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Organic sulfur compounds formed during early diagenesis in Black Sea sediments

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Abstract—Sediments from Units I and II of the Black Sea were analyzed to assess the early diagenetic formation of organic sulfur compounds (OSC). A series of isomeric C_{28}-2,4-dialkylthiophenes was found at low concentrations in surface sediments. OSC with C_{25}-highly branched isoprenoid (HBI) skeletons were not found in surface sediments even though there was a rapid decrease in the concentrations of C_{25}-HBI alkenes in the same sediments, nor were OSC found which contained carbon skeletons derived from C_{37}-C_{39} alkenes which are also abundant in the sediment. As depth increased in Unit I, concentrations of the C_{28}-2,4-dialkylthiophenes decreased while C_{25}- and C_{30}-HBI thiolanes appeared at the bottom of Unit I and increased in concentration into Unit II. Treatment of macromolecular material from sediment extracts with Raney nickel yielded phytane as the dominant hydrocarbon as well as series of C_{25}- and C_{30}-HBI hydrocarbons, β-carotane, and isorenieratane. These latter two components were generally absent from the free hydrocarbon fractions. These results indicate that sulfur incorporation into functionalized lipids can occur during the very early stages of sedimentary diagenesis, even at the sediment-water interface. On the other hand, the rapid loss of C_{25}-HBI alkenes in surface sediments could not be accounted for by sulfur quenching, and other potential OSC-precursors, such as C_{37}-C_{39} alkenes, also apparently did not incorporate sulfur into readily-analyzable material.

INTRODUCTION

A variety of organic sulfur compounds (OSC) have been identified in immature sediments and crude oils (see review by Sinninghe Damsté and de Leeuw, 1990). The available evidence indicates that inorganic species, such as H_2S or hydrogen polysulfides, react with functionalized lipids during diagenesis to yield low-molecular-weight OSC and S-bound moieties in macromolecules (e.g., Valisolalao et al., 1984; Brassell et al., 1986; Sinninghe Damsté et al., 1988, 1989, 1990; Kohnen et al., 1990a,b, 1991a,b; ten Haven et al., 1990; Adam et al., 1993). It has recently been reported that natural sulfurization can be simulated in the laboratory, and the results show that alkenes, aldehydes, and ketones can incorporate sulfur (Fukushima et al., 1992; de Graaf et al., 1992; Rowland et al., 1993; Schouten et al., 1993, 1994). Although the mechanism(s) involved in incorporation of sulfur into lipids in nature is still not completely understood, a diverse group of OSC with mono-, di-, and polysulfide bonds are generated, resulting in the removal of lipids from the low molecular weight portion of bitumen and in the preservation of the carbon skeletons of reactive precursors by protecting them from transformation or mineralization. Thus, structural features of labile compounds may be preserved in the fossil sedimentary record owing to the formation of their more stable sulfur-containing counterparts. Recovery of these remnant carbon skeletons by S-selective chemolysis provides an alternative means of assessing paleoenvironmental conditions of sediment deposition, the results of which may be significantly different from those obtained by analysing the free lipids (Kohnen et al., 1991a, 1992a,b; Sinninghe Damsté et al., 1993).

The majority of studies of sedimentary OSC have dealt with sediments of Pleistocene or older ages and have concluded that incorporation of sulfur into organic compounds takes place relatively early during diagenesis. An unresolved question regarding the diagenetic formation of OSC, however, is the extent to which these reactions occur in very young, contemporary sediments, at the sediment-water interface or in anoxic water columns. The few studies of contemporary environments involve evaporatic settings (Barbe et al., 1990; Kening and Huc, 1990) or humic substances from reducing salt marsh sediments (Al-Lihaibi and Wolff, 1991). The requirement of reduced sulfur as a reactant means that an anoxic basin, such as the Black Sea, may be a suitable environment to examine this issue. Potential precursor lipids for OSC, in particular highly branched isoprenoid (HBI) alkenes, long-chain normal alkenes, and long-chain alkenones, are abundant in the Black Sea and its sediments (Wakeham et al., 1991; Freeman and Wakeham, 1992). Moreover, a number of C_{25}- and C_{30}-HBI thiolanes have been identified in Black Sea sediment 3–6 × 10^3 yr old (Black Sea Unit II: Kohnen et al., 1990a), so at least over a several-thousand-year time frame conditions were conducive for formation of OSC.

In this paper we report the results of a study investigating the formation of OSC in young sediments from the Black Sea, ranging in age from several decades at the sediment surface to 6 × 10^3 years in Unit II. Three specific questions are addressed. First, given that OSC are present in Unit II sediments, Are these or other OSC generated in younger sediments of Unit I, perhaps even near the water-sediment interface? Second, since certain functionalized compounds which may act as precursors for OSC decrease markedly in concentration near the water-sediment interface (e.g., C_{25}-HBI alkenes), Are these compounds sequestered as OSC? And third, if OSC are formed, How important quantitatively are they in paleoenvironmental interpretations?
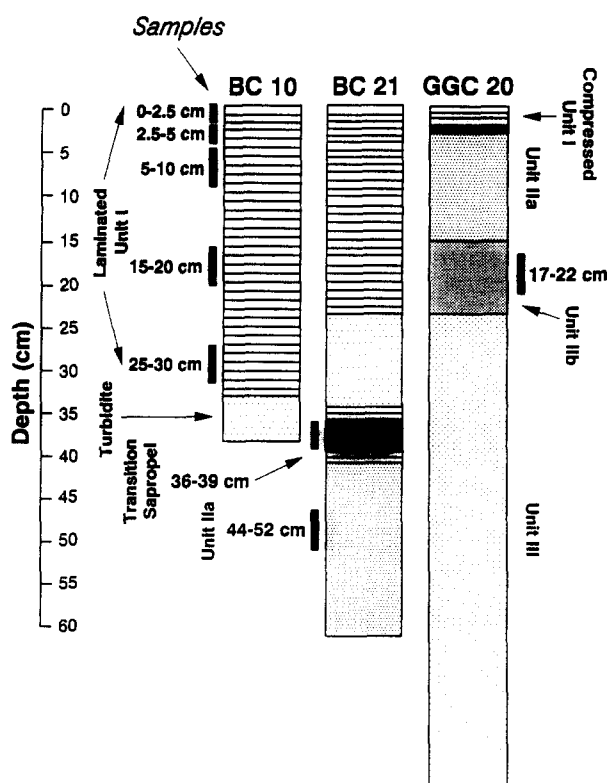
Samples

FIG. 1. Schematic diagram of the stratigraphic relationship between cores from which the sediment samples were taken, including the laminated Unit I samples from box core 10, transition sapropel and Unit IIa samples from box core 21, and Unit IIb from giant gravity core 20. Approximate ages for each section are given in Table 1.

EXPERIMENTAL

Samples

Sediments were collected in the central basin of the Black Sea during the 1988 expedition of R/V Knorr. Unit I sediments were obtained from a 30-cm box core (BC 10; 43°N, 34°E; 2200 m water depth; Wakeham et al., 1991). The transition sapropel at the base of

Unit I and Unit IIa sediment were obtained from a 60-cm box core (BC 21; 43°N, 32°E; 2100 m depth; Hay et al., 1991a). Sediments from Unit IIb were taken from a giant gravity core (GGC 20; 43°N, 32°E; 2100 m depth). A schematic overview of the sediment sections analysed is illustrated in Fig. 1 and further details are given in Table 1. The BC 10 sediment was frozen upon recovery; sediments from BC 21 and GGC 20 were stored at 4°C in the sediment repository at Woods Hole Oceanographic Institution.

A detailed description of sedimentation in the Black Sea during the past 5×10^3 years is given by Hay (1988) and Hay et al. (1991a). Unit I consists of a modern finely laminated coccolith ooze deposited in the present highly anoxic marine basin. Organic carbon contents range between 3–5%; the white laminae contain over 90% by weight of coccoliths of the marine alga, Emiliania huxleyi, deposited during spring and fall blooms while the dark laminae are enriched in terrigenous material (Hay et al., 1991b). The 5-cm thick transition sapropel lies at the base of Unit I, is relatively rich in organic matter (TOC ≈ 9%), and consists largely of terrigenous material (Hay et al., 1991a). The transition sapropel was deposited after the first invasion by E. huxleyi but before the final invasion of this alga. Unit II is an organic-rich sapropel deposited during an early stage of the present basin as the Black Sea and Mediterranean Sea were reconnected. Unit II has been divided stratigraphically into two subunits: the brownish Unit IIa contains more terrigenous material deposited under a period of lower primary productivity while the black Unit IIb of higher organic carbon content was deposited during a period of higher productivity (Hay, 1988). The relative "terrestrial vs. marine" distinction between Units IIa and IIb is supported by stable carbon isotope results (Calvert and Fontugne, 1987).

The chronology of Black Sea Unit I sediments is controversial, with varying sedimentation rates being determined by varve counting (Degens et al., 1980; Hay, 1988; Hay et al., 1991a), radiocarbon dating (Ross and Degens, 1974; Jones, 1990; Calvert et al., 1991; Jones and Gagnon, 1994), and ^{210}Pb measurements (Crusius and Anderson, 1992). The major question is whether the light-dark laminae used in varve counting are actually annual pairs. In summary, sediment accumulation rates of 130–160 g/m² y are obtained from counting the laminae compared to 35–50 g/m² y obtained by the two radiochronological methods. Thus, the Unit I/II boundary is estimated at about 1600 or 3200 y depending on the method used; the age of the Unit II/III boundary is generally accepted as 5000–7000 y. In this paper we use ^{14}C radiochronology.

Extraction and Fractionation

Wet samples (yielding 8–40 g dry weight) were ultrasonically extracted with methanol (4×), CH_2Cl_2/methanol (1:1, v/v; 3×) and

Table 1. Sediment samples and analytical yields.

Sample		Approximate Age Range (yr)*	C_{org} (%)	Extracted Sediment (g)‡	Lipid Extract		Apolars		Polars		Yield from Al_2O_3 column (%)	Apolar Yield from Desulfurization		
					(mg)	(mg/g)	(mg)	(mg/g)	(mg)	(mg/g)		(mg)	(mg/g)	(%)
Unit I														
BC 10	0-2.5 cm	0-100	3.2	8.0	107	13.4	3.6	0.45	24	3.0	26	0.3	0.05	1.8
BC 10	2.5-5 cm	100-300	3.6	11.7	68	5.8	1.7	0.15	25	2.1	39	0.3	0.04	1.7
BC 10	5-10 cm	300-600	3.7	27.4	130	4.7	2.5	0.09	66	2.4	53	0.5	0.07	3.0
BC 10	15-20 cm	1000-1200	4.1	28.2	210	7.5	2.7	0.10	64	2.3	32	0.5	0.06	2.7
BC 10	25-30 cm	1600-1900	5.4	33.2	195	5.9	2.6	0.08	67	2.0	37	1.0	0.09	4.5
Transition Sapropel														
BC 21	36-39 cm	2800-3100	8.6	44.2	264	6.0	3.3	0.07	69	1.6	27	0.3	0.03	1.8
Unit IIa														
BC 21	44-52 cm	3300-4400	11.7	18.5	231	12.5	3.5	0.19	119	6.4	53	0.6	0.23	3.6
Unit IIb														
GGC 20	17-22 cm	5200-6200	15.1	7.7	332	43.1	3.9	0.51	85	11.0	27	0.7	0.47	4.2

* Ages are estimated based on ^{14}C radiochronology of Ross and Degens (1974), Jones (1990), Calvert et al. (1991), and Jones and Gagnon (1994).

‡ - grams dry weight

CH_2Cl_2 (5×), respectively. The combined solvents were partitioned with 5% NaCl solution and the CH_2Cl_2 phase concentrated by rotary evaporation. Excess water in the extract was removed by passing the extract through a column (2 × 15 cm) of pre-extracted anhydrous Na_2SO_4 using CH_2Cl_2 as eluent. Extract yields were determined gravimetrically (Table 1). The entire extract was fractionated on a column (2 × 25 cm) of alumina (activated for 2 h at 150°C) by elution with 150 mL of hexane/CH_2Cl_2 (9:1, v/v) yielding an apolar fraction, followed by 150 mL of CH_2Cl_2/methanol (1:1, v/v), yielding a polar fraction. The two fractions were concentrated and weighed (Fig. 2). The apolar fractions were analysed by GC and GC–MS before and after catalytic hydrogenation. 5α-Androstane was added immediately before GC analysis to aid in quantitation.

Apolar fractions from the 0–2.5 cm and 25–30 cm sections of Unit I (BC 10), Unit IIa (BC 21, 44–52 cm), and Unit IIb (GGC 20, 17–22 cm) samples, and later from a composite of the top 0-5 cm of Unit I, were further fractionated by thin-layer chromatography on silica gel plates using hexane as the mobile phase. Standards were used to determine separations, where hydrocarbons had an $R_f = 0.8-$1.0, thiophenes had $R_f = 0.5-0.8$, and thiolanes had $R_f = 0-0.5$. The bands were scraped off the TLC plates and extracted ultrasonically with ethylacetate. Subsequent GC analysis of the thiophene and thiolane bands obtained from sediment extracts showed overlaps in composition so the two fractions were combined into a composite thiophene/thiolane fraction.

Desulfurization

Aliquots of the polar fractions obtained from Al_2O_3 chromatography were desulfurized using Raney nickel (Sinninghe Damsté et al., 1988). Typically, 16–20 mg of the polar fraction were reacted with Raney Ni in 2 mL of ethanol at reflux under N_2 for 2 h. Prior to reaction, 5-(1,1-d_2-hexadecyl)-2,3-dimethylthiophene was added to the reaction mixture as an internal standard. The desulfurization products were isolated by centrifugation and extraction with CH_2Cl_2. The combined ethanol/CH_2Cl_2 extracts were washed in a separatory fun-

nel with saturated NaCl solution and a few drops of acetic acid were added to break any emulsion due to the presence of $Al(OH)_3$. The CH_2Cl_2 extracts were washed with distilled water to remove residual acetic acid and dried over Na_2SO_4. The desulfurization products were isolated by chromatography on an alumina column (0.5 × 4 cm) and the apolar and polar fractions eluted with hexane/CH_2Cl_2 (9:1) and CH_2Cl_2/methanol (1:1), respectively. The apolar desulfurization products were weighed (Table 1) and analysed by GC and GC–MS before and after catalytic hydrogenation.

Catalytic Hydrogenation

Samples were dissolved in 0.5 mL of ethyl acetate in a 10 mL tube, 2 drops of acetic acid were added, and the mixture purged with H_2. Hydrogenation was performed with PtO_2 as catalyst and bubbling H_2 for 2 h, after which the tube was capped and stirred overnight. The reaction was terminated and the PtO_2 removed by passing the solution through a disposable pipette containing a small amount of Al_2O_3.

Gas Chromatography and Mass Spectrometry

Gas chromatography (GC) was performed on a Carlo Erba 5300 instrument with on-column injector and flame ionization and flame photometric detectors. The effluent splitter was set with a split ratio FID:FPD = 1:2. Separations were achieved using a fused silica column coated with CP Sil-5 (25 m × 0.32 mm i.d.; film thickness 0.12 µm). Samples dissolved in ethyl acetate were injected at 70°C and the oven programmed to 130°C at 10°C/min and then by 4°C/min to 320°C followed by a 20 min isothermal period. Hydrogen was used as carrier gas.

Gas chromatography–mass spectrometry (GC–MS) was carried out with a Hewlett Packard 5840 gas chromatograph connected to a VG-70S mass spectrometer. Operating conditions were: mass range m/z 40-800 with a cycle time of 1.8 s (resolution 1000); 70 eV ionizing energy. A CP Sil-5 capillary (25 m × 0.32 mm i.d.; 0.1 µm
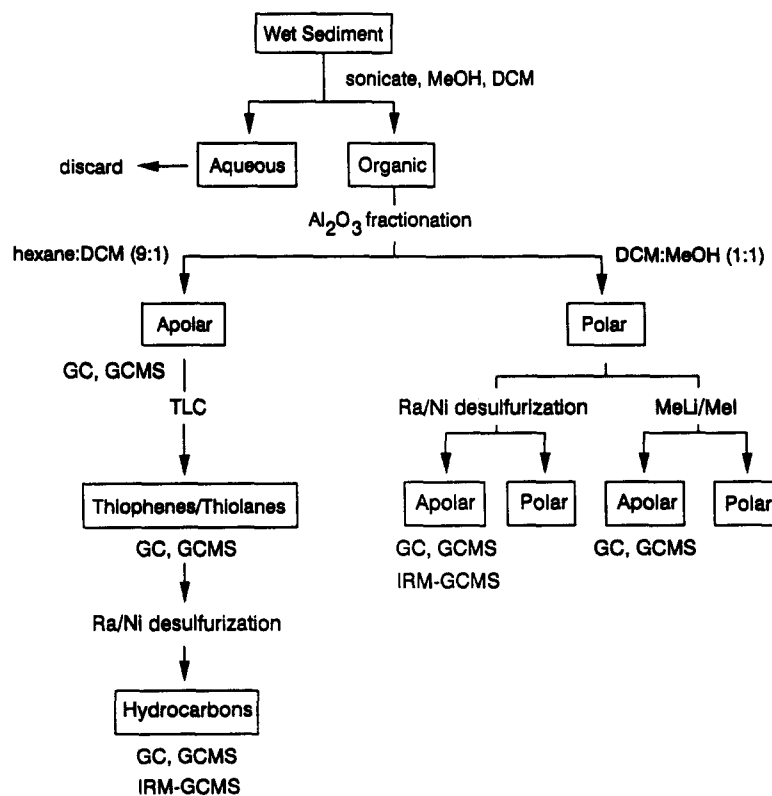

FIG. 2. Flow chart of the analytical workup.

film thickness) was used for separations with helium as carrier gas. Samples were injected at 50°C, followed by temperature programming at 20°C/min to 130°C and then at 4°C/min to 300°C.

Total Organic Carbon

Dried sediments were acidified to remove carbonate and organic carbon was measured using a Perkin Elmer CHN analyzer.

RESULTS

Free Hydrocarbons in Apolar Fractions

The apolar fractions of the Black Sea sediments contained a complex suite of linear and isoprenoid alkanes and alkenes, with distributions varying with depth (Fig. 3). C_{25}-HBI alkenes containing two to four double bonds dominated in surface sediments, but their concentrations decreased rapidly with depth in the upper 5 cm (Fig. 3a and b and Table 2). (In another core from a nearby location in the Black Sea, high resolution analyses showed concentrations of C_{25}-HBI alkenes decreasing by a factor of 100 in the upper 2 cm; Sun and Wakeham, 1994). C_{25}-HBI alkenes were also abundant in the transition sapropel and Unit II sections (Fig. 3c and d), possibly reflecting changes in the supply of organic matter or preservation through time. C_{30}-HBI hydrocarbons were not detected in Unit I, but compounds containing four and six double bonds were important constituents of the Unit IIb sediment (Fig. 3d). Other isoprenoid hydrocarbons, notably pristane, phytane, 2,6,10,15,19-pentamethyleicosane and squalene, were present in all sections, with the highest concentrations in the surface sediment. The Unit IIa sediment contained a relatively high concentration of 7-methylheptadecane. Long-chain C_{23}-C_{35} n-alkanes of terrestrial higher plant origin and with CPI's of 5–10 were abundant in all sediment sections. Long-chain C_{37}-C_{39} alkenes containing two to four double bounds and possibly produced by E. huxleyi (Volkman et al., 1980; Wakeham et al., 1991) were abundant, except in the Unit IIb section which was deposited before the initial invasion of E. huxleyi into the Black Sea (Hay et al., 1991a). The abundance of long-chain alkenes in Unit IIa sediment (Table 2) is puzzling. This sediment section also was deposited prior to the invasion by E. huxleyi, which is consistent with the absence of long-chain alkenones, implying that the biogenic source of the corresponding alkenes may not be the E. huxleyi of contemporary oceans. In the case of the transition sapropel, there is evidence of early, short-term and temporary invasions of E. huxleyi (Hay et al., 1991a). Small amounts of several bicyclic-carotenes were detected in the Unit IIb sediment; catalytic hydrogenation reduced these compounds to β-carotane. The large peak in the chromatogram of the apolar fraction of Unit IIb eluting just before the C_{30}-HBI alkenes (Fig. 3d) has a molecular mass of 326 daltons and was identified as a des-A-arboradiene (positions of the double bonds unknown). Hydrogenation of this component in pure acetic acid yielded des-A-arborane (J. M. Trendel, pers. commun.) with a molecular mass of 330 daltons.

Hydrocarbons Obtained by Raney Nickel Treatment of Polar Fractions

Treatment of polar fractions of sediment extracts with Raney nickel yielded a hydrocarbon mixture dominated by phy-

tane, C_{25}- and C_{30}-HBI hydrocarbons, and β-carotane and isorenieratane (Fig. 4 and Table 3). Phytane concentrations in the desulfurized fractions were approximately 2–3 orders of magnitude greater than in the free hydrocarbon fraction (25–112 μg/g vs. 0.1–0.3 μg/g dw). 2,6,10,15,19-Pentamethyleicosane was absent from the desulfurization products, pristane and squalene were present at very low levels, but farnesane was relatively more abundant. (Concentrations of farnesane should be considered lower limits as recoveries were poor due to its volatility). C_{25}-HBI alkenes or alkanes were recovered at low abundances from the upper Unit I sediments (Fig. 4a) but higher abundances of monoenes, dienes, and trienes were recovered in the 25–30 cm section and below, especially in Unit IIb. Upon further catalytic hydrogenation (the desulfurization reaction with Raney Ni may in itself partially hydrogenate unsaturated components; cf. Smith and Simpson, 1987; Schaeffer et al., 1993), the C_{25}-HBI alkenes collapsed into a single C_{25}-HBI alkane (Fig. 4b and c). Several C_{30}-HBI alkenes were recovered only in the Unit IIb sediment; further hydrogenation yielded a C_{30}-alkane and a C_{30}-monoene (Fig. 4c). Long-chain n-alkanes were only minor components, except for n-C_{31} in the upper sections and n-C_{31} and n-C_{33} in the deepest section. Long-chain C_{37}-C_{39} alkanes or alkenes were absent from all desulfurized fractions.

A cluster of partially hydrogenated β-carotene isomers was recovered from all sediments treated with Raney Ni but were of greatest abundance in the transition sapropel and Unit IIa and of significantly lower abundance in Unit IIb. Catalytic hydrogenation of these compounds produced β-carotane (Fig. 4 and Table 3). A diaryl isoprenoid hydrocarbon was also recovered and identified as isorenieratane by coinjection with an authentic standard. Upon further hydrogenation, fully hydrogenated isorenieratane (MW 558 daltons; A in Fig. 4) and a partially hydrogenated isorenieratane (MW 552; B in Fig. 4) were obtained. The concentration of isorenieratane in the Black Sea sediments showed the opposite trend to that of β-carotane, being lowest in the transition sapropel and in Unit IIa and highest in Unit IIb.

C_{28}-Dialkylthiophenes

GC–FPD analyses of the apolar fractions of Unit I sediments revealed a cluster of three OSC eluting in the vicinity of n-C_{29} to n-C_{31} (Fig. 5a). The concentrations of these compounds were too low to produce visible signals next to the more abundant aliphatic hydrocarbons in the GC–FID chromatograms, but from GC–MS data we estimate concentrations to be approximately 0.1 μg/g dw (0.005 mg/g OC). Concentrations decreased with increasing depth in the sediment, such that the compounds were no longer clearly detected in the transition sapropel (Fig. 5b). GC–MS analysis of the "composited" thiophenes + thiolanes obtained after removal of the aliphatic hydrocarbons by TLC indicated that these OSC possess mass spectra similar to those of the long-chain C_{36}-C_{54} 3,4-di-n-alkylthiophenes in various Pleistocene to Cretaceous sediments described by Kohnen et al. (1990b). Mass spectra of all three S-containing compounds from the Black Sea surface sediment (e.g., Fig. 6a) are characterized by molecular ions at m/z 420, a base peak at m/z 266, and a major fragment ion at m/z 111. The molecular weights indi-
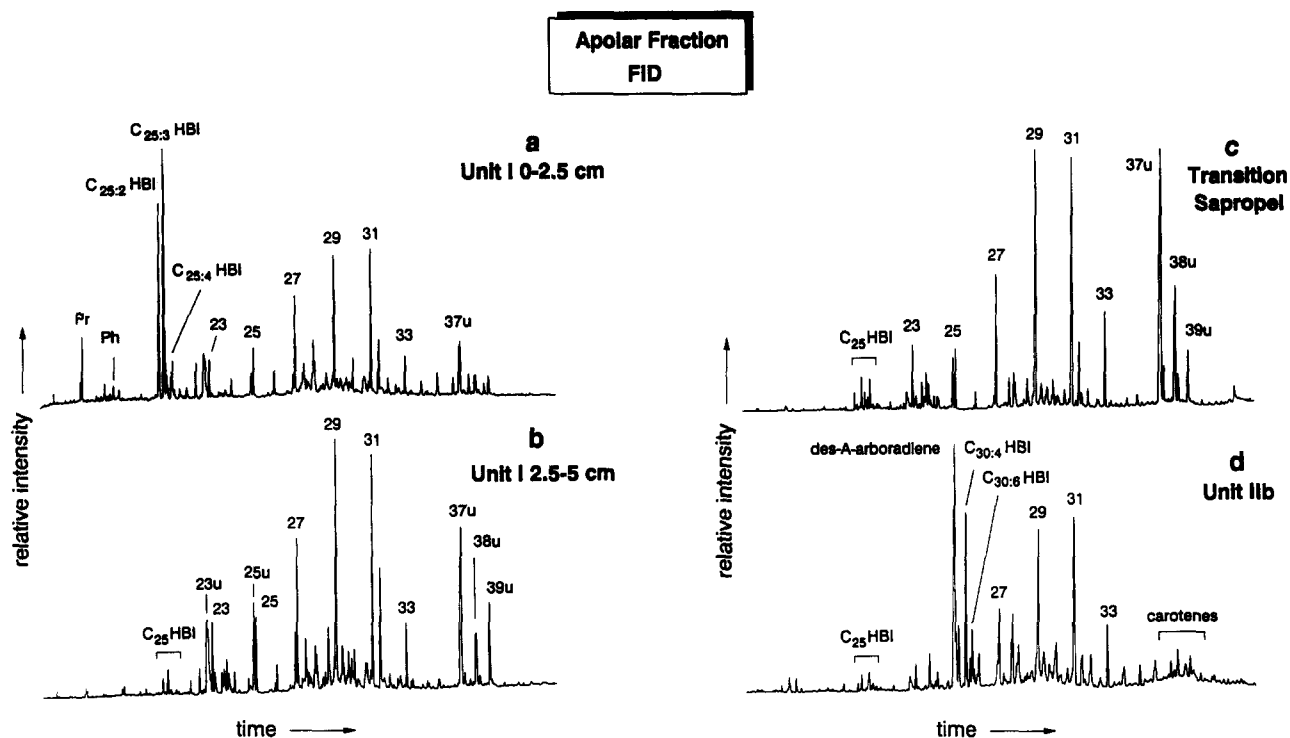
FIG. 3. Gas chromatograms (FID) of apolar fractions from Unit I (BC 10 0–2.5 cm and 2.5–5 cm), the transition sapropel (BC 21 36–39 cm) and Unit IIb (GGC 20 17–22 cm) sediments. Numbers above peaks refer to total numbers of carbon atoms of n-alkanes; 23u, 25u, 37u, 38u, and 39u = long-chain alkenes; Pr = pristane; Ph = phytane; $C_{25:x}$ HBI = highly branched isoprenoid alkenes with twenty-five carbon atoms and x double bonds; $C_{30:x}$ HBI = HBI alkenes with thirty carbon atoms and x double bonds.

cate the compounds to be C_{28}-alkylthiophenes and the fragment at m/z 266 indicates a C_{12}-dialkyl substitution.

To elucidate further the structures of these dialkylthiophenes, desulfurization using Raney Ni followed by hydrogenation was performed on a TLC fraction (R_f = 0.2–0.5) of a composited apolar fraction of surface sediment (0–5 cm) containing these OSC. Three isomeric methyl-branched C_{28}-alkanes were detected in the FID chromatogram in the vicinity of squalane in the hydrogenated sample (peaks A, B, and C in Figs. 7 and 8). The mass spectrum of the last eluting component (peak C) is characterized by fragment ions at m/z 196/197 and 224/225 with the fragments with an even mass predominating (Fig. 6b). No molecular ion is observed but a large m/z 379 thought to be M^+-15 suggests an elemental composition of $C_{28}H_{58}$. These data would suggest 13-methylheptacosane as a possible structure. The Kovats retention index (2733 on CP Sil-5) of this component is in agreement with this assignment (Kissin et al., 1986). Component B gave a similar mass spectrum, except that m/z 225 is now more abundant than m/z 224. This suggests additional branching which would be consistent with the lower Kovats retention index (2700). In fact, the shift in retention time (Δ = 33 Kovats units) would suggest that this component has an additional methyl group in the iso position (Kissin et al., 1986). Combination of these data would suggest that 2,14-dimethylhexacosane is the structure of this component. We favor 2,14-dimethylhexacosane over 2,12-dimethylhexacosane based on the relative intensities of the odd and even fragment ions (McCarthy et al., 1968). The first eluting component (peak A;

Figs. 7 and 8) coelutes with squalane (Kovats index = 2667) which hampers its characterization by mass spectrometry. It is noteworthy, however, that the shift in retention index relative to the last eluting component is 66 Kovats units, exactly twice as the shift between peak B and C. This suggests an additional methyl group in the iso position, which would lead to its very tentative identification as 2,12,24-trimethylpentacosane.

Combination of the mass spectral data of the C_{28}-thiophenes and the proposed structures of their desulfurization products leads us to hypothesize the C_{28}-thiophenes to be 2,4-di(dodecyl)thiophene, 2-(10-methylundecyl)-4-dodecylthiophene, and 2,4-di(10-methylundecyl)thiophene. 2,4-Dialkylthiophenes have not been reported before in sediments.

C_{25}- and C_{30}-Thiolanes

Unsaturated C_{25}-HBI thiolanes were present in the 25–30 cm Unit I and deeper sediment sections (Fig. 5b and c). C_{30}-HBI thiolanes were present only in the Unit II sediments (Fig. 5c). These HBI-thiolanes are the same as compounds in the slightly older Black Sea Unit II sediment described by Kohnen et al. (1990a). The C_{25}-HBI thiolanes had molecular ions at m/z 378 and 380 indicating that they possess two double bonds and one double bond, respectively. A major fragment ion at m/z 115 suggests that the double bonds are positioned in the long alkyl-side-chain (Fig. 5 of Kohnen et al., 1990a). The C_{30}-HBI thiolanes exhibited molecular ions at m/z 444, 446, and 448 and thus would contain two to four double

Table 2. Concentrations (µg/gdw and mg/g OC) of selected biomarkers in the "free" hydrocarbon fraction.

| | µg/gdw (mg/g OC) | | | | | | | |
| | Unit I | | | | | Transition sapropel | Unit IIa | Unit IIb |
	0-2.5 cm	2.5-5 cm	5-10 cm	15-20 cm	25-30 cm	36-39 cm	44-52 cm	17-22 cm
farnesane	—* (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
n-C₁₇	1.0 (0.03)	0.3 (0.01)	0.2 (0.01)	0.2 (0.005)	0.2 (0.004)	0.2 (0.002)	3.2 (0.03)	1.5 (0.01)
pristane	3.2 (0.10)	0.2 (0.01)	0.2 (0.01)	0.1 (0.002)	0.2 (0.004)	0.2 (0.002)	— (—)	1.4 (0.01)
7-methyl-heptadecane	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	11.4 (0.10)	— (—)
phytane	0.9 (0.03)	0.1 (0.03)	0.1 (0.005)	0.1 (0.002)	0.1 (0.002)	0.1 (0.001)	0.3 (0.003)	0.2 (0.001)
25:2 HBI	17.5 (0.55)	0.5 (0.01)	0.3 (0.01)	0.1 (0.002)	0.2 (0.004)	1.0 (0.01)	1.7 (0.01)	1.2 (0.01)
25:3 HBI	26.2 (0.82)	0.9 (0.03)	0.4 (0.01)	0.1 (0.002)	0.2 (0.004)	0.6 (0.01)	0.8 (0.005)	2.9 (0.02)
PME‡	3.0 (0.09)	0.8 (0.02)	0.4 (0.01)	0.2 (0.005)	0.1 (0.002)	0.1 (0.001)	— (—)	— (—)
30:4 HBI	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	31.2 (0.21)
30:6 HBI	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	11.4 (0.08)
trisnorhopene	0.6 (0.02)	1.7 (0.05)	1.4 (0.04)	0.4 (0.01)	0.4 (0.01)	0.9 (0.01)	2.4 (0.02)	17.1 (0.11)
squalenes	6.1 (0.19)	1.5 (0.04)	0.5 (0.01)	0.3 (0.01)	0.4 (0.01)	1.0 (0.01)	3.5 (0.03)	15.2 (0.11)
n-C₂₉	14.2 (0.44)	12.1 (0.34)	7.3 (0.20)	7.5 (0.18)	3.3 (0.06)	12.5 (0.15)	14.2 (0.12)	36.6 (0.24)
n-C₃₁	11.8 (0.37)	10.2 (0.28)	7.9 (0.21)	8.9 (0.22)	4.2 (0.08)	13.7 (0.16)	16.0 (0.14)	47.0 (0.31)
diploptene	5.9 (0.18)	5.1 (0.14)	3.0 (0.08)	3.3 (0.08)	2.3 (0.04)	3.4 (0.04)	5.7 (0.05)	9.4 (0.06)
C₃₇u	9.2 (0.29)	11.7 (0.33)	10.8 (0.29)	13.4 (0.33)	7.4 (0.14)	23.3 (0.27)	15.8 (0.14)	— (—)
C₃₈u	3.1 (0.10)	4.1 (0.11)	4.2 (0.11)	5.3 (0.13)	3.1 (0.06)	8.1 (0.09)	5.3 (0.05)	— (—)
Σ β-carotene isomers	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	3.5 (0.02)
Σ diaryl carotenes	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)

* not detected

‡ 2,6,10,15,19-pentamethyleicosane

bonds. We estimate that concentrations of the C_{25}-HBI thiolanes in the transition sapropel and the C_{25}-HBI thiolanes and C_{30}-HBI thiolanes in the Unit IIb sediments were approximately 0.2 µg/g dw (0.02 mg/g OC). Our samples did not contain HBI thiophenes which are common in older and more mature sediments (Sinninghe Damsté et al., 1988, 1989; ten Haven et al., 1990). The sediments we investigated also did not contain the unsaturated C_{25}- and C_{30}-HBI sulfoxides reported in the Black Sea sediment described by Kohnen et al. (1990a). This absence of sulfoxides supports the conclusion of Kohnen et al. (1990a) that sulfoxides may be artifacts formed by the oxidation of HBI thiolanes during long-term storage of the sediments or their extracts.

DISCUSSION

Our investigation was designed to address three questions related to the incorporation of inorganic sulfur into labile lipids during early diagenesis. Answers to these questions can be summarized as follows. First, How early during sedimentary diagenesis are OSC produced in Black Sea sediments? The presence of C_{28}-dialkylthiophenes and compounds released by Raney Ni desulfurization in even the uppermost sediments of Unit I show that both intramolecular and intermolecular sulfur incorporation can occur near the water-sediment interface. Second, Can the early diagenetic loss of functionalized precursors, such as C_{25}-HBI alkenes, be attributed to sulfur quenching? We did not recover C_{25}-HBI carbon skeletons, as OSC containing either intermolecular or intramolecular C-S-bonds, in the upper Unit I sediments, although they were recovered in deeper and older sections. However, we cannot rule out that other OSC fractions, either higher molecular weight material in the bitumen or in the kerogen, were not amenable to the desulfurization techniques used. Finally,

Quantitatively how important is diagenetic sulfur quenching? For some compounds, S-quenching as we have tested it preserves more material than is found in the free form (e.g., phytane and C_{25}- and C_{30}-HBI in Unit IIb). For the carotenoids, the amounts of material in the free and OSC forms were often similar. Yet for the C_{25}-HBI and C_{37}-C_{39} alkenes which were present in surface sediment, S-quenching was apparently not important.

Early Diagenetic Formation of C_{28}-Dialkylthiophenes

The presence of isomeric C_{28}-dialkylthiophenes, albeit at low concentrations, is evidence that sulfur quenching has occurred in the surface sediment of the Black Sea, although an external source of these compounds cannot be excluded. Their presence in the "free hydrocarbon" fraction means that they have been produced by an intramolecular incorporation of sulfur and are not bound by C-S-linkages to macromolecular material. Mechanisms have been postulated for the formation of long-chain 2,5-dialkylthiophenes from symmetrical methylene-interrupted alkadienes (Sinninghe Damsté et al., 1989) and long-chain 3,4-dialkylthiophenes from mid-chain dimethyl hydrocarbons with two methylenic double bonds (Kohnen et al., 1990b). By analogy, C_{28}-methyldienes containing double bonds located in the middle of the molecule might produce 2,4-didodecylthiophenes which upon desulfurization and hydrogenation would yield C_{28}-alkanes of the type we found. Recent experiments by Schouten et al. (1993) suggest unsaturated dialdehydes as hypothetical precursors for dialkyl thiophenes in immature S-rich sediments. However, appropriate precursor alkenes and aldehydes are unknown in sediments, and in any event thiolanes (which we did not find) should form preferentially. Therefore, the origin of these OSC remains uncertain.
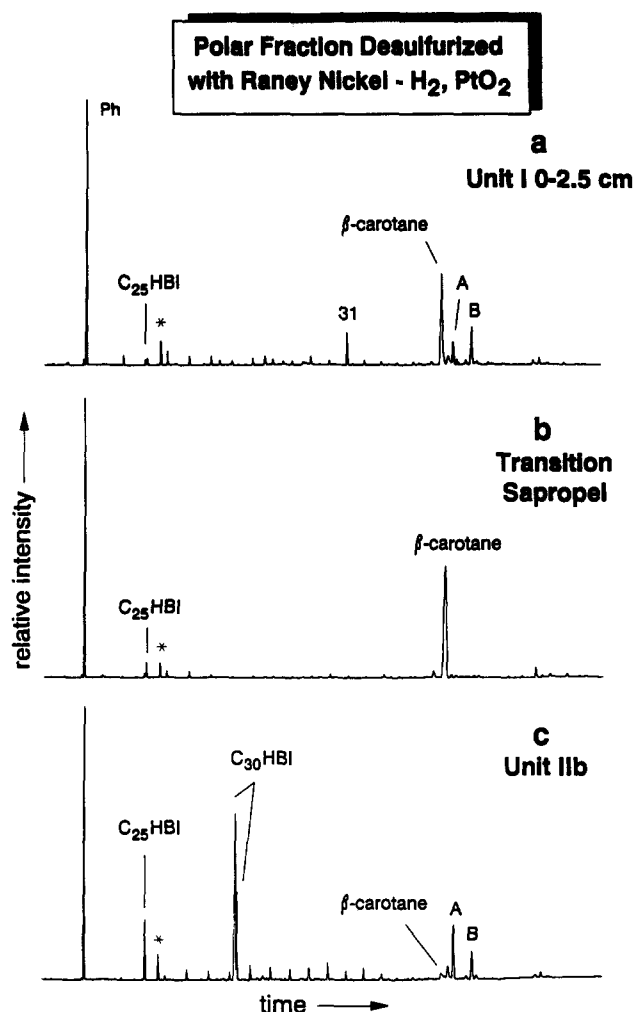
FIG. 4. Gas chromatograms (FID) of hydrocarbons released by Raney Ni desulfurization of polar fractions and hydrogenated using PtO_2 catalyst from Unit I (BC 10 0–2.5 cm), the transition sapropel (BC 21 36–39 cm) and Unit IIb (GGC 20 17–22 cm) sediments. * = internal standard; Ph = phytane; 31 = 31-carbon n-alkane; A = completely hydrogenated isorenieratane (MW 558); B = partially hydrogenated isorenieratane (MW 552).

The concentrations of the C_{28}-dialkylthiophenes decrease with depth in Unit I and these compounds are absent in sediments deeper than the transition sapropel. If they were formed in surface sediments (rather than in the anoxic water column), then decreasing concentrations with depth imply either (1) lower supply of precursor molecules in the past compared to the present, (2) past sedimentary conditions which were less conducive to incorporation of sulfur into the (unknown) precursors, or (3) thiophenes were degraded or transferred from the apolar fraction to other more polar fractions which we did not examine. Since we are unable to identify precursor compounds, we cannot confidently address the issue of changing supply of precursors over time, but this factor cannot be discounted. It is, however, unlikely that with precursors being available, sulfur incorporation could not occur because of unfavorable conditions within the sediment since other OSC compounds (C_{25}- and C_{30}-HBI thiolanes) did indeed form. Finally, degradation of OSC generally is thought

to be relatively unimportant over the timescales covered by these sediments (a major assumption in using S-bound carbon skeletons in paleo-environmental interpretations), and without the presence of additional functional groups, incorporation of "free" thiophenes into polar macromolecules is unlikely.

C_{25}- and C_{30}-HBI Organic Sulfur Compounds

A major contribution to the organic matter in the Black Sea water column and sediments arises from diatoms. They account for 10–40% of the annual particle mass flux in the Black Sea (Hay et al., 1991b), and C_{25}-HBI alkenes, presumed to be of diatom origin, are abundant in particulate matter (Wakeham et al., 1991) and surface sediments (Table 2). In this context, it is interesting to note that the major diatom in sediment trap material in the Black Sea is a *Rhizosolenia* *sp.* (Hay et al., 1991b) and that Volkman et al. (1994) recently identified C_{25} and C_{30} HBI alkenes in two diatoms, although in *Rhizosolenia setigera*, C_{30} rather than C_{25} alkenes were present. A working hypothesis in this investigation was that the rapid loss of C_{25}-HBI alkenes from the upper sediment layers resulted from their removal from the free hydrocarbon pool due to incorporation of inorganic sulfur into an OSC pool outside of the normal hydrocarbon analytical window. If true, then an increase in the abundances of C_{25}-HBI OSC might accompany the loss of C_{25}-HBI alkenes. Although intramolecular incorporation of sulfur yielding HBI thiolanes and intermolecular linkages between HBI alkenes and macromolecules did occur within sediments at the bottom of Unit I and in Unit II, a parallel loss of HBI alkenes coupled with production of HBI OSC was not apparent in the upper sediment sections.

C_{25}-HBI remnants were not abundant in surface sediments either as free OSC in the apolar fraction or in Raney Ni desulfurization products of the polar fraction, even though these alkenes apparently were rapidly lost from surface sediments. This indicates that neither intramolecular incorporation of sulfur into HBI alkenes nor intermolecular cross-linking with polar material which we analysed are prevalent in these very young Black Sea sediments. It is important to note that our analytical scheme accounts for a relatively small fraction of total extract initially charged to the Al_2O_3 column (26–53%) and an unknown fraction of total organic sulfur in the sediment (attempts at constructing a sulfur mass balance were unsuccessful). It is possible that the HBI alkenes might simply be sequestered in fractions we did not examine, including asphaltenes or kerogen. On the other hand, the identification of C_{25}-HBI thiolanes in sediments older than the 25–30 cm section of Unit I ($\geq 2 \times 10^3$ y BP) and of C_{30}-HBI thiolanes in Unit IIb (5.2–6.2 $\times$ 10^3 y BP) shows that intramolecular incorporation of sulfur can indeed occur with the HBI alkenes in Black Sea sediments younger than the Unit IIb sample previously studied by Kohnen et al. (1990a) ($\approx 2 \times 10^3$ vs. 3–6 $\times$ 10^3 y BP) and the Quaternary and Pleistocene samples described by ten Haven et al. (1990). The HBI thiolanes in the Black Sea samples of this study possess two double bonds fewer than the corresponding precursor alkenes (1–2 double bonds for C_{25}-thiolanes vs. 3–4 double bonds for C_{25}-alkenes; 2–4 double

Table 3. Concentrations (μg/gdw and *mg/g OC*) of selected biomarkers in the hydrocarbon fraction obtained by Raney Ni desulfurization and following hydrogenation.

	μg/gdw (mg/g OC)							
	Unit 1					Transition sapropel	Unit IIa	Unit IIb
	0-2.5 cm	2.5-5 cm	5-10 cm	15-20 cm	25-30 cm	36-39 cm	44-52 cm	17-22 cm
farnesane	—* (—)	2.1 (0.06)	0.1 (0.003)	1.0 (0.02)	1.6 (0.03)	0.4 (0.005)	0.7 (0.01)	— (—)
n-C$_{17}$	0.1 (0.003)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
pristane	0.2 (0.006)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
7-methyl-heptadecane	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
phytane	53.2 (1.66)	26.2 (0.73)	28.1 (0.76)	39.1 (0.95)	27.8 (0.51)	26.1 (0.31)	64.5 (0.55)	112.0 (0.74)
Σ 25 HBI	— (—)	— (—)	— (—)	— (—)	0.3 (0.006)	0.9 (0.010)	2.1 (0.02)	17.8 (0.11)
PME‡	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
Σ 30 HBI	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	90.1 (0.61)
trisnorhopene	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
squalane	— (—)	— (—)	0.1 (0.003)	— (—)	0.1 (0.002)	0.1 (0.001)	0.3 (0.003)	— (—)
n-C$_{29}$	0.9 (0.03)	0.7 (0.02)	0.3 (0.001)	0.6 (0.01)	0.2 (0.004)	0.1 (0.001)	0.5 (0.004)	1.8 (0.01)
n-C$_{31}$	4.1 (0.13)	1.2 (0.03)	0.8 (0.02)	0.6 (0.01)	0.4 (0.01)	0.2 (0.002)	0.4 (0.003)	4.2 (0.03)
diploptene	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
n-C$_{37}$	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
n-C$_{38}$	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)	— (—)
β-carotane	9.6 (0.30)	5.6 (0.16)	16.2 (0.44)	17.4 (0.42)	17.2 (0.32)	26.4 (0.31)	32.1 (0.27)	1.9 (0.01)
Isorenieratane†	12.9 (0.40)	8.3 (0.36)	13.0 (0.35)	10.8 (0.26)	11.4 (0.21)	0.4 (0.005)	0.4 (0.003)	50.0 (0.33)

* not detected

‡ 2,6,10,15,19-pentamethyleicosane

† Isorenieratane = sum of fully hydrogenated and partially hydrogenated isorenieratane (peaks A and B in Fig. 4)

bonds for C$_{30}$-thiolanes vs. 4–6 double bonds for C$_{30}$-alkenes; cf. Kohnen et al. 1990a). That abundant C$_{25}$-HBI hydrocarbons were recovered after desulfurization from the 25–30 cm and deeper sediments and C$_{30}$-HBI hydrocarbons from Unit IIb indicates that crosslinking via acyclic C-S-linkages with macromolecular material has occurred. We presume that the difference between the apparent time of formation of C$_{25}$-HBI OSC and C$_{30}$-HBI OSC is related to the availability of appropriate precursors or depositional environment, but there are no solid experimental data to address this. The Black Sea sediments studied here apparently have not undergone diagenesis to the extent required to produce HBI thiophenes.

Biodegradation has been suggested as a mechanism for rapid loss of algal HBI alkenes in the water column (Wakeham, 1990; Wakeham et al., 1991) and sediments (Volkman et al., 1983). The fact that we could not demonstrate a precursor-OSC-product coupling means that biodegradation cannot be ruled out as a potential mechanism for the loss of the HBI alkenes. On the other hand, our analytical scheme does not account for all of the organic sulfur in either the sediment or in the extract. In order to try to address this problem to some extent, we treated the polar fractions of selected samples (BC 10 0–5 cm and 25–30 cm, BC 21 36–39 cm, and GGC 20 17–22 cm sections) with MeLi/MeI to check on incorporation of HBI skeletons into di- and polysulfide-bound compounds (Kohnen et al., 1991b). MeLi/MeI will react better than Raney Ni with the larger macromolecular aggregates in the polar fraction because of the better solubility of this reagent. Reaction yields were very low and a very complex mixture of HBI-sulfides was obtained. Identification of these components presents a very difficult task. Concentrations of HBI-sulfides were low and would not account for the missing HBI hydrocarbons.

Formation of Macromolecular Aggregates by Sulfur-Crosslinking

Treatment of the polar fraction with Raney Ni releases biomarker skeletons which were bound via sulfur linkages to high molecular weight macromolecules (Schmid, 1986; Sinninghe Damsté et al., 1988, 1990). The fractions we obtained following treatment with Raney Ni variously contained farnesane, phytane, C$_{25}$- and C$_{30}$-HBI isoprenoids, and β-carotane and isorenieratane, and these compounds occur almost exclusively in the desulfurized polar fraction. Except for farnesane, all were also the dominant hydrocarbons released by desulfurization of polar fractions from older Messinian Vena del Gesso and Pleistocene Peruvian upwelling sediments (Kohnen et al., 1991a). High ratios of "sulfur-bound" to "free" hydrocarbons (cf. Tables 2 and 3) show that the precursors of these compounds react selectively with inorganic sulfur, thereby being preferentially crosslinked to macromolecular material. Precursors for the sulfur bound C$_{25}$- and C$_{30}$-HBI's are the corresponding HBI alkenes, as discussed above. The probable precursors for S-bound phytane and nonaromatic carotenes are the phytol side-chain of chlorophyll-*a* and various carotenoid pigments, respectively. The ubiquity of chlorophyll-*a* and carotenoids among the pigments of photosynthetic organisms explains the generally covarying abundance of phytane and β-carotane in the desulfurization products, although the concentration of phytane rises in Unit IIb while that of β-carotane decreases.

Isorenieratane and farnesane have different precursors than phytane and β-carotane. Both are likely derived from anoxygenic photosynthetic bacteria inhabiting the chemocline in the water column of the Black Sea. The molecular structure of isorenieratane is highly specific and points to an origin from isorenieratene, a characteristic pigment of green photo-
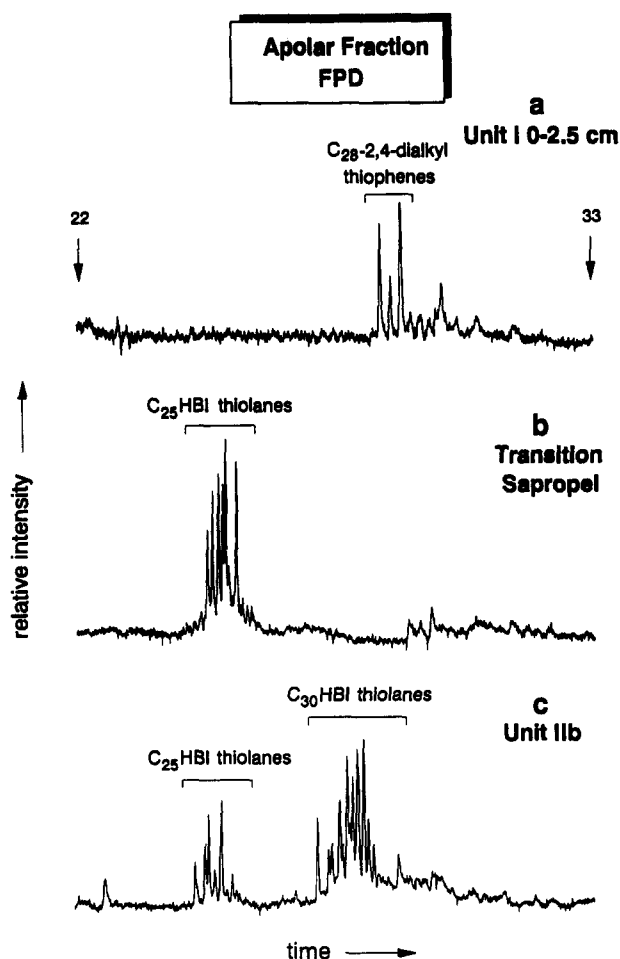
FIG. 5. Partial gas chromatograms (FPD; between C_{22} and C_{33} alkanes) of apolar fractions from Unit I (BC 10 0–2.5 cm), the transition sapropel (BC 21 36–39 cm); and Unit IIb (GGC 20 17–22 cm) sediments.

synthetic bacteria of the genus Chlorobiaceae. Farnesane may derive from the farnesol side-chain of 4-*i*-butylbacteriophaeo-farnesyl-*e*, also a diagnostic chlorin-pigment for green sulfur bacteria. Detailed pigment analyses across the chemocline in the Black Sea (Repeta et al., 1989; Repeta and Simpson, 1991), demonstrated the presence of these bacterial pigments at the top of the anoxic zone and carbon isotopic analysis of isorenieratane (Sinninghe Damsté et al., 1993) indicated an origin from green photosynthetic bacteria.

The concentration of isorenieratane in the sediments from the Black Sea is relatively constant throughout Unit I, low in the transition sapropel and Unit IIa, and high in Unit IIb. Farnesane may well display similar concentration trends, although its quantification is less rigorous because of poorer recoveries during analysis. This observation, along with high resolution analysis of pigments in Units I and II by Repeta (1993) show that the depth of the chemocline and hence the extent of anoxygenic primary production has varied greatly during much of the Holocene. The data of Repeta (1993) further show that transitions from high to low periods of anoxygenic primary production occurred on the scale of a decade (a timescale which our sample intervals cannot address).

Although a direct comparison of our data and that of Repeta (1993) is complicated by the fact that different cores were analyzed by different techniques, down-core distributions of free isorenieratene in the cores studied by Repeta (1993) parallel the depth profiles of isorenieratane in our samples, including the absence of both compounds in the transition sapropel and Unit IIa. Concentrations of isorenieratane were 8–13 μg/g dw sediment (0.2–0.4 mg/g OC) in Unit I and 50 μg/g dw (0.33 mg/g OC) in Unit IIb. (Note that concentrations reported earlier by Sinninghe Damsté et al., 1993, are in error. The concentration axis of Fig. 2 should read mg/g C.) For comparison, Repeta (1993) reported concentrations of free isorenieratene of 2–70 μg/g dw (0.05–1.3 g/g OC) in Unit I and up to 1.5 μg/g dw in Unit IIb.

Our results indicate that sequestration of isorenieratene via S-bonds with macromolecular material in the polar fraction we analysed and its subsequent recovery as isorenieratane is important in Unit IIb sediments. In Unit IIb, the concentration of isorenieratane we obtained appears to be significantly greater than free isorenieratene obtained by the direct pigment analyses of Repeta (1993). On the other hand, the Unit I sediments we analyzed contained concentrations of isorenieratane roughly similar to those of free isorenieratene reported in the samples of Repeta (1993). It is thus uncertain whether the isorenieratane we obtained by desulfurization of the polar fraction of Black Sea sediment extracts was indeed S-bound or whether it resulted from hydrogenation by Raney Ni of isorenieratene initially present as the free pigment in the polar fraction from our Al_2O_3 fractionation. A similar question may also be raised regarding the β-carotane generated upon desulfurization and free β-carotene in the sediment (although Repeta, 1993, did not give concentration data for β-carotene).

We conducted several experiments in order to address this problem. In the absence of sufficient quantities of authentic isorenieratene, we used β-carotene and determined (1) that β-carotene does indeed elute in the polar fraction from Al_2O_3 and (2) that reaction with Raney Ni produces a series of partially hydrogenated β-carotene isomers which can be further hydrogenated to β-carotane, as we observed in our samples. Isorenieratene is assumed to behave in a similar manner. Thus, any free isorenieratene and β-carotene in our extracts could be recovered as isorenieratane and β-carotane by our desulfurization and hydrogenation procedures. We are therefore uncertain how much of the isorenieratane and β-carotane we found in Unit I sediments had been sequestered via C-S linkages to macromolecular material.

Sulfurization of Long-Chain C_{37}-C_{39} Alkenes and C_{37}-C_{39} Alkenones

Long-chain C_{37}-C_{39} alkenes and C_{37}-C_{39} alkenones are major components in Black Sea sediments. Sulfur incorporation into these functionalized lipids has been suggested (Sinninghe Damsté et al., 1989) as the mechanism by which long-chain C_{37}-C_{38}-2,5-dialkylthiophenes, thiolanes, and thiophenes (Kohnen et al., 1990c) and macromolecularly S-bound C_{37}-C_{38} OSC are formed in some S-rich sediments (Sinninghe Damsté et al., 1988, 1990; Rullkötter and Michaelis, 1990; Schouten et al., 1993). Yet we found no evidence for this processes in the Black Sea sediments we examined, since no
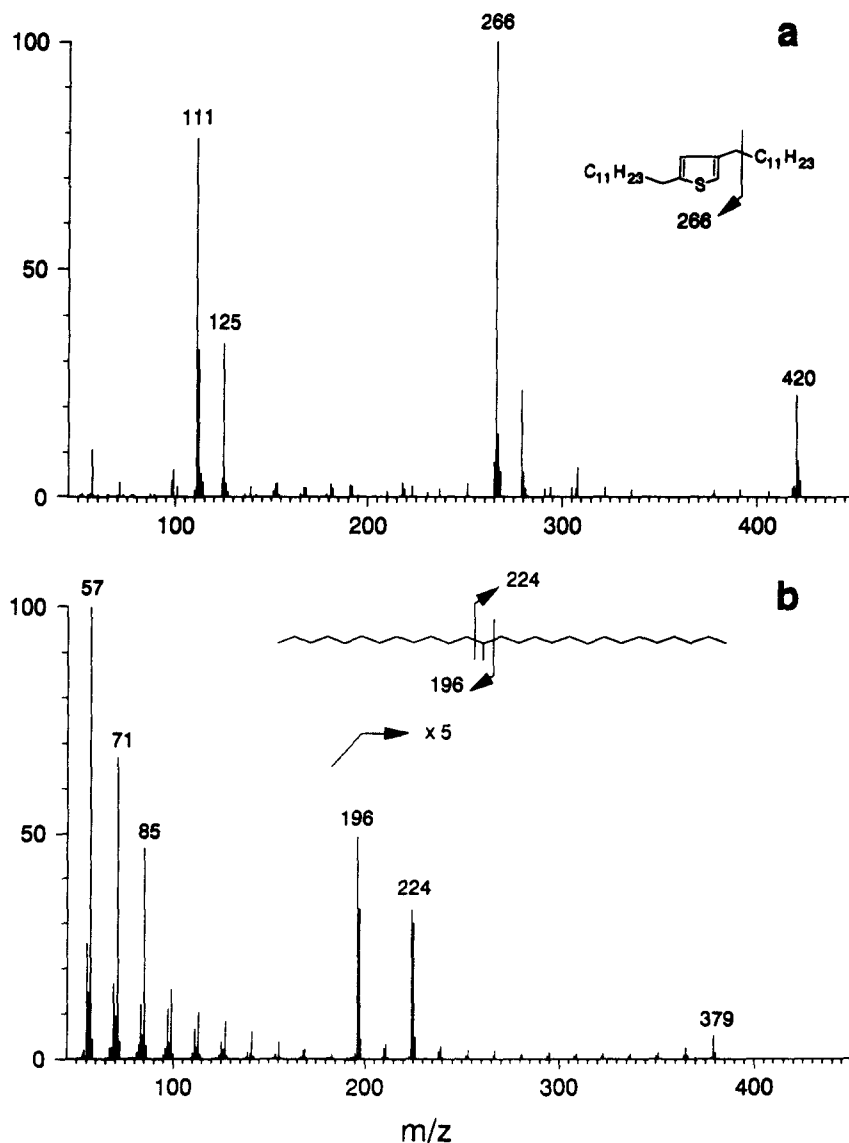
FIG. 6. Mass spectra, subtracted for background, of proposed 2,4-didodecylthiophene and its desulfurization product, postulated to be 13-methylheptacosane.

C_{37}-C_{39} hydrocarbons were obtained by desulfurization experiments. It is apparently not the lack of suitable precursor molecules which is responsible for the absence of corresponding OSC. Perhaps the incorporation of sulfur into molecules having widely separated double bonds requires much greater timescales than were covered by our Black Sea sediment samples; this interpretation is in line with experimental results of Schouten et al. (1994). Alternatively, sulfur incorporation may indeed occur in our sediments but the OSC produced were outside our analytical "window."

CONCLUSIONS

1) Hydrocarbons recovered in apolar fractions of sediment extracts from Unit I and II sediments of the Black Sea contained C_{25}- and C_{30}-highly branched isoprenoid alkenes, long-chain C_{37}-C_{39} alkenes, and long-chain n-alkanes. C_{25}-HBI alkenes were rapidly lost from the upper

5 cm of sediment, and C_{30}-HBI alkenes were only found in Unit IIb. Desulfurization using Raney nickel revealed that phytol or phytenes, C_{25}- and C_{30}-HBI alkenes, and β-carotene and isorenieretene have been sequestered in S-containing macromolecules during early diagenesis.

2) Isomeric C_{28}-2,4-didodecylthiophenes were present in surface sediments but decreased in concentrations with increased depth in Unit I. Based on mass spectra and Raney nickel desulfurization, we postulate that these thiophenes are 2,4-di(dodecyl)thiophene, 2-(10-methylundecyl)-4-dodecylthiophene, and 2,4-di(10-methylundecyl)thiophene.

3) C_{25}-HBI thiolanes were detected in sediment at the bottom of Unit I; C_{30}-HBI thiolanes were only present in Unit IIb. We did not observe a precursor-OSC-product relationship between HBI alkenes and HBI thiolanes in Unit I sediment which would account for the rapid loss of C_{25}-HBI alkenes.

4) A comparison of concentrations of OSC containing intramolecular S-linkages (0.1–0.2 μg/g sediment for C_{28}-thiophenes and C_{25}- and C_{30}-HBI thiolanes) with concentrations of OSC containing intermolecular linkages (up to 50 μg/g sediment for compounds released by Raney nickel desulfurization) indicates that in these sediments most sulfur incorporation apparently occurs via intermolecular bonds to macromolecules. However, it should be stressed that an unknown quantity of OSC may be bound in forms not amenable to presently available analytical techniques, but this would only increase the fraction associated with macromolecules.

5) Sequestration of β-carotene and isorenieratene into macromolecular OSC can be an important preservation mechanism, especially in Unit IIb. In the case of the isorenieratene, concentrations recovered by desulfurization of polar material from surface sediments were approximately 15% of the maximum concentrations of solvent-extractable, free carotenoid pigments, but in Unit IIb sediments the diaromatic carotenoid released by desulfurization was 25 times more abundant than the free form. On the other hand, we did not observe a correspondence between loss
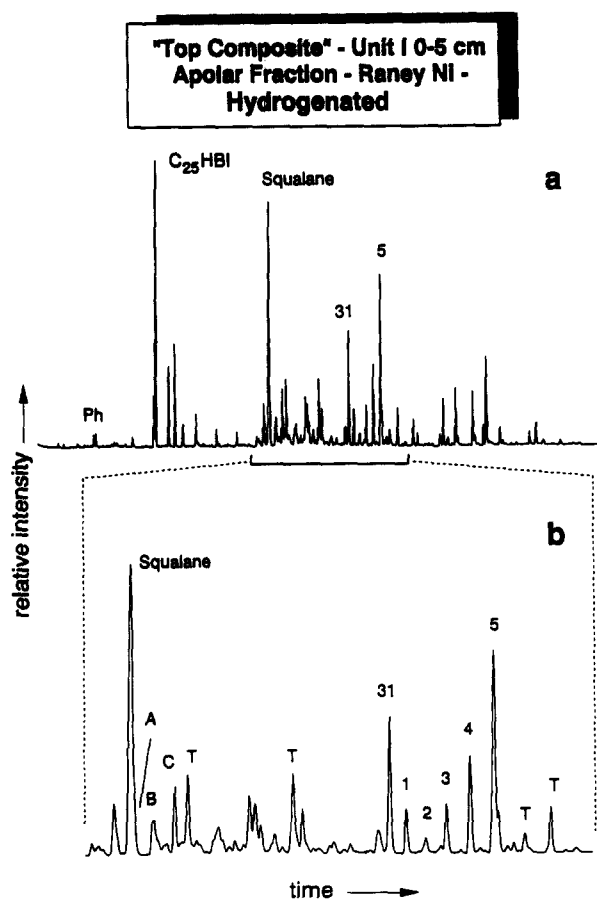

FIG. 7. Gas chromatogram (FID) of hydrogenated hydrocarbons recovered by Raney Ni desulfurization of the thiophene + thiolane fraction (R_f = 0.2–0.5) of composited apolar components from surface sediment samples (0–5 cm). Ph = phytane; A-C = C_{28}-polymethylated alkanes (see text); T = triterpenoids; 31 = n-C_{31}; 1–5 = C_{32}-C_{36}-polymethylated alkanes (Sinninghe Damsté et al., 1995).
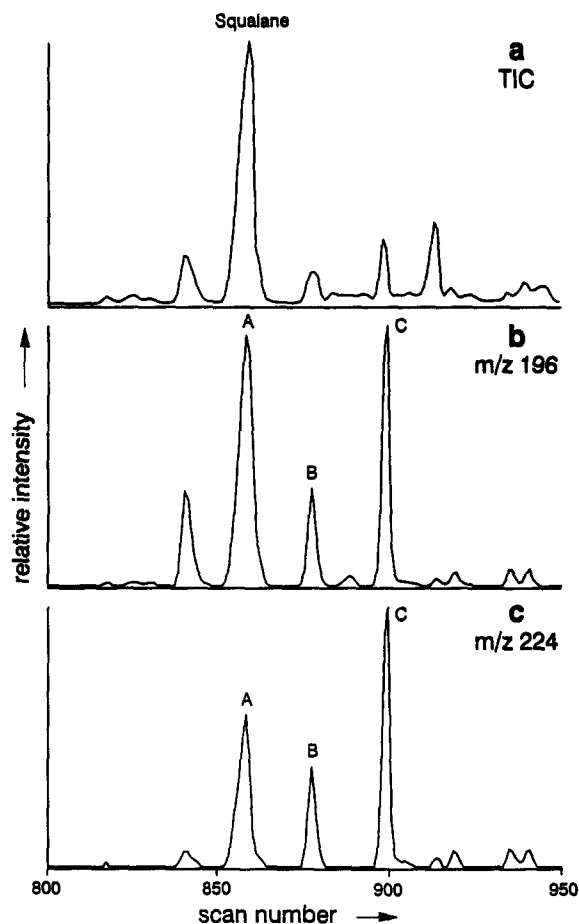

FIG. 8. Partial total ion current and partial mass chromatograms of m/z 196 and m/z 224, characteristic fragment ions of the branched C_{28}-hydrocarbons formed upon desulfurization of the sedimentary C_{28}-2,4-dialkylthiophenes.

of precursor C_{25}-HBI alkenes from surface sediments and the appearance of either C_{25}-HBI thiolanes or thiophenes if intramolecular sulfur incorporation occurred or C_{25}-HBI hydrocarbons released by desulfurization if intermolecular sulfur incorporation occurred. Quenching of C_{25}-HBI alkenes into other forms of OSC cannot be ruled out, nor can some degree of biodegradation.

REFERENCES

Adam P., Schmid J. C., Mycke B., Strazielle C., Connan J., Huc A., Riva A., and Albrecht P. (1993) Structural investigation of non-polar sulfur cross-linked macromolecules in petroleum. *Geochim. Cosmochim. Acta* **57**, 3395–3419.

Al-Lihaibi S. S. and Wolff G. A. (1991) The role of sulphur in humic acid diagenesis: reductive degradation studies. In *Organic Geochemistry. Advances and Applications in the Natural Environment* (ed. D. A. C. Manning), pp. 399–402. Univ. Manchester Press.

Barbe A., Grimalt J. O., Pueyo J. J., and Albaiges J. (1990) Characterization of model evaporitic environments through study of lipid components. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 815–828.

Brassell S. C., Lewis C. A., de Leeuw J. W., de Lange F., and Sinninghe Damsté J. S. (1986) Isoprenoid thiophenes: novel products of sediment diagenesis? *Nature* **320**, 160–162.

Calvert S. E. and Fontugne M. R. (1987) Stable carbon isotopic evidence for the marine origin of the organic matter in the Holocene Black Sea sapropel. *Chem. Geol.* (Isotope Geosci.) **66**, 315–322.

Calvert S. E., Karlin R. E., Toolin L. J., Donahue D. J., Southon J. R., and Vogel J. S. (1991) Low organic carbon accumulation rates in Black Sea sediments. *Nature* **350**, 692–695.

Crusius J. and Anderson R. F. (1992) Inconsistencies in accumulation rates of Black Sea sediments inferred from records of laminae and ^{210}Pb. *Paleoceanogr.* **7**, 215–227.

Degens E. T., Stoffers P., Olubic S., and Dickman M. D. (1980) Varve chronology: Estimated rates of sedimentation in the Black Sea deep basin. In *Initial Report of the Deep Sea Drilling Project* (ed. D. A. Ross et al.), Vol. **42B**, pp. 499–508. U.S. Govnt. Printing Office.

de Graaf W., Sinninghe Damsté J. S., and de Leeuw J. W. (1992) Laboratory simulation of natural sulphurization: 1. Formation of monomeric and oligomeric isoprenoid polysulphides by low-temperature reactions of inorganic polysulphides with phytol and phytadienes. *Geochim. Cosmochim. Acta* **56**, 4321–4328.

Freeman K. H. and Wakeham S. G. (1992) Variations in the distributions and isotopic compositions of alkenones in Black Sea particles and sediments. In *Advances in Organic Geochemistry 1991* (ed. C. B. Eckhardt et al.); *Org. Geochem.* **19**, 277–285.

Fukushima K., Yasukawa M., Muto N., Uemura H., and Ishitawari R. (1992) Formation of C_{20} isoprenoid thiophenes in modern sediments. *Org. Geochem.* **18**, 83–91.

Hay B. J. (1988) Sediment accumulation in the central western Black Sea over the past 5100 years. *Paleoceanogr.* **3**, 491–508.

Hay B. J., Arthur M. A., Dean W. E., Neff E. D., and Honjo S. (1991a) Sediment deposition in the Late Holocene abyssal Black Sea with climatic and chronological implications. *Deep-Sea Res.* **38**(Suppl. 2), S1211–S1235.

Hay B. J., Honjo S., Kempe S., Ittekkot V. A., Degens E. T., Konuk T., and Izdar E. (1991b) Interannual variability in particle flux in the southwestern Black Sea. *Deep-Sea Res.* **37**, 911–928.

Jones G. (1990) AMS radiocarbon dating of sediments and waters from the Black Sea: An integrated approach. *Eos Trans. Amer. Geophys. Union* **37**, 152 (abstr.).

Jones G. and Gagnon A. (1994) Radiocarbon chronology of Black Sea sediments. *Deep-Sea Res.* **41**, 531–557.

Kening F. and Huc A. Y. (1990) Incorporation of sulfur in recent organic matter in a carbonate environment (Abu Dahbi, United Arab Emirates). In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series 249*, pp. 170–185. Amer. Chem. Soc.

Kissin Y. V., Feulmer G. P., and Payne W. B. (1986) Gas chromatographic analysis of polymethyl-substituted alkanes. *J. Chromatogr. Sci.* **24**, 164–169.

Kohnen M. E. L., Sinninghe Damsté J. S., Kock-van Dalen A. C., ten Haven H. L., Rullkötter J., and de Leeuw J. W. (1990a) Origin and diagenetic transformations of C_{25} and C_{30} highly branched isoprenoid sulphur compounds: Further evidence for the formation of organically bound sulphur during early diagenesis. *Geochim. Cosmochim. Acta* **54**, 3053–3063.

Kohnen M. E. L., Peakman T. M., Sinninghe Damsté J. S., and de Leeuw J. W. (1990b) Identification and occurrence of novel C_{36}-

C_{54} 3,4-dialkylthiophenes with an unusual carbon skeleton in immature sediments. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 1103–1113.

Kohnen M. E. L., Sinninghe Damsté J. S., Rijpstra W. I. C., and de Leeuw J. W. (1990c) Alkylthiophenes as sensitive indicators of paleoenvironmental changes. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series 249*, pp. 444–485. Amer. Chem. Soc.

Kohnen M. E. L., Sinninghe Damsté J. S., and de Leeuw J. J. (1991a) Biases from natural sulfurization in paleoenvironmental 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- and polysulphide-bound biomarkers in sulphur-rich geomacromolecules as revealed by selective chemolysis. *Geochim. Cosmochim. Acta.* **55**, 1375–1394.

Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merritt D. A., and Hayes J. M. (1992a) Recognition of paleobiochemicals by a combined molecular sulfur and isotope geochemical approach. *Science* **256**, 358–362.

Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merritt D. A., and Hayes J. M. (1992b) The combined application of organic sulphur and isotope geochemistry to assess multiple sources of paleobiochemicals with identical carbon skeletons. In *Advances in Organic Geochemistry 1991* (ed. C. B. Eckhardt et al.); *Org. Geochem.* **19**, 403–419.

McCarthy F. D., Han J., and Calvin M. (1968) Hydrogen atom transfer in mass spectrometric fragmentation patterns of saturated aliphatic hydrocarbons. *Anal. Chem.* **40**, 1475–1480.

Overmann J., Cypionka H., and Pfenning N. (1992) An extremely low-light adapted phototrophic sulfur bacterium from the Black Sea. *Limnol. Oceanogr.* **37**, 150–155.

Repeta D. J. (1993) A high resolution historical record of Holocene anoxygenic primary production in the Black Sea. *Geochim. Cosmochim. Acta* **57**, 4337–4342.

Repeta D. J. and Simpson D. J. (1991) The distribution and recycling of chlorophyll, bacteriochlorophyll and carotenoids in the Black Sea. *Deep-Sea Res.* **38**(Suppl 2), S969–S984.

Repeta D. J., Simpson D. J., Jørgenson B. B., and Jannasch H. W. (1989) Evidence of anoxygenic photosynthesis from the distribution of bacteriopigments in the Black Sea. *Nature* **342**, 69–72.

Ross D. A. and Degens E. T. (1974) Recent sediments of the Black Sea. In *The Black Sea—Geology, Chemistry and Biology* (ed. E. T. Degens and D. A. Ross); *Mem. AAPG* **20**, 183–199.

Rowland S., Rickey C., Al-Lihaibi S. S., and Wolff G. A. (1993) Incorporation of sulphur into phytol derivatives during simulated early diagenesis. *Org. Geochem.* **20**, 1–5.

Rullkötter J. and Michaelis W. (1990) The structure of kerogen and related materials. A review of recent progress and future trends. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 829–852.

Schaeffer P., Ocampo R., Callot H. J., and Albrecht P. (1993) Extraction of bound porphyrins from sulphur-rich sediments and their use for reconstruction of paleoenvironments. *Nature* **364**, 133–136.

Schmid J. C. (1986) Marqueurs biologiques soufrés dan les pétroles. Ph.D. dissertation, Univ. of Strasbourg, France.

Schouten S., Van Driel G., Sinninghe Damsté J. S., and de Leeuw J. W. (1993) Natural sulphurization of ketones and aldehydes: A key reaction in the formation of organic sulfur compounds. *Geochim. Cosmochim. Acta.* **57**, 5111–5116.

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 organic polysulphides at low temperatures. In *Advances in Organic Geochemistry 1993* (ed. K. Øygard et al.) (In press).

Sinninghe Damsté J. S. and de Leeuw J. W. (1990) Analysis, structure and geochemical significance of organically-bound sulphur in the geosphere: State of the art and future research. In *Advances in Organic Geochemistry 1989* (ed. B. Durand and F. Behar); *Org. Geochem.* **16**, 1077–1101.

Sinninghe Damsté J. S., Rijpstra W. I. C., de Leeuw J. W., and 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* (ed. L. Mattavelli and L. Novelli); *Org. Geochem.* **13**, 593–606.

Sinninghe Damsté J. S., Rijpstra W. I. C., Kock-van Dalen A. C., de Leeuw J. W., and Schenck P. A. (1989) Quenching of labile functionalized lipids by inorganic sulphur species: Evidence for the formation of sedimentary organic sulphur compounds at the early stages of diagenesis. *Geochim. Cosmochim. Acta* **53**, 1343–1355.

Sinninghe Damsté J. S., Eglinton T. I., Rijpstra W. I. C., and de Leeuw J. W. (1990) Molecular characterization of organically-bound sulfur in high molecular weight sedimentary organic matter using flash pyrolysis and Raney Ni desulfurization. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series 249*, pp. 486–528. Amer. Chem. Soc.

Sinninghe Damsté J. S., Wakeham S. G., Kohnen M. E. L., Hayes J. M., and de Leeuw J. W. (1993) A 6000-year sedimentary record of chemocline excursions in the Black Sea. *Nature* **362**, 827–829.

Sinninghe Damsté J. S., Erkes A.-M. W. E. P., Rijpstra W. I. C., de Leeuw J. W., and Wakeham S. G. (1995) C_{32}-C_{36} Polymethyl alkenes in Black Sea sediments. *Geochim. Cosmochim. Acta* **59**, 347–353.

Smith K. M. and Simpson D. J. (1987) Raney nickel reductions of chlorophyll derivatives: Hydroporphyrins in the anhydro series. *J. Amer. Chem. Soc.* **109**, 6326–6333.

Sun M.-Y. and Wakeham S. G. (1994) Molecular evidence for degradation and preservation of organic matter in the anoxic Black Sea basin. *Geochim. Cosmochim. Acta* **58**, 3395–3406.

ten Haven H. L., Rullkötter J., Sinninghe Damsté J. S., and de Leeuw J. W. (1990) Distribution of organic sulfur compounds in Mesozoic and Cenozoic sediments from the Atlantic and Pacific Oceans and the Gulf of California. In *Geochemistry of Sulfur in Fossil Fuels* (ed. W. L. Orr and C. M. White); *ACS Symposium Series 249*, pp. 170–185. Amer. Chem. Soc.

Valisolalao J., Perakis N., Chappe B., and Albrecht P. (1984) A novel sulphur-containing C_{35} hopanoid in sediments. *Tetrahedron Lett.* **14**, 555–567.

Volkman J. K., Eglinton G., Corner E. D. S., and Forsberg T. E. V. (1980) Long chain alkenes and alkenones in the marine coccolithophorid *Emiliania huxleyi*. *Phytochem.* **19**, 2619–2622.

Volkman J. K., Farrington J. W., Gagosian R. B., and Wakeham S. G. (1983) Lipid composition of coastal marine sediments from the Peru upwelling region. In *Advances in Organic Geochemistry 1981* (ed. M. Bjorøy et al.), pp. 228–240. Wiley.

Volkman J. K., Barnett S. M., Dunstan G. A., and Jeffrey S. W. (1994) C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. *Org. Geochem.* **21**, 407–413.

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., Beier J. A., and Clifford C. H. (1991) Organic matter sources in the Black Sea as inferred from hydrocarbon distributions. In *Black Sea Oceanography* (ed. E. Izdar and J. W. Murray), pp. 319–341. Kluwer.