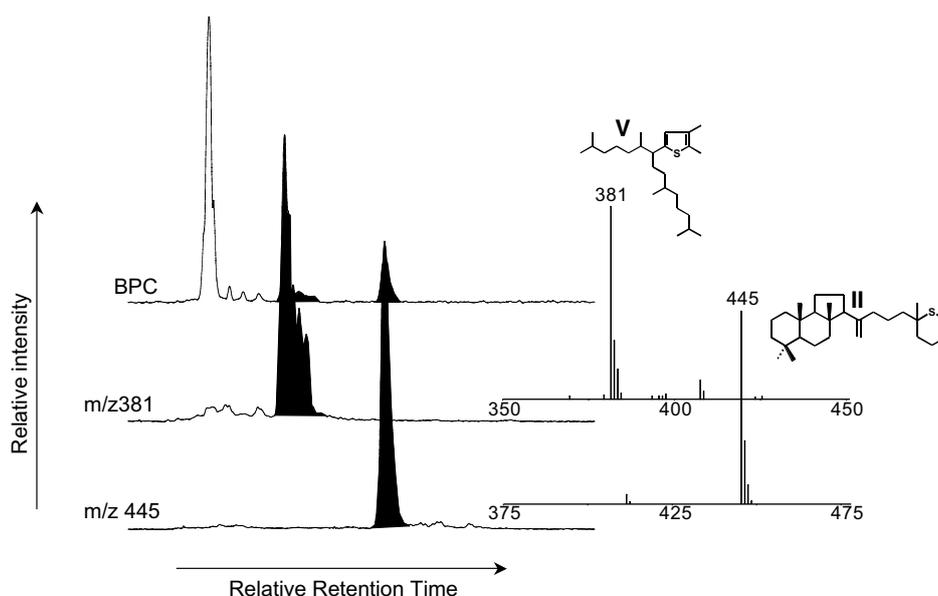


Fig. 5. Mass chromatograms and mass spectra of selected compounds from the apolar fraction of the total lipid extract from the Cariaco Basin. Basepeak chromatogram shows both OSC, and the specific ion monitoring chromatograms show only the HBIs (*m/z* 381, upper mass spectrum, **V**) and triterpenoid thiane (*m/z* 445, lower mass spectrum, **II**), respectively. Mass spectra both show the [M+H]<sup>+</sup> molecular ions. The two compounds are clearly separable by semi-preparative HPLC.

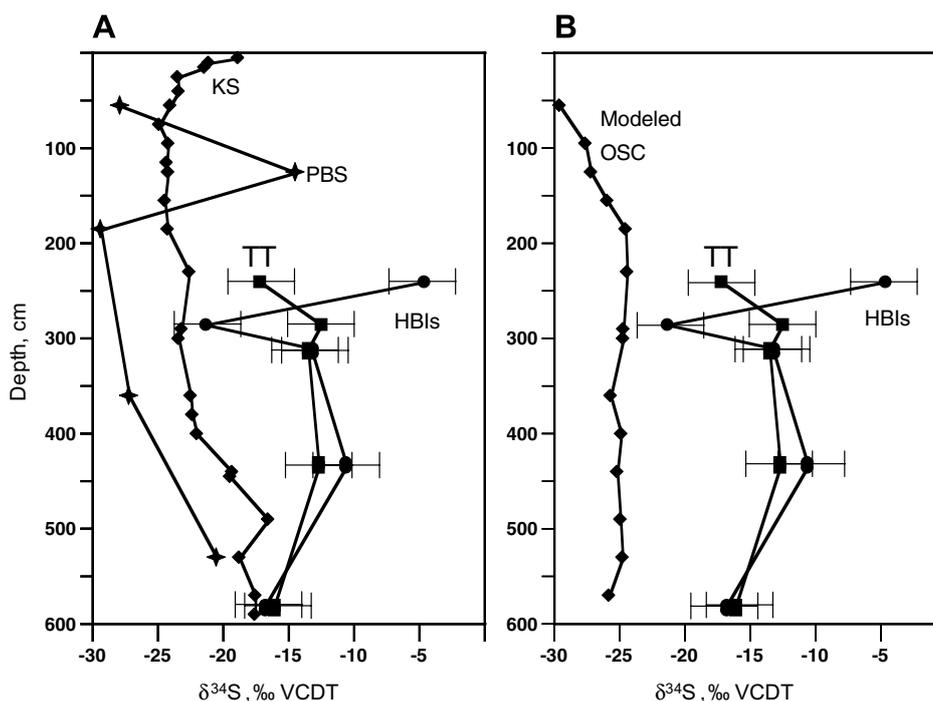


Fig. 6. (A) Sulfur isotopic composition of specific OSC measured by CSSIA and bulk organic sulfur pools in Cariaco Basin sediments. Diamonds, kerogen sulfur (KS); stars, polar bitumen sulfur (PBS); squares, triterpenoid thiane (TT); circles, HBI thiophenes and thiolanes combined (HBIs). Extracted compounds are  $^{34}\text{S}$  enriched relative to bulk phases. The large error for specific compounds is related to their lower concentrations and responses at the lower limit of the instrumentation. KS and PBS data from [Werne et al. \(2003\)](#). (B) Sulfur isotope composition of specific OSC measured by CSSIA (squares, triterpenoid thiane (TT); circles, HBI thiophenes and thiolanes combined (HBIs)) and model results of [Werne et al. \(2003\)](#) (diamonds, modeled OSC). Individual OSC are  $^{34}\text{S}$  enriched relative to model results by  $\sim 10\%$ , suggesting that the assumptions made by [Werne et al. \(2003\)](#) were not correct, and that other processes must be affecting the  $\delta^{34}\text{S}$  value of individual OSC.

then incorporated), and (3) there was no isotopic fractionation associated with the incorporation of  $\text{HS}^-$  into the organic compounds. The measured  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  values were used to estimate the  $\delta^{34}\text{S}$  of the OSC formed at each depth, and the cumulative  $\delta^{34}\text{S}_{\text{OSC}}$  was integrated over the entire depth of formation in the Cariaco Basin sediments. The  $\delta^{34}\text{S}_{\text{OSC}}$  values predicted by the model were very close to the bulk sulfur pools analyzed, suggesting that the assumptions were generally correct, though the model was likely not sensitive enough to identify contributions from S intermediates ([Werne et al., 2003](#)). [Werne et al. \(2003\)](#) hypothesized that the OSC formed from pore waters via direct incorporation into OM with no S-isotope fractionation. Here we test these hypotheses through compound-specific sulfur isotope analyses (CSSIA) of the same sediments from the Cariaco Basin. Relevant data from previous studies ([Lyons et al., 2003](#); [Werne et al., 2003, 2004](#)) are displayed in [Fig. 7B](#) to provide context for the present study.

#### 4.1. Diversity of organic $\delta^{34}\text{S}$ values and data-model offset

There are two major observations to note in the CSSIA data from the Cariaco Basin. First, the  $\delta^{34}\text{C}$  values for the different OS pools vary substantially ([Fig. 6](#)). The individual OSC are generally  $^{34}\text{S}$  enriched relative to co-occurring bulk OS pools by up to  $15\%$ , and the  $\delta^{34}\text{S}$  values of the individual bulk pools also differ (polar bitumen sulfur

(PBS) is  $^{34}\text{S}$  depleted relative to kerogen sulfur (KS) and TOS by  $\sim 5\%$ ). Second, the measured  $\delta^{34}\text{S}$  values of both the triterpenoid thiane and the combined HBIs are consistently  $^{34}\text{S}$  enriched by almost  $10\%$  relative to values predicted by a simple isotopic mass balance model for molecular sulfurization that assumed that  $\text{HS}^-$  was incorporated directly into OM with no fractionation ([Fig. 6](#); cf. [Werne et al., 2003](#) for model details). The clear differences between the model and the data described herein suggest that these assumptions were too simple, that these compounds do not represent bulk OS pools, or both.

Several possible explanations exist for the observed sulfur isotopic heterogeneity among different OS forms, as well as the data-model offset. First, the timing of OM sulfurization results in sequestration of inorganic S at differing stages of its isotopic evolution (e.g., variations reflecting down-core  $^{34}\text{S}$  enrichment trends as observed in pore water sulfide), which would result in earlier formed OSC with a relatively  $^{34}\text{S}$  depleted isotopic signature compared to later formed OSC. Second, there could be additional S-isotope fractionations associated with incorporation of inorganic sulfur species into the various pools of OS. Third, the inorganic S source for the OSC could be different from that of the bulk OS phases, and distinguishing among the various potential S sources for formation of OSC is critical to understanding the mechanism(s) of OS sulfurization. These three mechanisms are discussed in detail below.

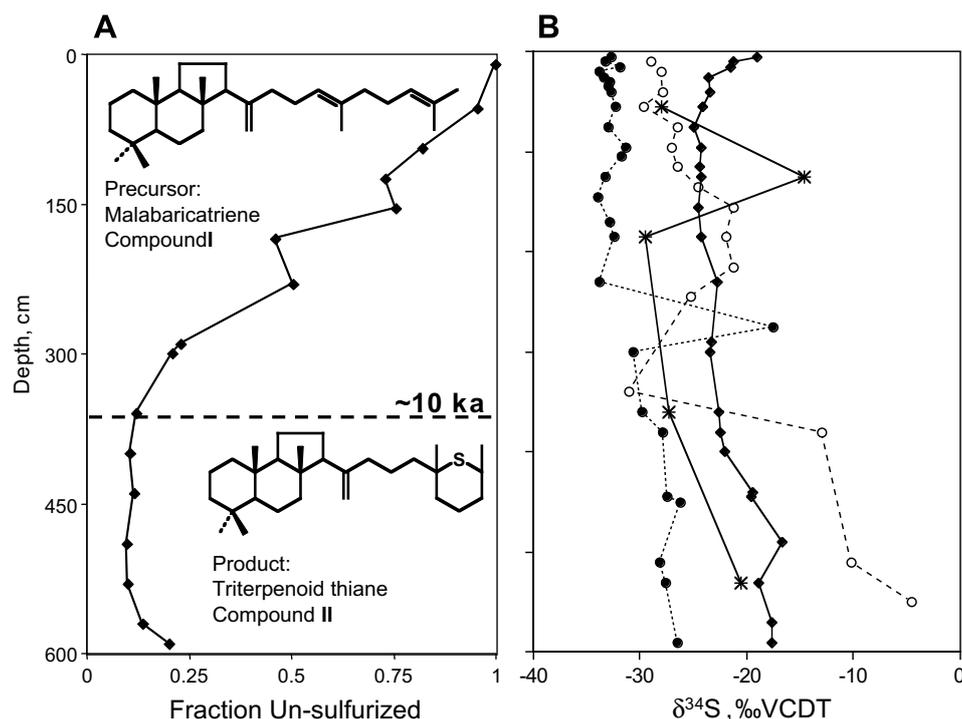


Fig. 7. (A) Precursor/product relationship demonstrated for sulfuration reaction in Cariaco Basin sediments, converting malabaricatriene to triterpenoid thiane (compound I) (after Werne et al., 2000b). (B)  $\delta^{34}\text{S}$  of bulk sulfur species in Cariaco Basin sediments. Solid circles, pyrite sulfur ( $\text{S}_{\text{py}}$ ); open circles, dissolved sulfide ( $\text{HS}^-$ ); solid diamonds, kerogen sulfur (KS); asterisks, polar bitumen sulfur (PBS). All species display a trend of  $^{34}\text{S}$  enrichment with depth. KS is enriched relative to  $\text{S}_{\text{py}}$  and PBS. Data from Werne et al. (2003).

#### 4.2. Timing of sulfuration

One possible mechanism that could contribute to the observed isotopic heterogeneity is the timing of S incorporation into the individual compounds relative to PBS and KS. It has been well documented that different OSC form at different rates in marine sediments (Wakeham et al., 1995; Kok et al., 2000b; Werne et al., 2000b; Sinninghe Damsté et al., 2007). The  $\delta^{34}\text{S}$  trends of all major organic and inorganic sulfur species show increasing  $^{34}\text{S}$  enrichment with depth in the Cariaco Basin (Fig. 7 and Lyons et al., 2003; Werne et al., 2003). Such trends are observed commonly in sediments due to an isotopically evolving sulfide pool reflecting progressive sulfate reduction under conditions of diffusion-controlled sulfate renewal (Hartmann and Nielsen, 1969; Jørgensen, 1979, 2004; Zaback et al., 1993; Lyons, 1997; Donahue et al., 2008). Assuming that the species of inorganic sulfur incorporated into OM follows this trend, regardless of what the specific inorganic sulfur species is, the OS formed later during diagenesis deeper in the sediments would be enriched in  $^{34}\text{S}$  relative to that formed during earlier diagenesis in shallow sediments.

If this factor is the primary control on the  $\delta^{34}\text{S}$  of all of the OS pools (with the exception of any biogenic sulfur present), it suggests that the individual OSC analyzed in this study formed later during diagenesis than either PBS or KS. Furthermore, the trends in PBS and KS towards  $^{34}\text{S}$  enrichment at depth suggest continued diagenetic addition of increasingly heavy inorganic S to these OS pools, which approach the value of the individual OSC at a depth

of 6 meters in the sediments (Fig. 6). While these arguments make some sense, the deepest sample in the Cariaco Basin sediments appears to be  $^{34}\text{S}$  depleted relative to those immediately above it. While the  $\delta^{34}\text{S}$  values are indistinguishable within our estimated error, it could indicate that other factors besides timing of sulfuration are influencing the  $\delta^{34}\text{S}$  of OSC.

The observed trends are in direct contrast with previously published data indicating that the individual OSC formed early during diagenesis (in the upper 3 m of sediment) and incorporated their  $\delta^{34}\text{S}$  composition from the inorganic sulfur species available at shallower depths, which are  $^{34}\text{S}$  depleted relative to those at depth. It has been demonstrated previously that HBIs are sulfurized in the uppermost Cariaco Basin sediments on  $\sim 100$ -year timescales, and malabaricatriene is sulfurized in the upper 3 m over a period of 10 kyr (Werne et al., 2000b). Other studies have also indicated that HBIs are sulfurized on 100- to 500-year timescales (Wakeham et al., 1995; Sinninghe Damsté et al., 2007), while other compounds are sulfurized more slowly or resist sulfuration completely (Werne et al., 2000b). Thus, while differences in the timing of sulfuration of different pools of OM in the sedimentary diagenetic sequence could lead to variability in the  $\delta^{34}\text{S}$  values of the different pools of OM, this mechanism cannot explain the observed  $^{34}\text{S}$  enrichment of the individual OSC relative to bulk OS pools in the Cariaco Basin sediments. It should be noted that if OSC were to exchange S with pore water sulfur species, then the  $\delta^{34}\text{S}$  values could effectively be “reset” many times during diagenesis as a result of such reac-

tions. However, at present we cannot demonstrate whether or not such exchange is occurring, and therefore limit our discussion to factors we can assess.

#### 4.3. Isotopic fractionations during sulfurization

It has generally been accepted that any initial sulfur isotope fractionation associated with the formation of OSC is negligible, particularly when compared to the magnitude of the isotope discrimination associated with dissimilatory sulfate reduction; however, a kinetic fractionation mechanism has been proposed that would lead to  $^{34}\text{S}$  depleted OSC relative to inorganic sulfur pools (Brüchert and Pratt, 1996). In addition, a laboratory study of OM sulfurization demonstrated a clear sulfur isotope fractionation of 4–5‰ associated with the incorporation of polysulfides into OM (Amrani and Aizenshtat, 2004b), with the resulting OSC being  $^{34}\text{S}$  enriched relative to the starting sulfides and polysulfides. Such a fractionation could explain at least part the  $^{34}\text{S}$  enrichment observed in Cariaco Basin OSC relative to the isotopic mass balance model results of Werne et al. (2003).

#### 4.4. Sources of organic sulfur

We can use the sulfur isotope relationships observed among the various OS pools examined in the Cariaco Basin sediments to assess the likelihood of various sources of inorganic sulfur. For example, Werne et al. (2003) used the KS isotope trends to suggest that 10–25% of the organic sulfur in the KS pool is biogenic sulfur in the Cariaco Basin sediments. The isotopic  $^{34}\text{S}$  enrichment of the OSC analyzed relative to the two bulk pools (KS and PBS) cannot be explained by the presence of biogenic sulfur because these OSC are not biologically produced compounds, but formed via abiotic incorporation of inorganic sulfur into OM during diagenesis. The triterpenoid thiane was demonstrated to form diagenetically in the sediments via incorporation of dissolved inorganic sulfur into malabaricatriene (Werne et al., 2000b), and the HBI thiophenes are thought to derive similarly from diatom HBI alkenes that are sulfur free when produced by the organisms (cf. Sinnighe Damsché et al., 1988, 2007; Kohnen et al., 1991c). It is possible that the OSC are formed from biogenic sulfur via conversion of amino acid sulfur to dissolved sulfide during OM remineralization, followed by immediate incorporation of that amino acid-derived sulfide into the precursor organic compounds. However, we view this possibility as unlikely due to the relatively slow kinetics of OM sulfurization compared to those of sedimentary cycling (both microbial and abiotic) of inorganic sulfur species. Furthermore, if there was a significant addition of biogenic sulfur to the dissolved sulfide pool from degradation of amino acids, we would expect to see  $^{34}\text{S}$  enrichment of the sulfide pool in the uppermost 50 cm of the sediments, where the KS  $\delta^{34}\text{S}$  profile suggests rapid remineralization of biogenic S containing OM (Werne et al., 2003). Such  $^{34}\text{S}$  enrichment is not observed.

A second possible explanation for the observed variability in  $\delta^{34}\text{S}$  values in the sedimentary OM is the incorpora-

tion of varying amounts of S derived from terrestrial higher plants, which would have a  $^{34}\text{S}$  enriched isotopic signature relative to OSC formed from dissolved sulfide because the terrestrial OS is formed via assimilatory sulfate reduction. However, there is not a significant present-day input of terrestrial OM to the waters of the Cariaco Basin, as evident in studies of water-column particulates (Wakeham, 1990; Freeman et al., 1994) and sediment-trap materials (Thunell et al., 2000). Furthermore, the accumulation rate of the  $\text{C}_{29}$  *n*-alkane, a terrestrial plant biomarker, is more than an order of magnitude lower than that of aquatic biomarkers (Werne et al., 2000b), and the BIT index, a terrestrial/aquatic ratio index based on microbial tetraether lipids, is <0.1 (Hopmans et al., 2004). These proxies indicate that terrestrial OM is not a significant portion of the sedimentary OM in the Cariaco Basin and that the proportion of terrestrial organic matter has not changed significantly over the period of the present study. Thus, we can rule out significant changes in the delivery of terrestrially derived OS to the Cariaco as a mechanism for varying sedimentary  $\delta^{34}\text{S}$  values of OS.

As the contributions of biogenic sulfur appear to be minimal, we restrict our discussion of the source(s) of inorganic sulfur for diagenetic OM sulfurization to the species present in the sediment pore waters. The primary species of dissolved inorganic sulfur typically present in pore waters are sulfide (present as bisulfide,  $\text{HS}^-$ , at typical marine sediment pH; (Boulegue, 1978; Vairavamurthy and Mopper, 1987), polysulfides ( $\text{S}_x^{2-}$ ), elemental sulfur, and oxidized reactive intermediates such as thiosulfate or sulfite). At present, we rule out more oxidized reactive intermediates like thiosulfate as immediate sources of S for the Cariaco Basin OSC because these sulfur species would likely lead to other types of OSC, such as sulfonates or sulf oxides (cf. Vairavamurthy et al., 1994, 1997; Schouten et al., 1995a). Such oxidized forms of OS may very well be a component of the PBS and/or the KS pools in Cariaco Basin sediments, but we have not yet analyzed these forms at a molecular level.

Thus, the most likely candidates for the source for sulfur in the OSC are pore water sulfide ( $\text{HS}^-$ ), polysulfides ( $\text{S}_x^{2-}$ ), and elemental sulfur ( $\text{S}^0$ ). Unfortunately, we cannot at this time unequivocally determine which of these three sources is the source of sulfur to the OSC in the Cariaco Basin sediments, in part because the  $\delta^{34}\text{S}$  of polysulfides and elemental sulfur in this system have not been measured. However, the inorganic S source must explain the  $^{34}\text{S}$  enrichment measured in the OSC relative to that predicted using isotopic mass balance of Werne et al. (2003), and we can use this requirement and knowledge of the mechanisms of OM sulfurization to try to rule out one or more of these inorganic sulfur pools.

Thermodynamic equilibrium calculations (Tudge and Thode, 1950) and studies of natural systems (Anderson and Pratt, 1995) indicate that elemental sulfur is typically  $^{34}\text{S}$ -enriched relative to dissolved sulfide (e.g., by 3‰ at 25 °C). It has also recently been demonstrated that there is complete isotopic mixing among sulfide and polysulfides (Fossing et al., 1992; Amrani and Aizenshtat, 2004b; Amrani et al., 2006). The result of this isotopic exchange is that at

equilibrium, polysulfides are  $^{34}\text{S}$  enriched relative to sulfide by up to 3.9‰ (for polysulfide  $\text{S}_7$ ), and the degree of  $^{34}\text{S}$  enrichment increases with chain length (Amrani et al., 2006). Thus, if either elemental sulfur or polysulfides are the source of inorganic sulfur we should expect to see moderate  $^{34}\text{S}$  enrichment up to  $\sim 4\%$  in the OSC formed relative to what we would expect from incorporation of sulfide with no fractionation, suggesting that these two inorganic species are more likely to be the immediate source of inorganic sulfur to the OSC in the Cariaco Basin sediments than sulfide.

Polysulfides and sulfide are strong nucleophiles (LaLonde et al., 1987), and at the slightly basic pH conditions normally found in natural sedimentary environments ( $\sim 7$ – $9$ ) polysulfides and bisulfide are the predominant inorganic sulfur species present (LaLonde et al., 1987; Vairavamurthy and Mopper, 1989; Krein, 1993; Loch et al., 2002). Both laboratory- (LaLonde et al., 1987; Moers et al., 1987; de Graaf et al., 1992, 1995; Schouten et al., 1994; Amrani and Aizenshtat, 2004a) and field-based molecular (non-isotopic) OS studies (Aizenshtat et al., 1983; Kohnen et al., 1989, 1991b; Vairavamurthy et al., 1992; Filley et al., 2002) have demonstrated that polysulfides can be incorporated into OM during early diagenesis. In addition, the nucleophilic addition of polysulfides to OM is favored over the addition of either bisulfide (Vairavamurthy and Mopper, 1989) or elemental sulfur (Giggenbach, 1972; LaLonde et al., 1987; Aizenshtat et al., 1995) under normal marine conditions.

There are two primary arguments against polysulfides as the primary source of inorganic sulfur to OSC during diagenesis. The first is a lack of sedimentary oxidants in euxinic systems such as the Cariaco Basin; however, there are two potential sources of oxidation in the sediments of the Cariaco Basin. Reactive iron in the Cariaco sediments has been shown to be low to the point of limiting pyrite formation (Lyons et al., 2003; Werne et al., 2003). While the 'reactive' iron determined using boiling 12 N HCl extraction is much greater than that determined using a dithionite extraction, both are very low in the Cariaco Basin sediments (Lyons et al., 2003; Werne et al., 2003). Typically, the differences in these two methods are attributed to Fe present in silicates that can be reactive towards sulfide on timescales of  $10^3$ – $10^5$  years (Canfield, 1989; Canfield et al., 1992; Raiswell and Canfield, 1996). In addition, the degree of pyritization (DOP) in the Cariaco Basin sediments is intermediate, suggesting the presence of slow-reacting silicate Fe (Lyons et al., 2003). Thus, there is the possibility of sulfide oxidation through reaction with Fe from these silicates on longer timescales in the Cariaco. In addition, it has recently been demonstrated that dissolved organic matter (DOM) can react with dissolved sulfide to produce thiosulfate and possibly aryl polysulfides (Heitmann and Blodau, 2006). Thus, there may be sufficient oxidizing power present in the oxidized iron in silicates and the DOM to react with sulfides to produce polysulfide. The second argument against polysulfides as the source of OS is the very low concentrations of polysulfides typically encountered relative to (bi)sulfide, except at the oxic/anoxic boundary. While we cannot refute this argument entirely,

we consider the fact that polysulfides were demonstrated to be 1–2 orders of magnitude more reactive than bisulfide towards OM (Vairavamurthy and Mopper, 1989) strong evidence in favor of polysulfides. It is reasonable to believe that, given the reaction times of minutes to hours observed for polysulfide incorporation into OM (Amrani and Aizenshtat, 2004a), the concentration of polysulfides in sedimentary pore waters is lowered, in part, through their rapid reaction with OM immediately upon formation, as well as through pyrite formation.

The identification of polysulfides as the major source of inorganic sulfur for OM sulfurization is not conclusive, although the incorporation of  $^{34}\text{S}$  enriched polysulfides associated with a S-isotopic fractionation upon OM sulfurization is consistent with the observed compound-specific sulfur isotope data from the Cariaco Basin. If the 4–5‰ fractionation associated with polysulfide incorporation (Amrani and Aizenshtat, 2004b) is added to the  $\sim 2$ – $4\%$   $^{34}\text{S}$  enrichment in polysulfides relative to sulfide at equilibrium (Amrani et al., 2006), it explains the observed  $^{34}\text{S}$  enrichment of  $\sim 10\%$  in the OSC relative to the model results of Werne et al. (2003). Thus, polysulfides appear to be the most likely source of inorganic sulfur to the Cariaco OSC.

## 5. CONCLUSIONS

Our study represents the first use of compound-specific sulfur isotope analysis to investigate OM sulfurization in marine sediments. We have demonstrated that CSSIA has the potential to provide substantial information related to sedimentary sulfur cycling in both modern and ancient environments. In the Cariaco Basin this approach provides insight into the timing and mechanism(s) of diagenetic sulfur formation.

The  $\delta^{34}\text{S}$  values of OSC from the Cariaco Basin sediments appears to be set during the period of initial formation in shallow sediments, and can therefore be used in other systems as an indication of the timing of diagenetic OM sulfurization. For example, OSC that are  $^{34}\text{S}$  enriched relative to other organic sulfur pools/compounds would be expected to have formed later during diagenesis, because, in the absence of other contributing factors, the sulfides available for incorporation into organic matter are generally  $^{34}\text{S}$  enriched deeper in the sediments.

$^{34}\text{S}$  enrichment in specific OSC from the Cariaco Basin sediments indicates that organic sulfur is not derived from only one inorganic sulfur source but instead reflects sulfur derived from a mixture of sources. These sources likely include primary biogenic sulfur from amino acids, reduced sources such as sulfide, and reactive intermediates such as polysulfides and thiosulfate. It is anticipated that each of these different sulfur sources would impart a distinct S-isotope signature to the OSC.

Comparison of our CSSIA results with earlier modeling efforts applied to the same sediments indicates that the sulfur in these OSC is most likely derived from polysulfides that are enriched relative to sulfide and that there is a fractionation associated with the polysulfide incorporation. While we cannot rule out the possibility that bisulfide is

incorporated into the Cariaco Basin OSC, our interpretation is consistent with other recent studies (Amrani and Aizenshtat, 2004b; Amrani et al., 2006). It is also possible (and perhaps likely) that both bisulfide and polysulfides contribute to the  $\delta^{34}\text{S}$  of the OSC. Most importantly, the results discussed here demonstrate that CSSIA is feasible on marine sediment extracts, thus providing a methodological foundation for future studies devoted to S cycling and molecular approaches to paleoenvironmental reconstruction.

#### ACKNOWLEDGMENTS

We thank Marianne Baas general analytical support, Steve Studley and Jon Fong for analytical assistance with S-isotope measurements, and Margaret Ricci for assistance with data processing and error estimation. We thank the ODP for samples. Financial assistance for this work was provided to TWL by NSF award EAR-9875961 (CAREER) and to JSSD by the PIONIER grant of the Dutch Organization for Scientific Research (NWO). Rodger Harvey, Mike Engel and three anonymous reviewers are gratefully acknowledged for constructive criticisms on earlier drafts that greatly improved the manuscript.

#### REFERENCES

- Adam P., Schmid J. C., Albrecht P. and Connan J. (1991)  $2\alpha$  and  $3\beta$  steroid thiols from reductive cleavage of macromolecular petroleum fraction. *Tetrahedron Lett.* **32**, 2955–2958.
- Adam P., Phillippe E. and Albrecht P. (1998) Photochemical sulfurization of sedimentary organic matter: a widespread process occurring at early diagenesis in natural environments? *Geochim. Cosmochim. Acta* **62**, 265–271.
- Aizenshtat Z., Krein E., Vairavamurthy M. and Goldstein, T. (1995) Role of sulfur in the transformations of sedimentary organic matter: a mechanistic overview. In *Geochemical Transformations of Sedimentary Sulfur* (eds. M. A. Vairavamurthy and M. A. A. Schoonen). ACS Symposium Series 612, Washington, DC, pp. 378–396.
- Aizenshtat Z., Stoler A., Cohen Y. and Nielsen H. (1983) The geochemical sulphur enrichment of recent organic matter by polysulfides in the Solar-Lake. In *Advances in Organic Geochemistry 1981* (ed. Bjoroy et al.), pp. 279–288. Advances in Organic Geochemistry 1981. John Wiley & Sons Limited.
- Amrani A. and Aizenshtat Z. (2004b) Mechanisms of sulfur introduction chemically controlled:  $\delta^{34}\text{S}$  imprint. *Org. Geochem.* **35**, 1319–1336.
- Amrani A. and Aizenshtat Z. (2004a) Reaction of polysulfide anions with a, b unsaturated isoprenoid aldehydes in aquatic media: simulation of oceanic conditions. *Org. Geochem.* **35**, 909–921.
- Amrani A., Kamyshtny A., Lev O. and Aizenshtat Z. (2006) Sulfur stable isotope distribution of polysulfide anions in an  $(\text{NH}_4)_2\text{S}_n$  aqueous solution. *Inorg. Chem.* **45**, 1427–1429.
- Anderson T. F. and Pratt L. M. (1995) Isotope evidence for the origin of organic sulfur and elemental sulfur in marine sediments. In *Geochemical Transformations of Sedimentary Sulfur* (eds. M. A. Vairavamurthy and M. A. A. Schoonen). ACS Symposium Series 612, Washington, DC, pp. 378–396.
- Baskin D. and Peters K. (1992) Early generation characteristics of a sulfur-rich Monterey kerogen. *Am. Assoc. Petrol. Geol. Bull.* **76**, 1–13.
- Berner R. A. and Raiswell R. (1983) Burial of organic carbon and pyrite sulfur in sediments over Phanerozoic time: a new theory. *Geochim. Cosmochim. Acta* **47**, 855–862.
- Böttcher M. E., Schale H., Schnetger B., Wallmann K. and Brumsack H.-J. (2000) Stable sulfur isotopes indicate net sulfate reduction in near-surface sediments of the deep Arabian Sea. *Deep-Sea Res. II* **47**, 2769–2783.
- Boulegue J. (1978) Solubility of elemental sulfur in water at 298 K. *Phosphorous Sulfur Rel. Elem.* **5**, 127–128.
- Brassell S. C., Lewis C. A., de Leeuw J. W., de Lange F. and Sinninghe Damsté J. S. (1986) Isoprenoid thiophenes in recent sediments. *Nature* **320**, 160–162.
- Brüchert V. (2004) Physiological and ecological aspects of sulfur isotope fractionation during bacterial sulfate reduction. In *Sulfur Biogeochemistry—Past and Present* (eds. J. P. Amend, K. J. Edwards and T. W. Lyons). GSA Special Paper 379, pp. 1–16.
- Brüchert V., Knoblauch C. and Jørgensen B. B. (2001) Controls on stable sulfur isotope fractionation during bacterial sulfate reduction in Arctic sediments. *Geochim. Cosmochim. Acta* **65**, 763–776.
- Brüchert V. and Pratt L. M. (1996) Contemporaneous early diagenetic formation of organic and inorganic sulfur in estuarine sediments from St. Andrew Bay, Florida, USA. *Geochim. Cosmochim. Acta* **60**, 2325–2332.
- Brunner B. and Bernasconi S. M. (2005) A revised isotope fractionation model for dissimilatory sulfate reduction in sulfate reducing bacteria. *Geochim. Cosmochim. Acta* **69**, 4759–4771.
- Canfield D. E. (1989) Reactive iron in marine sediments. *Geochim. Cosmochim. Acta* **53**, 619–632.
- Canfield D. E. (2001) Biogeochemistry of sulfur isotopes. In *Stable Isotope Geochemistry*, vol. 43 (eds. J. W. Valley and D. R. Cole), pp. 607–636. Reviews in Mineralogy and Geochemistry. The Mineralogical Society of America, Washington, DC.
- Canfield D. E., Raiswell R. and Bottrell S. (1992) The reactivity of sedimentary iron minerals toward sulfide. *Am. J. Sci.* **292**, 818–834.
- Canfield D. E., Boudreau B. P., Mucci A. and Gundersen J. K. (1998) The early diagenetic formation of organic sulfur in the sediments of Mangrove Lake, Bermuda. *Geochim. Cosmochim. Acta* **62**, 767–781.
- Chambers L. A. and Trudinger P. A. (1979) Microbiological fractionation of stable sulfur isotopes: a review and critique. *Geomicrobiol. J.* **1**, 249–293.
- de Graaf W., Sinninghe Damsté J. and de Leeuw J. (1992) Laboratory simulation of natural sulphurization: I. Formation of monomeric and oligomeric isoprenoid polysulfides by low-temperature reactions of inorganic polysulfides with phytol and phytadienes. *Geochim. Cosmochim. Acta* **56**, 4321–4328.
- de Graaf W., Sinninghe Damsté J. and de Leeuw J. (1995) Low-temperature addition of hydrogen polysulfides to olefins: formation of 2,2'-dialkyl polysulfides from alk-1-enes and cyclic (poly)sulfides and polymeric organic sulfur compounds from  $\alpha,\omega$ -dienes. *J. Chem. Soc. Perkin Trans. I*, 634–640.
- Detmers J., Brüchert V., Habicht K. S. and Kuever J. (2001) Diversity of sulfur isotope fractionations by sulfate-reducing prokaryotes. *Appl. Environ. Microbiol.* **67**, 888–894.
- Dinur D., Spiro B. and Aizenshtat Z. (1980) The distribution and isotopic composition of sulfur in organic-rich sedimentary rocks. *Chem. Geol.* **31**, 37–51.
- Donahue M., Werne J., Meile C. and Lyons T. (2008) The sulfur isotope signature of sulfate reduction in Cariaco Basin sediments: modeling effects of fractionation and differential diffusion. *Geochim. Cosmochim. Acta* **72**, 2287–2297.
- Eglinton T., Sinninghe Damsté J., Kohnen M., de Leeuw J., Larter S. and Patience R. (1990) Analysis of maturity-related changes in organic sulfur composition of kerogens by flash pyrolysis-gas chromatography. In *Geochemistry of Sulfur in Fossil Fuels* (eds.

- W. Orr and C. White). ACS Symposium Series 429, American Chemical Society, Washington, DC, pp. 529–565.
- Filley T., Freeman K., Wilkin R. and Hatcher P. (2002) Biogeochemical controls on reaction of sedimentary organic matter and aqueous sulfides in Holocene sediments of Mud Lake, Florida. *Geochim. Cosmochim. Acta* **66**, 937–954.
- Fossing H., Thode-Andersen S. and Jørgensen B. (1992) Sulfur isotope exchange between  $^{35}\text{S}$ -labeled inorganic sulfur compounds in anoxic marine sediments. *Mar. Chem.* **38**, 117–132.
- Freeman K. H., Wakeham S. and Hayes J. (1994) Predictive isotopic biogeochemistry: hydrocarbons from anoxic marine basins. *Org. Geochem.* **21**, 629–644.
- Fry B., Gest H. and Hayes J. M. (1984) Isotope effects associated with the anaerobic oxidation of sulfide by the purple photosynthetic bacterium, *Chromatium vinosum*. *FEMS Microbiol. Lett.* **22**, 283–287.
- Fry B., Gest H. and Hayes J. M. (1988)  $^{34}\text{S}/^{32}\text{S}$  fractionation in sulfur cycles catalyzed by anaerobic bacteria. *Appl. Environ. Microbiol.* **54**, 250–256.
- Fry B., Cox J., Gest H. and Hayes J. (1986) Discrimination between  $^{34}\text{S}$  and  $^{32}\text{S}$  during bacterial metabolism of inorganic sulfur compounds. *J. Bacteriol.* **165**, 328–330.
- Garrels R. M. and Lerman A. (1984) Coupling of the sedimentary sulfur and carbon cycles—an improved model. *Am. J. Sci.* **284**, 989–1007.
- Giggenbach W. (1972) Optical spectra and equilibrium distribution of polysulfide ions in aqueous solution at 20 °C. *Inorg. Chem.* **11**, 1201–1207.
- Gug S., Schaeffer P., Adam P. and Albrecht P. (2007) Synthesis of a sulfurized bicyclic squalene derivative: geochemical implications. *Proc. Int. Meeting Org. Geochem.* **23**, 1021–1022.
- Habicht K. S. and Canfield D. E. (1997) Sulfur isotope fractionation during bacterial sulfate reduction in organic-rich sediments. *Geochim. Cosmochim. Acta* **61**, 5351–5361.
- Habicht K. S. and Canfield D. E. (2001) Isotope fractionation by sulfate-reducing natural populations and the isotopic composition of sulfide in marine sediments. *Geology* **29**, 555–558.
- Hanin S., Adam P., Kowalewski I., Huc A., Carpentier B. and Albrecht P. (2002) Bridgehead alkylated 2-thiaadamantanes: novel markers for sulfurisation processes occurring under high thermal stress in deep petroleum reservoirs. *Chem. Commun.* 1750–1751. doi:10.1039/b203551k.
- Hartmann M. and Nielsen H. (1969)  $\delta^{34}\text{S}$ -Werte in rezenten meeressedimenten und ihre Deutung am Beispiel einiger Sedimentprofile aus der westlichen Ostsee. *Geol. Rundschau* **58**, 621–655.1.
- Heitmann T. and Blodau C. (2006) Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. *Chem. Geol.* **235**, 12–20.
- Henneke E., Luther G., de Lange G. and Hoefs J. (1997) Sulphur speciation in anoxic hypersaline sediments from the eastern Mediterranean Sea. *Geochim. Cosmochim. Acta* **61**, 307–321.
- Hopmans E. C., Weijers J. W. H., Schefuss E., Herfort L., Sinninghe Damsté J. S. and Schouten S. (2004) A novel proxy for terrestrial organic matter in sediments based on branched and isoprenoid tetraether lipids. *Earth Planet. Sci. Lett.* **224**, 107–116.
- Hughen K. A., Overpeck J. T., Peterson L. C. and Anderson R. F. (1996a) The nature of varved sedimentation in the Cariaco Basin, Venezuela, and its palaeoclimatic significance. In *Palaeoclimatology and Palaeoceanography from Laminated Sediments* (ed. A. E. S. Kemp). Geol. Soc. London, pp. 171–183.
- Hughen K. A., Overpeck J. T., Peterson L. C. and Trumbore S. (1996b) Rapid climate changes in the tropical Atlantic region during the last deglaciation. *Nature* **380**, 51–54.
- Hughen K. A., Overpeck J. T., Lehman S. J., Kashgarian M., Southon J., Peterson L. C., Alley R. and Sigman D. M. (1998) Deglacial changes in ocean circulation from an extended radiocarbon calibration. *Nature* **391**, 65–68.
- Jørgensen B. B. (1979) A theoretical model of the stable sulfur isotope distribution in marine sediments. *Geochim. Cosmochim. Acta* **43**, 363–374.
- Jørgensen B. B., Böttcher M. E., Lüschen H., Neretin L. N. and Volkov I. I. (2004) Anaerobic methane oxidation and a deep  $\text{H}_2\text{S}$  sink generate isotopically heavy sulfides in Black Sea sediments. *Geochim. Cosmochim. Acta* **68**, 2095–2118.
- Kaplan I. and Rittenberg S. (1964) Microbiological fractionation of sulphur isotopes. *J. Gen. Microbiol.* **34**, 195–212.
- Kohnen M. E. L., Sinninghe Damsté J. S., ten Haven H. L. and de Leeuw J. W. (1989) Early incorporation of polysulphides in sedimentary organic matter. *Nature* **341**, 640–641.
- Kohnen M. E. L., Sinninghe Damsté J. S. and de Leeuw J. W. (1991a) Biases from natural sulphurization in palaeoenvironmental reconstruction based on hydrocarbon biomarker distributions. *Nature* **349**, 775–778.
- Kohnen M. E. L., Sinninghe Damsté J. S., Kock-van Dalen A. C. and de Leeuw J. W. (1991b) Di- or polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. *Geochim. Cosmochim. Acta* **55**, 1375–1394.
- Kohnen M. E. L., Sinninghe Damsté J. S., Rullkötter J., ten Haven H. L. and de Leeuw J. W. (1991c) Origin and diagenetic transformations of  $\text{C}_{25}$  and  $\text{C}_{30}$  highly branched isoprenoid sulfur-compounds—further evidence for the formation of organically bound sulfur during early diagenesis. *Geochim. Cosmochim. Acta* **55**, 3053–3063.
- Kok M. D., Schouten S. and Sinninghe Damsté J. S. (2000a) Formation of insoluble, nonhydrolyzable, sulfur-rich macromolecules via incorporation of inorganic sulfur species into algal carbohydrates. *Geochim. Cosmochim. Acta* **64**, 2689–2699.
- Kok M. D., Rijpstra W. I. C., Robertson L., Volkman J. and Sinninghe Damsté J. S. (2000b) Early steroid sulfurisation in surface sediments of a permanently stratified lake (Ace Lake, Antarctica). *Geochim. Cosmochim. Acta* **64**, 1425–1436.
- Koopmans M., Rijpstra W., de Leeuw J., Lewan M. and Sinninghe Damsté J. (1998) Artificial maturation of immature sulfur- and organic matter-rich limestone from the Ghareb Formation, Jordan. *Org. Geochem.* **28**, 503–521.
- Krein E. (1993) The phase-transfer catalyzed reactions between polysulfide anions and  $\alpha,\beta$ -unsaturated carbonyl compounds. *J. Org. Chem.* **58**, 6103–6108.
- Krein E. B. and Aizenshtat Z. (1995) Proposed thermal pathways for sulfur transformations in organic macromolecules: laboratory simulation experiments. In *Geochemical Transformations of Sedimentary Sulfur* (eds. M. A. Vairavamurthy and M. A. A. Schoonen). ACS Symposium Series 612, Washington, DC, pp. 110–137.
- LaLonde R., Ferrara L. and Hayes M. (1987) Low-temperature, polysulfide reactions of conjugated ene carbonyls: a reaction model for the geologic origin of S-heterocycles. *Org. Geochem.* **11**, 563–571.
- Le Faou A., Rajagopal B., Daniels L. and Fauque G. (1990) Thiosulfate, polythionates, and elemental sulfur assimilation and reduction in the bacterial world. *FEMS Microbiol. Rev.* **75**, 351–382.
- Lewan M. (1993) Laboratory simulation of petroleum formation: hydrous pyrolysis. In *Organic Geochemistry, Principles and Applications, Topics in Geobiology* (eds. M. H. Engel and S. A. Macko), Plenum, NY, vol. 11, pp. 419–444.
- Lewan M. D. (1998) Sulphur-radical control on petroleum formation rates. *Nature* **391**, 164–166.

- Lin H. L., Peterson L. C., Overpeck J. T., Trumbore S. E. and Murray D. W. (1997) Late Quaternary climate change from  $\delta^{18}\text{O}$  records of multiple species of planktonic foraminifera: high-resolution records from the anoxic Cariaco Basin, Venezuela. *Paleoceanography* **12**, 415–427.
- Loch A., Lippa D., Carlson D., Chin Y., Trina S. and Roberts A. (2002) Nucleophilic aliphatic substitution reactions of propachlor, alachlor, and metolachlor with bisulfide ( $\text{HS}^-$ ) and dipolysulfides ( $\text{Sn}^{2-}$ ). *Environ. Sci. Technol.* **36**, 4065–4073.
- Lyons T. W. (1997) Sulfur isotope trends and pathways of iron sulfide formation in upper Holocene sediments of the anoxic Black Sea. *Geochim. Cosmochim. Acta* **61**, 3367–3382.
- Lyons T. W., Werne J. P., Hollander D. J. and Murray R. W. (2003) Contrasting sulfur geochemistry and Fe/Al and Mo/Al ratios across the last oxic-to-anoxic transition in the Cariaco Basin, Venezuela. *Chem. Geol.* **195**, 131–157.
- Moers M., de Leeuw J., Cox H. and Schenck P. (1987) Interaction of glucose and cellulose with hydrogen sulphide and polysulfides. *Org. Geochem.* **13**, 1087–1091.
- Mossman J. R., Aplin A. C., Curtis C. D. and Coleman M. L. (1991) Geochemistry of inorganic and organic sulphur in organic-rich sediments from the Peru Margin. *Geochim. Cosmochim. Acta* **55**, 3581–3595.
- Orr W. (1986) Kerogen/asphaltene/sulfur relationships in sulfur-rich Monterey oils. *Org. Geochem.* **10**, 499–516.
- Passier H. F., Luther, III, G. W. and de Lange G. J. (1997) Early diagenesis and sulphur speciation in sediments of the Oman Margin, northwestern Arabian Sea. *Deep-Sea Res. II* **44**, 1361–1380.
- Peterson L. C., Overpeck J. T., Kipp N. G. and Imbrie J. (1991) A high-resolution late Quaternary upwelling record from the anoxic Cariaco Basin, Venezuela. *Paleoceanography* **6**, 99–119.
- Price F. T. and Shieh Y. N. (1979) Fractionation of sulfur isotopes during laboratory synthesis of pyrite at low temperatures. *Chem. Geol.* **27**, 245–253.
- Putschew A., Schaeffer-Reiss C., Schaeffer P., Koopmans M. P., de Leeuw J. W., Lewan M. D., Sinninghe Damsté J. S. and Maxwell J. R. (1998) Release of sulfur- and oxygen-bound components from a sulfur-rich kerogen during simulated maturation by hydrous pyrolysis. *Org. Geochem.* **29**, 1875–1998.
- Pyzik A. J. and Sommer S. E. (1981) Sedimentary iron monosulfides: kinetics and mechanism of formation. *Geochim. Cosmochim. Acta* **47**, 687–698.
- Raiswell R. and Canfield D. E. (1996) Rates of reaction between silicate iron and dissolved sulfide in Peru Margin sediments. *Geochim. Cosmochim. Acta* **60**, 2777–2788.
- Rees C. E., Jenkins W. J. and Monster J. (1978) The sulfur isotopic composition of ocean water sulfate. *Geochim. Cosmochim. Acta* **42**, 377–381.
- Schouten S., Sinninghe Damsté J. S. and De Leeuw J. W. (1995b) A novel triterpenoid carbon skeleton in immature sulphur-rich sediments. *Geochim. Cosmochim. Acta* **59**, 953–958.
- Schouten S., Sinninghe Damsté J. S. and de Leeuw J. W. (1995a) The occurrence and distribution of low-molecular-weight sulphoxides in polar fractions of sediment extracts and petroleum. *Org. Geochem.* **23**, 129–138.
- Schouten S., de Graaf W., Sinninghe Damsté J. S., van Driel G. B. and de Leeuw J. W. (1994) Laboratory simulation of natural sulphurization. II. Reaction of multifunctionalized lipids with inorganic polysulfides at low temperatures. *Org. Geochem.* **22**, 825–834.
- Shipboard Scientific Party (1997) Site 1002, In *Proc. ODP. Init. Rep.*, 165 (eds. H. Sigurdsson, R. M. Leckie and G. D. Acton), College Station, TX.
- 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. In *Advances in Organic Geochemistry 1987* (eds. L. Mattavelli and L. Novelli). *Org. Geochem.* **13**, 593–606.
- Sinninghe Damsté J. S. and deLeeuw J. W. (1990) Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: state of the art and future research. *Org. Geochem.* **16**, 1077–1101.
- Sinninghe Damsté J. S., Rijpstra W. I. C., Coolen M. J. L., Schouten S. and Volkman J. K. (2007) Rapid sulfurisation of highly branched isoprenoid (HBI) alkenes in sulfidic Holocene sediments from Ellis Fjord, Antarctica. *Org. Geochem.* **38**, 128–139.
- Smittenberg R., Hopmans E., Schouten S. and Sinninghe Damsté J. (2002) Rapid isolation of biomarkers for compound specific radiocarbon dating using high-performance liquid chromatography and flow injection analysis-atmospheric pressure chemical ionization mass spectrometry. *J. Chromatogr. A* **978**, 129–140.
- Thauer R. and Kunow J. (1995) Sulfate-reducing Archaea. In *Sulfate-Reducing Bacteria* (ed. L. Barton). Plenum Press, New York, pp. 33–48.
- Thunell R., Varela R., Llano M., Collister J., Muller-Karger F. and Bohrer B. (2000) Organic carbon fluxes and regeneration rates in an anoxic water column: sediment trap results from the Cariaco Basin. *Limnol. Oceanogr.* **45**, 300–308.
- Trust B. A. and Fry B. (1992) Sulphur isotopes in plants: a review. *Plant Cell Environ.* **15**, 1105–1110.
- Tudge A. Pl. and Thode H. G. (1950) Thermodynamic properties of isotopic compounds of sulfur. *Can. J. Res. B Chem. Sci.* **28**, 56–578.
- Vairavamurthy A. and Mopper K. (1987) Geochemical formation of organic sulphur compounds (thiols) by addition of  $\text{H}_2\text{S}$  to sedimentary organic matter. *Nature* **329**, 623–625.
- Vairavamurthy A. and Mopper K. (1989) Mechanistic studies of organosulfur (thiol) formation in coastal marine sediments. In *Biogenic Sulfur in the Environment* (eds. E. Saltzman and W. Cooper). ACS Symposium Series 393. American Chemical Society, Washington, DC, pp. 231–242.
- Vairavamurthy A., Mopper K. and Taylor B. (1992) Occurrence of particle-bound polysulfides and significance of their reaction with organic matters in marine sediments. *Geophys. Res. Lett.* **19**, 2043–2046.
- Vairavamurthy A., Zhou W., Eglinton T. and Manowitz B. (1994) Sulfonates: a novel class of organic sulfur compounds in marine sediments. *Geochim. Cosmochim. Acta* **58**, 4681–4687.
- Vairavamurthy M., Maletic D., Want S., Manowitz B., Eglinton T. and Lyons T. (1997) Characterization of sulfur-containing functional groups in sedimentary humic substances by X-ray absorption near-edge structure spectroscopy. *Energy Fuels* **11**, 546–553.
- Vairavamurthy M., Orr W. and Manowitz B. (1995) Geochemical transformations of sedimentary sulfur: an introduction. In *Geochemical Transformations of Sedimentary Sulfur* (eds. M. A. Vairavamurthy and M. A. Schoonen). ACS Symposium Series 612. American Chemical Society, Washington DC, pp. 1–15.
- Valisollalao J., Perakis N., Chappe B. and Albrecht P. (1984) A novel sulfur containing C35 hopanoid in sediments. *Tetrahedron Lett.* **25**, 1183–1186.
- van Dongen B. E., Schouten S. and Sinninghe Damsté J. S. (2003b) Sulfurization of carbohydrates results in a S-rich, unresolved complex mixture in kerogen pyrolysates. *Energy Fuels* **17**, 1109–1118.
- van Dongen B. E., Schouten S., Baas M., Geenevasen J. A. J. and Sinninghe Damsté J. S. (2003a) An experimental study of the

- low-temperature sulfurization of carbohydrates. *Org. Geochem.* **34**, 1129–1144.
- van Kaam-Peters H., Schouten S., Köster J. and Sinninghe Damsté J. (1998) Controls on the molecular and carbon isotopic composition of organic matter deposited in a Kimmeridgian euxinic shelf sea: evidence for preservation of carbohydrates through sulfurisation. *Geochim. Cosmochim. Acta* **62**, 3259–3283.
- Wakeham S. G. (1990) Algal and bacterial hydrocarbons in particulate matter and interfacial sediment of the Cariaco Trench. *Geochim. Cosmochim. Acta* **54**, 1325–1336.
- Wakeham S. G., Sinninghe Damsté J. S., Kohlen M. E. L. and de Leeuw J. W. (1995) Organic sulfur compounds formed during early diagenesis in Black Sea sediments. *Geochim. Cosmochim. Acta* **59**, 521–533.
- Wakeham S. G., Lee C., Hedges J. I., Hernes P. J. and Peterson M. L. (1997) Molecular indicators of diagenetic status in marine organic matter. *Geochim. Cosmochim. Acta* **61**, 5363–5369.
- Werne J. P., Hollander D. J., Lyons T. W. and Peterson L. C. (2000a) Climate-induced variations in productivity and planktonic ecosystem structure from the Younger Dryas to Holocene in the Cariaco Basin, Venezuela. *Paleoceanography* **15**, 19–29.
- Werne J. P., Hollander D. J., Lyons T. W. and Sinninghe Damsté J. S. (2004) Organic sulfur biogeochemistry: recent advances and future directions for organic sulfur research. In *Sulfur Biogeochemistry: Past and Present* (eds. J. Amend, K. Edwards, T. Lyons). GSA Special Paper 379, Chapter 9, pp. 135–150.
- Werne J. P., Lyons T. W., Hollander D. J., Formolo M. and Sinninghe Damsté J. S. (2003) Reduced sulfur in euxinic sediments of the Cariaco Basin: sulfur isotope constraints on organic sulfur formation. *Chem. Geol.* **195**, 159–179.
- Werne J. P., Hollander D. J., Behrens A., Schaeffer P., Albrecht P. and Sinninghe Damsté J. S. S. (2000b) Timing of early diagenetic sulfurization of organic matter: a precursor–product relationship in Holocene sediments of the anoxic Cariaco Basin, Venezuela. *Geochim. Cosmochim. Acta* **64**, 1741–1751.
- Zaback D. A. and Pratt L. M. (1992) Isotope composition and speciation of sulfur in the Miocene Monterey Formation: reevaluation of sulfur reactions during early diagenesis in marine environments. *Geochim. Cosmochim. Acta* **56**, 763–774.
- Zaback D. A., Pratt L. M. and Hayes J. M. (1993) Transport and reduction of sulfate and immobilization of sulfide in marine black shales. *Geology* **21**, 141–144.
- Zehnder A. and Zinder S. (1980) The sulfur cycle. In *The Handbook of Environmental Chemistry* (ed. O. Hutzinger). Springer Verlag, Heidelberg, pp. 105–145.

Associate editor: H. Rodger Haevey