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1   **Sulfur-isotopic compositions of individual organic compounds from Cariaco Basin**

2   **sediments**

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13

14   **ABSTRACT**

15   Reactions between reduced inorganic sulfur and organic compounds are thought to be important

16   for the preservation of organic matter in sediments, but the sulfurization process is poorly

17   understood. Sulfur isotopes are potentially useful tracers of sulfurization reactions, which often

18   occur in the presence of a strong porewater isotopic gradient driven by microbial sulfate

19   reduction. Prior studies of bulk sedimentary organic matter indicate that sulfurized products are

20   ^{34}S-enriched relative to coexisting sulfide, and experiments have produced ^{34}S-enriched

21   organosulfur compounds. However, analytical limitations have prevented this relationship from

22   being tested at the molecular level in natural environments. Here we apply a new method,

23   coupled gas chromatography – inductively coupled plasma mass spectrometry, to measure the

24   compound-specific sulfur isotopic compositions of volatile organosulfur compounds over a six-

25   meter-long core of anoxic Cariaco Basin sediments. In contrast to current conceptual models,

26   nearly all extractable organosulfur compounds are substantially depleted in ^{34}S relative to

27   coexisting kerogen and porewater sulfide. We hypothesize that this ^{34}S depletion is due to a

28 normal kinetic isotope effect during the initial formation of a carbon-sulfur bond and that the

29 source of sulfur to this relatively irreversible reaction is most likely the bisulfide anion in

30 sedimentary pore water. The ^{34}S-depleted products of irreversible bisulfide addition alone cannot

31 explain the isotopic composition of total extractable or residual organic matter. Therefore, at

32 least two different sulfurization pathways must operate in Cariaco Basin, generating isotopically

33 distinct products. Compound-specific sulfur isotope analysis thus provides new insights into the

34 timescales and mechanisms of organic matter sulfurization.

35

36

1. Introduction

Organic matter is preserved in sediments as a complex macromolecular structure known as kerogen. However, many of the mechanisms for forming kerogen are still poorly understood. (Aizenshtat et al., 1995; Eglinton et al., 1994; Francois, 1987; Vandenbroucke and Largeau, 2007). Organic molecules are sometimes bound to the kerogen matrix by sulfur bridges, and organic sulfur in a variety of aromatic and aliphatic ring structures can also be extracted from immature sediments (Sinninghe Damsté et al., 2007; Vairavamurthy and Mopper, 1987; Vairavamurthy et al., 1994) and crude oils (e.g., Schmid et al., 1987; Sinninghe Damsté et al., 1987; Sinninghe Damsté and De Leeuw, 1990). Although some of the organic sulfur compounds (OSC) observed in marine sediments have been reproduced under laboratory conditions (Amrani and Aizenshtat, 2004a; 2004b; de Graaf et al., 1992; Kok et al., 2000; LaLonde et al., 1987; Rowland et al., 1993; Schouten et al., 1994), the relationship between sulfurization reactions in natural systems and the sulfur-isotopic composition of sedimentary organic matter has not yet been determined.

Organic sulfur (OS) in kerogen or proto-kerogen, which we refer to as 'residual' OS, is typically more enriched in ^{34}S than its likely sulfur sources, either bisulfide or polysulfide species in coexisting porewater (Anderson and Pratt, 1995; Bottrell and Raiswell, 2000). This phenomenon has been difficult to explain based on experimental (Amrani and Aizenshtat, 2004) and initial compound-specific δ^{34}S results (Werne et al., 2008). Moreover, only a subset of available functionalized organic compounds are affected by sulfurization reactions, while others persist in sediments without experiencing sulfurization. In this study, we investigate whether sulfur isotopes can provide a tracer for mechanisms of organic sulfur formation at the molecular level.

60   We use a newly developed method for compound-specific sulfur isotope analysis (Amrani et al.,

61   2009) to obtain $\delta^{34}S$ values for individual, GC-amenable organosulfur compounds from Cariaco

62   Basin sediments.

63

64   Sulfur isotopic compositions are a potentially powerful tool for exploring the timing and

65   mechanisms of organic sulfurization reactions due to the large down-core porewater sulfur

66   isotope gradients typically generated by microbial sulfate reduction in anoxic sediments. The

67   sulfur isotopic compositions of bulk pools have previously been used to constrain the timing of

68   kerogen and extractable OSC formation relative to pyrite and other sedimentary sulfur sinks.

69   However, because OSC form at different rates and during various stages of early diagenesis

70   (Sinninghe Damsté et al., 2007; Vairavamurthy et al., 1994; Vairavamurthy and Mopper, 1987;

71   Werne et al., 2008), bulk organic sulfur potentially represents a mixture of OSC with diverse

72    $\delta^{34}S$ values. Our results represent the first $\delta^{34}S$ measurements for individual organosulfur

73   compounds in complex lipid extracts from marine sediments.

74

75

76   **2. Site Background**

77   Samples for this study were taken from Cariaco Basin, located north of Venezuela. The Cariaco

78   Basin water column is anoxic and sulfidic below 300 m, which facilitates the delivery of

79   moderately high concentrations of reduced inorganic sulfur species and organic carbon (up to 6

80   wt%) to the sediments, making this an excellent site to study organic sulfurization reactions.

81   Sample material was obtained from Ocean Drilling Program (ODP) Core 1002B, which was

82   collected from the western side of Cariaco Basin at Site 165 at approximately 900 m water depth

83   (Shipboard Scientific Party, 1997). The core was frozen immediately following collection, with

84   the exception of the shallowest material (40 cm sample), which was squeezed to extract

85   porewater onboard and then frozen. The core has been stored frozen since that time. Eight

86   subsamples of the core were obtained from the frozen archive in 2011, and three additional

87   subsamples were collected in 2012. The subsamples represent approximately 2 cm of

88   homogenized material each and are spaced at eight roughly equal depth intervals between 40 and

89   535 cm, all within the upper laminated section of the core that appears to have experienced

90   continuous anoxic and sulfidic deposition (Lyons et al., 2003). Our deepest sample is near the

91   contact with underlying massive sediments at approximately 650 cm and represents nearly 12.6

92   ^{14}C kyr (14.5 calendar kyr) of deposition. The age model for this core is based on correlation of

93   magnetic susceptibility to a nearby core (PL07-39PC) that has been well-dated using radiocarbon

94   (Lin et al., 1997; Werne et al., 2000).

95

96   The lithology and geochemistry of Core 1002B have been studied extensively, providing

97   valuable context for our work. Werne and colleagues (2003) presented depth profiles of the

98   concentrations and sulfur-isotopic compositions of pyrite, reactive iron, extractable OS

99   ('bitumen'), residual OS ('kerogen'), total sulfur, and porewater sulfate and sulfide. Other

100   available data for this core include concentrations of organic and inorganic carbon, molybdenum,

101   iron, and aluminum (Lyons et al., 2003), and major biomarker distributions (Werne et al.,

102   2000b).

103

104   Porewater sulfate concentrations in Cariaco Basin decline steadily from more than 27 mM at the

105   sediment-water interface to 3.8 mM by 590 cm depth. Porewater sulfide concentrations increase

106   from water column-like concentrations of less than 0.1 mM near the surface to 9.0 mM at 215

107   cm and then decrease below. Both sulfate and sulfide become progressively ^{34}S-enriched in

108   deeper sediments, a common pattern associated with distillation of the porewater sulfate

109   reservoir by dissimilatory microbial sulfate reduction (Amrani and Aizenshtat, 2004b; 2004a; de

110   Graaf et al., 1992; Kok et al., 2000; LaLonde et al., 1987; Rowland et al., 1993; Schouten et al.,

111   1994; Werne et al., 2003). Pyrite sulfur is present at approximately 1.3 wt% in surface sediments.

112   Deeper diagenetic pyrite formation is relatively minor and likely iron-limited. Throughout the

113   profile, pyrite is similar in its δ^{34}S values to water column HS$^-$ and is ^{34}S-depleted relative to

114   both coexisting sulfide and kerogen, which is consistent with early formation of pyrite, including

115   formation in the water column (Aizenshtat et al., 1995; Anderson and Pratt, 1995; Lyons et al.,

116   2003). Organic matter is abundant (up to 6 wt% TOC) and sulfur rich (up to 0.6 wt% kerogen S)

117   (Werne et al., 2003). Organosulfur compounds have also been observed in the extractable lipid

118   fraction below 240 cm (Werne et al., 2000).

119

120

121   **3.   Methods and Instrumentation**

122   The data presented here were collected iteratively, as separation and analytical methods were

123   incrementally improved over the course of the study (described below). In all cases, two to five

124   grams of frozen sediment were dried under vacuum, and free lipids were obtained by microwave

125   extraction at 100°C for 15 minutes (Mars 5, CEM Corp.) in 9:1 v/v dichloromethane

126   (DCM):methanol (MeOH). Compounds were separated by polarity on a silica gel column (4g) by

127   sequential elution with 4:1 hexane:DCM (F1, 40 mL, all depths), DCM (F2, 40 mL, for 40, 70,

128   265, and 535 cm depths only), and 1:1 DCM:MeOH (F3, 40 mL, all depths). Initial analysis by

129   gas chromatography (GC) was conducted on a Varian CP-3800 GC equipped with a HP DB5-

130   MS (30 m x 320 μm ID x .25 μm film) capillary column and programmed temperature

131   vaporizing (PTV) injector. GC effluent was split between a Varian Saturn 2200 ion trap mass

132   spectrometer with electron impact (EI) ionization and a Sievers 355 sulfur chemiluminescence

133   detector (SCD) for simultaneous identification and quantification of organosulfur compounds.

134

135   **3.1   Compound-specific δ^{34}S analysis by GC-ICPMS**

136   The sulfur-isotopic compositions of individual GC-amenable organic compounds from all

137   fractions were measured with a multicollector inductively-coupled plasma mass spectrometer

138   (MC-ICPMS, Thermo Neptune[+]) using an Agilent 6890 GC for gas-phase sample introduction

139   and separation following the approach described by Amrani (2009). This GC was operated with

140   the same column type and operating conditions as the Varian instrument but differs in that it has

141   a split/splitless injector. Accuracy was established by repeated analyses of external standard

142   mixtures containing three to six OSC with known δ^{34}S values. Mean δ^{34}S values for the

143   combined data for each standard are within 1.4‰ of published EA-IRMS values (Amrani et al.,

7

144   2009) and have a root-mean-squared variance of 1.5‰. Due to improvements in chromatography

145   and baseline standardization, the 2012 data are all accurate to within 1.0‰ and have a root-

146   mean-squared variance of 0.9‰. Many of the polar fractions (F3, see below) of Cariaco extracts

147   were analyzed in triplicate to assess any additional uncertainty due to the complexity of sample

148   chromatograms. Triplicate analyses for most of the major peaks in this fraction achieved δ^{34}S

149   standard deviations of 1.3 to 1.8‰, although several peaks with less well-resolved baselines

150   returned a larger range of values. F2 chromatograms were much less crowded, and δ^{34}S values

151   for duplicate F2 runs varied by a maximum of 0.8‰. This performance is comparable to that

152   reported by Amrani and colleagues for complex environmental samples (Amrani et al., 2012;

153   2009).

154

155   A gas-phase standard (SF$_6$) with known δ^{34}S value was used for tuning and calibration of isotope

156   ratios. In our earliest analyses, the argon 'sample gas' for the plasma was preheated by flowing

157   through the GC oven before entering the custom-built heated transfer line. With this

158   arrangement, both sensitivity and δ^{34}S values drifted systematically (by nearly 10‰ in the latter

159   case) as the GC oven ramped from 80° to 300°C. We now understand that this effect arises from

160   an imperfect response of the Neptune mass flow controllers to changing flow resistance with GC

161   temperature. To account for such effects, sample δ^{34}S values were standardized to a linear

162   interpolation of frequent SF$_6$ reference gas peaks (Supplemental Figure 1). In later analyses, we

163   eliminated this drift by isolating the sample gas heating system from the GC oven. In this system,

164   the argon flow was preheated to a constant 320° outside the GC before entering the upstream end

165   of the transfer line to the plasma torch. An additional heating tape was also added at the ground-

166   glass connection between the transfer line and the injector torch to improve the transfer of

167 higher-boiling-point analytes into the plasma. With these modifications, good peak shapes were

168 obtained for compounds eluting at oven temperatures of up to 300°C, permitting sulfur-isotopic

169 analysis of a wider range of GC-amenable compounds. Because δ^{34}S values for SF_6 drift by less

170 than 1‰ across the GC temperature program under these conditions, drift correction is no longer

171 necessary and standard peaks are not required in the midst of the GC oven temperature program,

172 thus avoiding potential interferences between standard and analyte peaks.

173

174 GC-ICPMS data were exported from the Neptune Evaluation software into Isodat v. 3.1

175 (Thermo) for processing. Results are reported in the conventional δ^{34}S notation as permil (‰)

176 deviations from the VCDT standard. Accurate and precise δ^{34}S values were obtained for analytes

177 producing peak areas of at least ~1 Vs, representing approximately 50 pmol of analyte on-

178 column. Unlike for other light isotopes (H, C, N), we have not observed chromatographic

179 separation of sulfur isotopologues, meaning that $^{34}S/^{32}S$ ratios are invariant across even large

180 chromatographic peaks. This is consistent with previous observations (Amrani et al., 2012;

181 2009). Poor chromatographic peak shapes and/or coelutions were the dominant source of

182 measurement uncertainty, particularly during early analyses. Results were sometimes moderately

183 sensitive to the manually selected background interval due to the contribution of unresolved OSC

184 to the *m/z* 34/32 ratio of background signals, and peaks for which δ^{34}S values varied by more

185 than 1.5‰ for alternative baseline definitions were discarded. In sum, we conservatively

186 estimate that the data presented here have (1σ) uncertainties of 1.0‰ for F1 and F2 fractions and

187 1.5‰ for F3. Uncertainties are larger for F3 because the abundant OSC and other compounds in

188 this fraction generate complex chromatograms with poorer baseline resolution. Although these

189 analytical uncertainties are large compared to those typically achieved by conventional sulfur

190   isotope analyses, they are nevertheless much smaller than the scale of isotopic variability

191   observed in the Cariaco sulfur record and therefore still permit meaningful interpretations.

192

193   **3.2   Analysis of bulk organic fractions by ICPMS**

194   Aliquots of each fraction (F1, F2, F3) of the solvent extract were dried and then oxidized in 30%

195   H_2O_2 at 90° C for 24 hrs to convert organic sulfur to sulfate. Resulting sulfate was then purified

196   on AG1-X8 anionic exchange resin according to the method described in Paris et al. (2013).

197   Resin was washed with ten column volumes (CV) 10% HNO_3^-, conditioned with 10 CV 10%

198   HCl and 10 CV 0.5% HCl, loaded in trace HCl, and washed three times with 5 CV Milli-Q H_2O

199   before sulfate was eluted in 0.5N HNO_3. Sulfate samples were quantified by ion chromatography

200   (IC, Dionex ICS-2000) with an AS-19 anion column and AERS 500 ion regeneration.

201   Concentrations were used to intensity-match samples and the required Na^+ supplement for ICP-

202   MS analysis (Paris et al., 2013). Samples were injected into the plasma torch with a desolvating

203   nebulizer (Aridus) and bracketed with known $\delta^{34}S$ $NaSO_4$ standards. The Neptune was operated

204   in high resolution (M/ΔM ~ 10,000) to fully resolve oxygen interferences on masses 33 and 34.

205   Accuracy was assessed by repeated analyses of a seawater sulfate standard. Both accuracy and

206   precision for $\delta^{34}S$ values in this mode of analysis were typically better than ±0.2‰.

207

208   **4   RESULTS AND DISCUSSION**

209   **4.1   Abundance and δ^{34}S of bulk organic sulfur**

210   Concentrations of extractable OS polarity fractions and total residual OS ('kerogen' from Werne

211   et al. 2003) are shown in Figure 1. In sediments, extractable OS increases with depth from 17 to

212   77 nmol S/g_{OC} and represents about 0.1‰ of residual OS. The δ^{34}S of extractable and residual

213   OS are similar, especially below 40 cm, and range from -18.0‰ to -25.9‰. Polar (F3) material

214   represents 92 to 98% of total extractable OS.

215

216   [FIGURE 1]

217

218   **4.2   OSC identification and abundance**

219   A consistent suite of organosulfur compounds was observed by GC-MS-SCD and GC-ICP-MS at

220   all sample depths. Two OSC with well-constrained EI mass spectra are identified as isomers of a

221   C_{20} isoprenoid thiophene. They are inferred derive from the sulfurization of phytol and/or

222   phytadiene based on comparison to published mass spectra (Brassell et al., 1986; Putschew et al.,

223   1996). C_{20} isoprenoid thiophenes have been widely observed in non-polar fractions of extracts

224   from sediments (e.g., Sinninghe Damsté et al., 1987; Wakeham et al., 1995) and experimental

225   studies (de Graaf et al., 1992; Rowland et al., 1993; Krein and Aizenshtat, 1994; Gelin et al.,

226   1998) . In the current work, two identical C_{20} isoprenoid thiophenes were observed in multiple

227   polarity fractions and could not be isolated in a single fraction by column chromatography. The

228   sulfur-isotopic compositions of C_{20} isoprenoids in F1 and F3 fractions from the same sample are

229   similar but not identical, and their concentrations have different trends with depth (Figure 1,

230   shaded compounds in panels A and B), consistent with the C_{20} isoprenoid thiophenes in F1 and

231 F3 representing distinct precursor compounds. Those in the non-polar fraction (F1), which are

232 comparable to previous observations in the literature (e.g., Brassell et al., 1986, Fukushima et al.,

233 1992), are interpreted to represent C_{20} isoprenoid thiophenes present in the original sample.

234 Because thiophenes can form at elevated temperatures, we interpret the more abundant C_{20}

235 isoprenoid thiophenes observed in F3 as forming in the GC injector, for example by ring closure

236 or aromatization of a more polar precursor with a similar phytol-derived skeleton. We find no

237 evidence for either disulfide bonding or thiol groups in the precursors to the C_{20} isoprenoid

238 thiophenes in F3, and the original functional form of these molecules remains the subject of

239 ongoing investigation.

240

241 Peaks eluting at 28 min are identified as isomers of highly branched isoprenoid (HBI) thiolanes,

242 presumably derived from the sulfurization of diatom HBI lipids. Mass spectra do not decisively

243 establish whether the sulfur functionality is a thiolane or thiane, although the spectra share all

244 major fragment ions with previously published HBI thiolane spectra (Kohnen, 1991; Kohnen et

245 al., 1990). A single double bond in the molecule is indicated by the molecular ion at m/z = 380.

246 HBI thiolanes have previously been observed in Cariaco Basin (Werne et al., 2000), and they

247 were also physically isolated for conventional sulfur-isotopic analysis (Werne et al., 2008).

248 Similar to the C_{20} isoprenoid thiophenes, identical HBI thiolanes are observed in both the apolar

249 (F1) and polar (F3) fractions, with higher abundance in F3 (5 to 63 nmol/g OC) than in F1 (2 to

250 18 nmol/g OC).

251

252 We also observe the monounsaturated triterpenoid thiane that was previously described from

253 Cariaco Basin sediments based on published EI mass spectra (Wakeham et al., 1990; Werne et

254   al., 2000) The abundance of this triterpenoid thiane in our study (6 to 69 nmol/g OC) is in the

255   same range as observed previously (16 to 115 nmol/g OC) (Werne et al., 2000).

256

257   One of the only volatile OSC in F2 with a clear EI fragmentation spectrum (designated U-14) is

258   tentatively identified as a thiolane with a twelve-carbon skeleton ($C_{12}H_{20}S$). The fragment with a

259   m/z ratio of 127 may represent $C_7H_{11}S$, which contains two double bond equivalents and appears

260   to describe a thiane or thiolane aliphatic sulfide. Aliphatic sulfides are likely to fragment at the

261   α-β carbon bond, leading us to infer the existence of a five-carbon alkyl chain β to the sulfide

262   sulfur.

263

264   We were also able to measure $δ^{34}S$ values for nineteen other OSC in the Cariaco. These OSC are

265   observable by S-specific detectors like the ICPMS and GC-SCD but coelute with much more

266   abundant, non-S-bearing compounds in GC-MS analyses. We were thus unable to obtain useful

267   mass spectra despite attempting a variety of cleanup and fractionation schemes and different

268   ionization methods (EI, CI, MS/MS). Nevertheless, as shown in Figure 2, these unidentified

269   compounds have generally similar sulfur-isotopic compositions and suggest that our identified

270   compounds are representative of the larger population of volatile OSC.

271

272   The total amount of OSC observed by GC-ICP-MS was higher in F3 (83 to 306 nmol/g OC) than

273   in F1 (34 to 117 nmol/g OC) or F2 (31 to 178 nmol/g OC). Individual compounds were present

274   at concentrations of 1 to 100 nmol/g OC (Figure 1). The triterpenoid thiane, unidentified

275   compound U-14, and the C_{20} isoprenoid thiophene in F3 were particularly abundant at 535 cm,

276   with concentrations of 68 to 102 nmol/g OC. Their concentrations were consistently lower in the

277 shallower samples (4 to 20 nmol/g OC, Figure 1B). In contrast, the concentrations of HBI

278 thiolanes and C_{20} isoprenoid thiophenes in F1 drop to relatively low values below maxima at 40

279 cm.

280

281 **4.3   OSC sulfur-isotopic compositions**

282 Several compounds have consistently lower $\delta^{34}S$ values than pyrite and represent the most highly

283 [34]S-depleted species observed in Cariaco Basin to date. In the shallowest sample, the triterpenoid

284 thiane and HBI thiolane from F3 have $\delta^{34}S$ values of -43.6‰ and -40.9‰, respectively, and less

285 negative $\delta^{34}S$ values in deeper samples (between -32.3‰ and -28.2‰). C_{20} isoprenoid

286 thiophenes from both F1 and F3 are more [34]S-enriched than the triterpenoid thiane and HBI

287 thiolanes, with $\delta^{34}S$ values between -37.1‰ and -18.7‰. Depth trends for these compounds are

288 shown in Figure 2A. Figure 2B summarizes $\delta^{34}S$ values for all measured OSC at all depths,

289 including unidentified compounds. OSC $\delta^{34}S$ values have a unimodal distribution around

290 approximately -32 ± 10‰.

291

292 [FIGURE 2]

293

294 The sulfur-isotopic compositions of HBI thiolanes in F1 and the triterpenoid thiane were

295 measured in a previous study of Cariaco Basin sediment extracts by Werne and colleagues

296 (2008). Using preparative liquid chromatography (LC) followed by EA-IRMS, they obtained

297 $\delta^{34}S$ values between -21‰ and -5‰ for depths between 240 and 580 cm, an enrichment of

298 approximately 30‰ relative to our GC-ICP-MS results (Figure 3). As these results apply to the

299 same compounds, both cannot be correct. Given the improved specificity of our GC-ICPMS

300   technique, the LC-EA-IRMS results probably described a mixture of the target OSC and some

301   other coeluting (by LC) or background sulfur species, though there was no indication of

302   additional compounds in the fraction when analyzed by GC-FPD (Werne et al., 2008).

303   Nevertheless, the presence of a sulfur blank in the isolated fractions could potentially explain the

304   high δ^{34}S values for OSC in Werne et al. (2008), as these sulfur isotope compositions were

305   determined via EA-IRMS.

306

307   [FIGURE 3]

308

309   **4.4   Multiple timescales of OSC formation**

310   Volatile OSC in Cariaco Basin sediments have sulfur-isotopic compositions and depth profiles

311   that are similar to coexisting pyrite (Figure 3). A relatively large but variable proportion of

312   sedimentary pyrite forms in the water column (Lyons et al., 2003); this "syngenetic" pyrite forms

313   in an effectively open system with respect to sulfide, and its δ^{34}S value is affected by processes

314   at the oxygen-sulfide interface. A smaller component of pyrite in Cariaco Basin sediments is

315   diagenetic, forming in a diffusion-controlled regime within sediments in which sulfide δ^{34}S

316   values increase with depth (Werne et al., 2003). Like pyrite, Cariaco OSC are likely produced by

317   both syngenetic and diagenetic processes. Different individual compounds, however, may

318   preferentially form in one environment or the other. For example, Werne et al. (2000)

319   conclusively demonstrated diagenetic production of the triterpenoid thiane based on the extent of

320   precursor molecule conversion. Consistent with the conclusions of that study, we find that the

321   triterpenoid thiane increases in concentration from 5.7 to 69 nmol/g OC as its δ^{34}S value

322   increases from -43.6‰ to -32.3‰. Both concentration and sulfur-isotope data thus support

15

323 predominantly diagenetic formation of this compound. C_{20} isoprenoid thiophenes in F3 and

324 compound U-14 also have similar concentration profiles with only slightly smaller shifts in their

325 $\delta^{34}S$ values between 70 and 535 cm (Figure 1). Thus they are also probably products of

326 sedimentary diagenesis, forming on timescales of thousands of years.

327

328 In contrast, other OSC exhibit no concentration change with depth, thereby providing no

329 conclusive evidence for accumulation during diagenesis. Nevertheless, the $\delta^{34}S$ values of HBI

330 thiolanes from F3, C_{20} isoprenoid thiophene from F1, and compound U-1 also increase by 7‰ to

331 10‰ over the six-meter core, comparable to the pattern observed for diagenetic OSC and

332 implying ongoing formation. Concentration and $\delta^{34}S$ profiles for these compounds can be

333 reconciled in one of two ways. First, these OSC may have a significant syngenetic source, with

334 their concentration profiles reflecting variable production in the water column over time. Isotopic

335 variability on the scale of a few permil would then reflect dynamic sulfur cycling near the

336 chemocline, where sulfide $\delta^{34}S$ can vary due to changes in local redox state (Li et al., 2010;

337 2011). Alternatively, changes in the $\delta^{34}S$ values of non-accumulating OSC with depth could

338 result from slow equilibration between the OSC and (poly)sulfides. Given the scale of

339 depositional variability evident in this core, either scenario seems viable.

340

341 **4.5   Isotope effects during organic matter sulfurization**

342 The $\delta^{34}S$ profiles for individual OSC in Cariaco Basin reflect a combination of the $\delta^{34}S$ values of

343 the reactant sulfur species, the mechanism of sulfurization, and any associated fractionation

344 during incorporation. Our results help constrain the possible mechanisms of organic matter

345 sulfurization in Cariaco sediments.

346

347   Both bisulfide and polysulfide species have been considered probable reactants for organic

348   sulfurization (Anderson and Pratt, 1995). Under typical sedimentary conditions, the dominant

349   species available to react with organic matter are the bisulfide anion (HS^-) and dianionic

350   polysulfide (S_x^{-2}, Schwarzenbach and Fischer, 1960). These same species are involved in pyrite

351   formation (Rickard and Luther, 2007), which occurs at low rates in Cariaco sediments (Lyons et

352   al., 2003; Werne et al., 2003). In Cariaco sediment porewater, the $\delta^{34}S$ value of dissolved HS^-

353   increases from -29‰ near the sediment-water interface to greater than -5‰ at 550 cm depth.

354   Thus, all of the volatile OSC we observe are more ^{34}S-depleted than coexisting dissolved sulfide,

355   their likely sulfur source. The $\delta^{34}S$ difference between porewater sulfide and coexisting OSC is

356   as much as 17‰ (triterpenoid thiane, 70 cm). Polysulfide $\delta^{34}S$ has not been measured directly in

357   Cariaco Basin, but experimental work has documented a small ^{34}S enrichment in polysulfides

358   relative to total sulfur at equilibrium (Amrani et al., 2006). We thus predict that polysulfides are

359   slightly more ^{34}S-enriched than bisulfide in Cariaco Basin, and therefore the apparent kinetic

360   isotope effect associated with OSC formation from polysulfides would be even larger than for

361   bisulfide. We also note that oxidants are limiting in Cariaco sediments (Lyons et al., 2003),

362   which should maintain very low polysulfide concentrations and potentially favor organic matter

363   sulfurization reactions involving plentiful bisulfide.

364

365   If we assume that porewater (poly)sulfide is the source of sulfur to these ^{34}S-depleted OSC, their

366   formation would appear to involve a normal kinetic isotope effect, i.e., the reaction between

367   (poly)sulfide and an organic molecule proceeds more rapidly for the lighter S isotope. A normal

368 kinetic isotope effect has been previously predicted for initial C-S bond formation (Brüchert and

369 Pratt, 1996) during sedimentary organic matter sulfurization. In contrast, an equilibrium isotope

370 effect will result in ^{34}S-enrichment in the compound with the stronger bond and would be

371 expected to produce OSC that are enriched in ^{34}S relative to (poly)sulfides (Amrani et al., 2004).

372 Reversible reactions should lead to isotopic compositions that are governed by equilibrium

373 isotope effects, whereas irreversible reactions typically record kinetic isotope effects (Hayes,

374 1993). Therefore, the sulfurization reactions leading to extractable OSC monomers in Cariaco

375 sediments may be largely irreversible.

376

377 [FIGURE 4]

378

379 Sulfur addition by the bisulfide anion is more likely to be rapid and irreversible than sulfur

380 addition by polysulfide. Initial nucleophilic attack by a bisulfide anion on an organic functional

381 group produces a thiol. In contrast, the initial product of nucleophilic attack by a polysulfide

382 dianion has a chain of two sulfur atoms that retain a negative charge. These products may

383 subsequently undergo reaction with another functional group, either on the same molecule,

384 forming a ring, or on a different molecule, forming a (poly)sulfide bridge (Figure 4). Rapid

385 intramolecular addition would be more energetically favorable for a bisulfide-derived thiol group

386 than for a polysulfide chain due to the relative strength of the S-S bond compared to the S-H

387 bond. It is likely – although not certain for compounds appearing in multiple fractions – that all

388 of the volatile compounds we identified in Cariaco lipid extracts experienced intramolecular

389 addition for that second step. Therefore, if we assume that volatile OSC monomers in Cariaco

390   Basin derive from either porewater polysulfides or bisulfide, their highly ^{34}S-depleted isotopic

391   compositions appear to derive from the bisulfide anion and a strong kinetic isotope effect.

392

393   A large kinetic isotope effect has not been previously reported for organic matter sulfurization.

394   Total residual OS is typically more ^{34}S-enriched than its sulfur source, broadly indicating an

395   equilibrium isotope effect in its formation. This relationship holds both in a global compilation

396   of rocks, where pyrite δ^{34}S is taken to represent sulfide (Bottrell and Raiswell, 2000) and in

397   modern marine sediments (Dale et al., 2009). However, individual OSC need not conform to

398   models based on total OS. In Cariaco Basin, both extractable and residual OS, which are similar

399   below 70 cm, are significantly more ^{34}S-enriched than the GC-amenable OSC separated from the

400   extractable OS pool. Thus, extractable OS in Cariaco Basin must represent a mixture of OS with

401   distinct S-isotopic compositions.

402

403   Based on the preceding evidence, we propose that organic matter sulfurization may be associated

404   with equilibrium or kinetic isotope effects under different conditions (Figure 4). Although all of

405   the volatile OSC measured here by GC-ICP-MS fall in the second category, there is evidence for

406   both types of isotope effect in Cariaco Basin. The formation of GC-amenable OSC in Cariaco

407   Basin also appears to precede sulfur incorporation into residual OS, because all of the major

408   species of volatile OSC are abundant by 40 cm depth, whereas lipid sulfur cross-linking remains

409   minimal at 27 cm (Aycard et al., 2003). Different timescales for the onset of bitumen OSC and

410   sulfurized polymer formation are also consistent with a mechanistic distinction between

411   reversible and irreversible sulfurization pathways.

412

413   Alternatively, volatile OSC could potentially form with an equilibrium isotope effect if they

414   derive from a (poly)sulfide pool that has a different isotopic composition than porewater sulfide.

415   Such an isolated pool would need to be substantially more ^{34}S-depleted than porewater sulfide

416   and might be present within cells or in microenvironments in the sediment. Without

417   corroborating evidence for the existence of such an isolated reservoir, this explanation remains

418   highly speculative. Still, if their existence is supported by future work, non-porewater

419   (poly)sulfide reactants could explain OSC with δ^{34}S values that differ from porewater sulfide

420   δ^{34}S without invoking a large kinetic isotope effect during OSC formation.

421

422   The hypothesized distinction between the δ^{34}S values of the products of inter- and intramolecular

423   sulfurization reactions may provide a useful constraint on the processes controlling kerogen

424   formation and a path toward a better understanding of the preservation of organic carbon within

425   the kerogen matrix. Previous attempts at interpreting the sulfur isotopic composition of bulk

426   sedimentary organic matter have suffered from analytical limitations (Wakeham et al., 1995;

427   Werne et al., 2008), presenting a serious impediment to paleoenvironmental studies that rely on

428   sulfur isotopic signals or preserved biomarkers in kerogen to reconstruct paleoenvironmental

429   conditions. Different sulfurization mechanisms may yield distinct isotopic fractionations that will

430   have different effects on sulfur isotope mass balance and on the isotopic signals preserved in

431   sedimentary records. This study is, in part, a proof of concept that should guide future efforts.

432

433   **5.0   Conclusions**

434   The sulfur-isotopic compositions of GC-amenable organosulfur compounds in Cariaco Basin

435   sediments range from -43.6‰ to -18.7‰, similar to coexisting pyrite but more ^{34}S-depleted than

436   total extractable and residual organic sulfur. Organic matter sulfurization in Cariaco Basin

437   appears to occur on multiple timescales. Concentration increases and δ^{34}S enrichments with

438   depth reflect primarily diagenetic production on kyr-timescales for a triterpenoid thiane, C_{20}

439   isoprenoid thiophene in polar extracts (F3), and unidentified compound U-14, while either

440   variable syngenetic sources or diagenetic sinks exist for HBI thiolanes in both polar and non-

441   polar extracts (F1 and F3), C_{20} isoprenoid thiophene in non-polar extracts (F1), and compound

442   U-1. Regardless of their concentration patterns, all of the observed compounds exhibit a normal

443   kinetic isotope effect relative to their reactant sulfur source, which is most likely porewater

444   bisulfide. This hypothesis contrasts with the equilibrium isotope effects observed for the

445   formation of sulfur-cross-linked OSC polymers from polysulfide (Amrani et al., 2004) and with

446   the incorporation of (apparently) similar sulfur species into kerogen in Cariaco Basin (Aycard et

447   al., 2003). We hypothesize that at least two distinct pathways are at work in generating

448   sedimentary organosulfur molecules and that they may be distinguished by their sulfur isotopic

449   compositions. If correct, compound-specific δ^{34}S analysis of OSC should provide a powerful tool

450   for unraveling the complex pathways of kerogen formation.

451

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459

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Figure 1: Concentrations of individual OSC and OS pools with depth in Cariaco sediments.

Triangles, squares, and diamonds represent data associated with fractions F1, F2, and F3, respectively. Panels A and B: concentrations of individual OSC. Panel A shows C_{20} isoprenoid thiophene (F1, shaded triangles), triterpenoid thiane (open triangles), and HBI thiolane (diamonds); these compounds are also highlighted in Figure 2. Panel B shows C_{20} isoprenoid thiophene (F3, shaded diamonds) and two unidentified compounds, U-14 (squares) and U-1 (diamonds). Panel C shows total extractable OS in F1 (triangles), F2 (squares), and F3 (diamonds); note scale change. Panel D shows concentration of residual OS. Note that this concentration is expressed relative to organic carbon (OC) rather than sediment mass. OC concentrations average 4.7% wt% throughout the core (data from Werne et al. 2003).

Figure 2: Sulfur-isotopic compositions of OSC and extractable OS fractions. *Porewater sulfide (light grey) and residual OS (dark grey) data are from (Lyons et al., 2003; Werne et al., 2003). Panel A: Symbols with thick outlines indicate $\delta^{34}S$ values of total OS fractions F1 (triangles) and F3 (diamonds). Open symbols indicate $\delta^{34}S$ values of individual GC-amenable compounds in those fractions – triterpenoid thiane in F1 (open triangles), C_{20} isoprenoid in F1 (shaded triangles), and HBI thiolane in F3 (diamonds). Panel B: Histogram of all compound-specific measurements from Cariaco Basin sediment extracts, including both identified and unidentified compounds. The shaded area represents the range of $\delta^{34}S$ measured for total extractable OS.*

611   **Figure 3: Comparison of compound-specific δ^{34}S results by different analytical techniques.**

612   *Sulfur-isotopic compositions of triterpenoid thiane (triangles) and HBI thiolane (diamonds)*

613   *measured by two different analytical techniques. Symbols represent data obtained by GC-ICP-*

614   *MS (this study, smooth lines) and prep-LC and EA-IRMS (Werne et al., 2008, dashed lines).*

615   *Lines without symbols represent residual OS (dark grey), porewater sulfide (light grey), and*

616   *pyrite (black) δ^{34}S values (from Lyons et al., 2003; Werne et al., 2003).*

617

618   **Figure 4:** *Examples of intermolecular and intramolecular sulfurization pathways following*

619   *initial (poly)sulfide attack on a diene. Based on* Kohnen et al. (1990).

620

621   **Supplemental Figure 1: Examples of chromatography by GC-ICPMS.** *A: 70 cm, F2. B: 535*

622   *cm, F2. Grey circles mark SF_6 reference gas peaks; SF = solvent front; IS = internal standard.*

623   *Both examples include a large number of SF_6 peaks because they were run prior to modification*

624   *of the sample gas heating system (Section 3.1).*

625

A.

δ³⁴S (‰)$_{VCDT}$

depth (cm)

B.

\# OSC

Total
extractable
OS

HS$_x^-$

Intermolecular
Addition -
Reversible

Intramolecular
Addition -
Irreversible?

H$_x$

S$_x$

R

HS$_x$

R

S$_x$

R

A.

m/z = 32 (V)

SF

U-2

U-5

U-14

IS

Retention time

B.

m/z = 32 (V)

U-14

SF

U-5

IS

Retention time