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Carbon and sulfur isotopic composition of alkyl- and benzo-thiophenes provides insights to their origin and formation pathways

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Abstract:	Thiophenic compounds can provide significant geochemical information and may be used as paleoenvironmental indicators as long as their biological origin and formation pathways are understood. To this end, we investigated the structures, distribution, d 13 C and d 34 S values of specific thiophenic compounds extracted from immature sedimentary rocks from the Upper Cretaceous (Ghareb Formation, Shefela Basin, Israel). Isoprenoidal alkylthiophenes (ATs) show a general trend of depletion in their 13 C and 34 S relative to the normal (linear) ATs extracted from the studied samples. In addition, a consistent enrichment of up to 2‰ in 13 C and 8‰ in 34 S of methylated ATs (m/z= 111) relative to non-methylated ones (m/z= 97) was recorded. This suggests that AT precursors derived from different organisms and diagenetic pathways which later affected their sulfurization mechanisms. The large variation in d 34 S values of individual ATs, ~15 ‰, along with the general trend of 13 C depletion with increasing T max suggests that ATs formed by a set of diagenetic processes of mild thermochemical alteration. The d 13 C values of BTs are enriched in 13 C relative to the rest of OSCs, by ~3‰ in average. This in turn suggests that BTs were generated mainly by sulfurization and subsequent ring closure or aromatization of 13 C enriched aromatic/ alkyl cyclohexane compounds with unsaturation and/or functional groups in their alkyl chain. Such 13 C enriched precursors can be generated from carotenoids and terrestrial compounds (e.g., by lignin degradation). The large variation in d 34 S values of individual BTs, ~15 ‰, suggests that the BTs in our samples are formed during the diagenesis stage and did not experience advanced thermal alteration processes. Therefore, their occurrence in immature sedimentary rocks might be used as a proxy for early thermochemical alterations that cannot be detected using conventional geochemical indicators, such as T max . The combination of structural, 13 C and 34 S investigation of individual OSCs enhanced our understanding of their sulfurization pathways and origin which in turn will help develop new paleoenvironmental indicators.

Highlights

- Thiophenic compounds (benzothiophenes and alkylthiophenes) show unique ^{13}C and ^{34}S trends in the immature Ghareb Formation rocks.
- Benzothiophenes originated from ^{13}C enriched compounds such as carotenoids and terrestrial material.
- The presence of appropriate functionalized precursors and inorganic (poly)sulfides are essential for low temperature thiophenic compound formation.
- Thiophenic compounds formed as a result of multiple diagenetic sulfurization pathways followed by mild thermochemical alterations.
- Thiophenic compound distributions, and their associated S and C isotope composition can be used as a proxy for diagenetic thermochemical alteration.

Carbon and sulfur isotopic composition of alkyl- and benzo-thiophenes provides insights to their origin and formation pathways

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Abstract

Thiophenic compounds can provide significant geochemical information and may be used as paleoenvironmental indicators as long as their biological origin and formation pathways

are understood. To this end, we investigated the structures, distribution, $\delta^{13}C$ and $\delta^{34}S$ values of specific thiophenic compounds extracted from immature sedimentary rocks from the Upper Cretaceous (Ghareb Formation, Shefela Basin, Israel).

Isoprenoidal alkylthiophenes (ATs) show a general trend of depletion in their ^{13}C and ^{34}S relative to the normal (linear) ATs extracted from the studied samples. In addition, a consistent enrichment of up to 2‰ in ^{13}C and 8‰ in ^{34}S of methylated ATs (m/z= 111) relative to non-methylated ones (m/z= 97) was recorded. This suggests that AT precursors derived from different organisms and diagenetic pathways which later affected their sulfurization mechanisms. The large variation in $\delta^{34}S$ values of individual ATs, ~15 ‰, along with the general trend of ^{13}C depletion with increasing T_{max} suggests that ATs formed by a set of diagenetic processes of mild thermochemical alteration. The $\delta^{13}C$ values of BTs are enriched in ^{13}C relative to the rest of OSCs, by ~3‰ in average. This in turn suggests that BTs were generated *mainly* by sulfurization and subsequent ring closure or aromatization of ^{13}C enriched aromatic/ alkyl cyclohexane compounds with unsaturation and/or functional groups in their alkyl chain. Such ^{13}C enriched precursors can be generated from carotenoids and terrestrial compounds (e.g., by lignin degradation). The large variation in $\delta^{34}S$ values of individual BTs, ~15 ‰, suggests that the BTs in our samples are formed during the diagenesis stage and did not experience advanced thermal alteration processes. Therefore, their occurrence in immature sedimentary rocks might be used as a proxy for early thermochemical alterations that cannot be detected using conventional geochemical indicators, such as T_{max}.

The combination of structural, ^{13}C and ^{34}S investigation of individual OSCs enhanced our understanding of their sulfurization pathways and origin which in turn will help develop new paleoenvironmental indicators.

Keywords

Benzothiophenes, Alkylthiophenes, Organic S compounds, Compound specific S and C isotopes, Biomarkers, Ghareb Formation

1. Introduction

Organic sulfur compounds (OSCs) are generated during early diagenesis and linked to local and global paleo-environmental events (Sinninghe Damste and de Leeuw, 1990; Werne et al., 2004; Kutuzov et al., 2019). OSCs found in sedimentary rocks often resemble their biomolecular precursors and thus they can serve as biomarkers (Brassel et al., 1986; Schaeffer et al., 1995; Grice et al., 1998). They form as a result of reduced sulfur (e.g. HS^-, S_x^{-2}) incorporation into functionalized sites of biomolecules and this in turn enhances the preservation of their carbon skeleton by binding them to macromolecular fractions (Aizenshtat et al., 1983; Kohnen et al., 1989; Adam et al., 1993; Amrani, 2014). Moreover, saturation of double bonds with H_2S was suggested as a geochemical pathway that enhances the preservation of poly-unsaturated biomarkers such as carotenoids (Hebting et al., 2006).

Reduced S species, in anoxic environments, are generated by dissimilatory microbial sulfate reduction (MSR) (Kaplan et al., 1963). This process occurs at low temperatures (< 80 ° C) and it is associated with large S isotope fractionation (up to ~75‰) (Wortmann et al., 2001; Brunner and Bernasconi, 2005; Canfield et al., 2010; Sim et al., 2011). The S

isotope fractionation associated with MSR processes is recorded in the isotopic composition of organic and inorganic S species (e.g. pyrite) in sediments and sedimentary rocks (Anderson and Pratt, 1995; Canfield et al., 1998; 2010; Amrani, 2014).

Alkylthiophenes (ATs) and fused thiophenes (e.g., benzothiophenes (BTs)) are abundant sub-groups of OSCs (Sinninghe Damste and de Leeuw, 1990; Kutuzov et al., 2019). Many ATs, such as iC_{20} thiophenes, were detected in recent sediments, in mature and immature organic rich rocks, oils and natural gas (Brassell et al., 1986; de Leeuw and Sinninghe Damsté, 1990; Barakat and Rullkötter, 1995; Adam et al., 2000; Russell et al., 2000). The relative abundances of ATs and their isomers allow qualitative interpretations such as the environmental conditions involved during OM deposition. For instance, under hypersaline conditions, archaeal populations that produce polyunsaturated phytenols are favored. Sulfurization of these compounds yields isoprenoidal mid-chain thiophenes (Sinninghe Damsté et al., 1989b; Kohnen et al., 1990b; Barakat and Rullkötter, 1995; Schwark et al., 1998). Thus, the distribution of i-C20 thiophene (phytol thiophene) isomers is proposed as a proxy for paleo-salinity (Sinninghe Damsté et al., 1989b; de Leeuw and Sinninghe Damsté, 1990; Barakat and Rullkötter, 1995). Studies that investigated the $\delta^{13}C$ values of ATs have shown that a certain carbon skeleton of AT can have multiple biosynthetic precursors which are, in a number of cases, derived from several biological sources (Kohnen et al., 1992a; 1992b). Therefore, ATs product-precursor relation to study origin and formation process is sometimes limited. Recent studies have investigated $\delta^{34}S$ values of ATs, alongside another OSCs, in order to learn their formation process and the paleo-environmental conditions involved in their formation (Raven et al., 2015; 2016; Shawar et al., 2020). Raven et al. (2015) studied recent sediments from the Cariaco Basin. They

showed a large variation in $\delta^{34}S$ values of different OSCs, up to 25‰, with some OSCs (e.g. isoprenoid C_{20} thiophenes) that are ^{34}S depleted relative to the coexisting pyrite. They suggested that the sulfurization occurred through two different pathways, reversible and irreversible. Shawar et al. (2020) studied the ancient and immature organic-rich rocks from the Cretaceous Ghareb (Shefela Basin, Israel) and Miocene Monterey (Naples Beach, USA) Formations. Large variations in the $\delta^{34}S$ values of different OSCs, that reached up to 28‰ and 36‰, were observed in the Ghareb and Monterey samples, respectively. Additionally, they showed that $\delta^{34}S$ values of C_{25} highly branched isoprenoid thiophene might be used as a proxy for the euxinic sulfurization environment (Shawar et al., 2020).

Benzothiophenes (BTs) usually occur in oils but rarely in immature rocks (Orr, 1986; Schou and Myhr, 1988; Chakhmakhchev and Suzuki, 1995). They have been mainly used as a proxy for thermal maturation assessment in oils (e.g., Ho et al., 1974; Orr, 1986; Radke, 1988; Schou and Myhr, 1988; Chakhmakhchev and Suzuki, 1995). Only few studies have used BTs in oils as a proxy for depositional environment (siliceous versus carbonate rich environment; e.g., Hughes, 1984), most likely because their origin is still debated. Surprisingly, we could not find any study regarding the $\delta^{13}C$ values of BTs in the scientific literature. Sulfur isotope studies had used them as tracers for thermochemical sulfate reduction (TSR) as well as proxies for oil-oil and oil-source rock correlations (Amrani et al., 2012; Amrani, 2014; Cai et al., 2015; Meshoulam et al., 2016; Greenwood et al., 2018). However, a recent study has reported that BTs are abundant also in *immature* organic rich sedimentary rocks from the Ghareb Formation, Israel (Shawar et al., 2020). Moreover, these BTs vary in their $\delta^{34}S$ values by ~15‰ , much larger than the 2‰ variation reported in $\delta^{34}S$ values of BTs generated during artificial thermal maturation or the <5‰

variation of natural thermal maturation (non TSR) processes (Ellis et al., 2017; Rosenberg et al., 2017). The origin and formation pathways of these thermally "immature" BTs are not understood and may have important geochemical implications (Shawar et al., 2020).

The recent compound-specific sulfur isotope analysis (CSSIA) studies demonstrated the potential of OSCs as paleoenvironmental tools (Raven et al., 2015;2016; Shawar et al., 2020). Coupling $\delta^{13}C$ of individual OSCs to their $\delta^{34}S$ values may further improve our understanding of their precursors and formation pathways.

In the present study, we investigate the origin of OSCs and their mechanism of formation and attempt to draw possible sulfurization pathways and their associated paleo-environmental conditions. Immature rock samples from the Late Cretaceous Ghareb Formation (Shefela Basin, Israel) were used as a test case in continuation of our previous studies of these samples (Shawar et al., 2018; 2020). We applied compound specific C isotope analyses on saturates, biomarkers and OSC on these rock extracts. We then combined these results with the previously published (Shawar et al., 2020) CSSIA data of ATs and BTs.

2. Methods

2.1 Study sites

Nine core samples of the Late Cretaceous organic-rich chalk sequence were analyzed. These samples were drilled from 260 m to 600 m below the surface in the Ghareb Formation, Aderet borehole, the depo-center of the Shefela Basin (hereafter Ghareb Formation). These sediments represent a high-productivity succession developed at the Late Cretaceous outer shelf belt due to an extensive upwelling system along the southern

Tethyan margin (Almogi-Labin et al., 1993; Eshet and Almogi-Labin, 1996; Soudry et al., 2006; Meilijson et al., 2014). Detailed descriptions of these samples and their lithologies are given by Shawar et al. (2018).

2.2 Materials and standards

Analytical grade solvents used in this study (dichloromethane, hexane, methanol, etc.) were purchased from Biolab Ltd. (Jerusalem, Israel) and were distilled before use. Sulfur hexafluoride was purchased from Praxair Inc. (PA, USA). The S isotope reference materials NBS-127 ($BaSO_4$; $\delta^{34}S$ = 21.1‰), IAEA-S-1 (Ag_2S; $\delta^{34}S$ = -0.3‰), and IAEA-SO-6 ($BaSO_4$; $\delta^{34}S$ = -34.1‰) were purchased from the National Institute of Standards and Technology (NIST, USA) and used for calibration of in-house standards. All other chemicals (>99% purity) were purchased from Sigma-Aldrich (St. Louis, USA).

2.3 Organic extraction

Bitumen was extracted from 160 g of powdered rock, with a mixture of organic solvents (9:1, dichloromethane: methanol, 7 mL/g rock), for each core sample from the Ghareb Formation. The samples were extracted using a microwave accelerated solvent extractor (MARS5 Express, CEM Corporation, USA) at 120°C for 30 minutes. The microwave extraction was repeated twice to ensure complete extraction of the bitumen. Asphaltene separation from the dried and concentrated bitumen was achieved by dissolution of the bitumen with *n*-pentane (50:1 bitumen weight) overnight. The maltene fraction was evaporated and weighed. Detailed descriptions of this method are given by Shawar et al., (2020).

2.4 Sample preparation for OSC quantification and CSSIA

2.4.1 Liquid chromatography (LC)

About 140 mg of the maltenes were separated by liquid chromatography (LC) into five sub-fractions (saturated hydrocarbons, alkylthiophenes (ATs), benzothiophenes (BTs), sulfidic and polar compounds). A detailed description of this method is given by Shawar et al., (2020).

2.4.2 Identification and quantification of OSC (GC-MS, GC-FPD and GC-QQQ-MS analysis)

All details related to the analysis, quantification, and CSSIA of OSCs are published previously in Shawar et al., (2020).

For the identification and quantification of biomarkers (such as carotenoid biomarkers (isorenieratane, β-isorenieratane, β-carotene, γ-carotene, dimethyl/diethyl carotenoids, and dimethyl diaromatic carotenoids) and perylene) in non-polar fractions (the combination of saturates, ATs and BTs) a gas chromatograph coupled to triple quadrupole mass spectrometer (GC-QQQ-MS; Agilent 7890B/7010, USA) was used. 1µl of sample was injected into the device via multi-mode inlet using an auto-sampler. The inlet temperature was ramped from 60°C to 340°C at a rate of 70°C/min. The injection was carried out in a splitless mode. The carrier gas was at a constant rate flow of 1.5 mL/min. The GC oven temperature was programmed at 60°C for 2 min then 8°C/min to 220°C, then 2°C/min to 320°C min and held for 28 min. The transfer line temperature was held at 320°C. The quadrupole analyzer and the MS source were operated at 150°C and 270°C, respectively, in electron impact mode at 70 eV. The GC-MS system was equipped with an Agilent DB-5MS capillary column (60m × 250µm × 0.25µm).

2.5 Sample preparation for compound specific C isotope analysis

2.5.1 Molecular sieves

Approximately 1.2 mL of 5A° molecular sieve (Sigma Aldrich) was placed into 4 mL screwcap vials and activated overnight at 250 °C. Saturate fractions were washed into the vials with cyclohexane (Mallinckrodt, UltimAR grade). After agitation with a vortex mixer to fully wet the sieve, the vials were placed on a hotplate at 80 °C for 8 hours. After cooling, the sieve - cyclohexane slurry was transferred to a small glass Pasteur pipette (0.5 cm diameter) plugged with pre-extracted cotton wool and < 0.5 cm silica gel (Merck, 0.063-0.200 mm). The vial was rinsed with cyclohexane, and the sieve was washed thoroughly with cyclohexane to recover the branched and cyclic (B&C) fraction. The B&C fraction was evaporated to dryness and weighed.

2.5.2 Compound specific C isotope analysis using GC-IRMS

Saturate, B&C and OSC fractions were analyzed for compound specific $\delta^{13}C$ measurements on a Thermo Scientific Delta V Advantage isotope ratio mass spectrometer, coupled to a Trace GC Ultra via a GC Isolink and Conflo IV. Samples were dissolved in hexane (Honeywell, HPLC grade), and 1 µL was injected into the split/splitless injector operating in splitless mode at constant temperature of 280 °C. Helium carrier gas was used at a constant flow of 1.5 mL / min. For saturate and B&C fractions, the GC column was an Agilent DB-1MS ultra inert column (60m × 250µm × 0.25µm), while for OSC fractions the column was an Agilent DB-5MS ultra inert column (60m × 250µm × 0.25µm). For all fractions, the GC oven was held at 40 °C for 1 min after injection, then ramped at 3 °C/min to 325 °C, and held for 30 min. GC column outflow passed through the GC Isolink

combustion reactor (copper oxide and nickel oxide, 1000 °C) to combust hydrocarbons to CO_2. The CO_2 passed through the Conflo IV interface to the IRMS, which measured m/z 44, 45 and 46. $\delta^{13}C$ was calculated from the measured masses by Thermo Isodat software and calibrated to the VPDB scale by comparison with a mixture of n-alkane standards of known isotopic composition. All samples were measured in triplicate.

3. Results

3.1 Bulk Geochemical parameters

The bulk parameters (T_{max}, total organic carbon (TOC), total sulfur (TS), the S content and $\delta^{34}S$ values of kerogen and pyrite, and $\delta^{13}C$ of kerogen and bitumen) of the Ghareb Formation samples were previously reported by Shawar et al. (2018) and summarized in Table 1S. In short, the T_{max} ranges between 402 and 422°C (Table 1S). TOC and TS reach maximum concentrations of 22.4 wt% and 2.9 wt% at depths of 420m and 393m, respectively (Table 1S). The TOC and TS contents gradually decrease to minimum values (7.3 wt% and 0.4 wt%, respectively) at 595m (Table 1S).

The S occurs predominantly as kerogen S (KS), which represents up to 92.8 wt% of the TS at depth of 528m (Table 1S). Pyrite S reaches up to 28.4 wt% of the TS at 308m (Table 1S). The pyritic $\delta^{34}S$ values range between -39.5‰ at 308m and -9.8‰ at 420m. The $\delta^{34}S$ values of KS range between -8.7‰ at 450m and 3.4‰ at 265m (Table 1S). The S isotope composition of all the bulk organic S phases (namely bitumen, kerogen, asphaltenes and maltenes) is similar (Shawar et al., 2018). Therefore, hereafter $\delta^{34}S$ values of KS were used to represent the S isotope composition of the bulk organic S.

The $\delta^{13}C$ values range between -29.0‰ at 420m and -29.9‰ at 308m for kerogen, and between -29.4‰ at 420m and -31.7‰ at 450m for the bitumen (Table 1S).

3.2 Biomarker distribution

Pristane to phytane ratio (Pr/Ph) ranges between 0.3 at the depth of 393m to 1 at the depth of 341m (Fig. 1a).

Two biomarkers (mono-, di- and trimethylated 2-methyl-2-(4,8,12-trimethyltridecyl) chromans and perylene), that point to terrestrial contribution into the marine sediment, (Aizenshtat, 1973; Tulipani et al., 2015), were detected in our studied samples. The methyltrimethyltridecylchroman (MTTC) ratio (trimethyl-2-methyl-4,8,12-trimethyltridecyl chroman/ mono-, di- and trimethylated 2-methyl-2-4,8,12-trimethyltridecyl chromans) is almost constant throughout the studied section. This ratio is around 0.9 at the depth interval of 265 to 420m (Fig. 1b). Then, it decreased deeper in the section until it reached 0.7 at the depth of 595m (Fig.1b). Perylene concentration along the depth profile is presented in Fig. 1c. Perylene concentration increased deeper in the studied section to a maximum of 1.2µg/gr TOC at the depth 420m and then decreased again (Fig. 1c).

Carotenoid biomarkers were also identified in the studied samples, and the most abundant type is isorenieratane. It occurred in trace amounts, up to 0.04 µg/gr TOC (Fig. 1d).

3.3 $\delta^{13}C$ values of non-sulfurized individual organic compounds

The $\delta^{13}C$ values of individual non-sulfurized organic compounds from the saturated fraction of the Ghareb Formation samples (normal alkanes, isoprenoids, hopanes and

steranes) are presented in Table. 1. $\delta^{13}C$ values of normal alkanes vary from -37‰ to -30‰ with general trend of ^{13}C enrichment as the carbon number increases (Table. 1). The $\delta^{13}C$ values of pristane (Pr) and phytane (Ph) show different trends throughout the studied section (Fig. 1a). In general, Pr is enriched in ^{13}C relative to Ph by up to ~3‰ (Fig. 1a; Table. 1). Moreover, the $\delta^{13}C$ values of Pr are very variable, between -35‰ at 528m to -31‰ at 341m, while the $\delta^{13}C$ values of Ph are more consistent and vary between -35.4‰ at 450m to -33.5‰ at 265m (Fig. 1a; Table. 1).

In general, steranes are depleted in ^{13}C relative to the hopanes extracted from the studied samples (Table. 1). This depletion reaches down to 7.1‰ between C_{28} sterane-2 and C_{31} hopane-2 at 450m (Table. 1).

3.4 Organic S compounds distribution and their associated S and C isotope composition

The $\delta^{34}S$ values of kerogen and individual OSCs from aromatic fractions (ATs and BTs) and detailed description of their molecular distributions were previously reported by Shawar et al. (2020). For simplicity and ease of comparison with the C isotope data the $\delta^{34}S$ values of OSCs and their molecular distribution data are presented again in this manuscript (Fig., 2a-d and 3a-d; Tables 2S and 3S).

The $\delta^{13}C$ values of bulk phases (bitumen and kerogen) and individual ATs and BTs are presented in Figs. 2e-f and 3e-f, respectively, and summarized in Table. 4S. A detailed description of $\delta^{13}C$ of different OSCs is provided in the following sections.

3.4.1 $\delta^{13}C$ of OSCs from the alkylthiophenes fraction

The $\delta^{13}C$ values of ATs are presented in Fig. 2e-f and Table. 4S and they ranged between -35 to -28‰. Unlike the normal ATs, the isoprenoid ATs (such as iC_{20} thiophene and its isomers) show a moderate change in their $\delta^{13}C$ values throughout the studied section (Fig. 2e and 2f). In addition, isomers of the same AT show different C isotope composition (Fig. 2e and 2f). ATs with methyl substituted thiophenes (m/z 111) are ^{13}C enriched relative to those with non-methyl substituted isomers (m/z 97), by up to 2‰ (Fig. 2e and 2f). Similarly, most of the methyl substituted ATs (m/z 111) are ^{34}S enriched relative to the non-methyl substituted isomer (m/z 97) of the same AT, by up to 8‰ (Figs. 2a and 2b; Table. 2S).

3.4.2 $\delta^{13}C$ of OSCs from the benzothiophenes fraction

BTs were also measured for their $\delta^{13}C$ values along the depth profile and presented in Fig. 3e-f and Table 4S. In general, most of the BTs are ^{13}C enriched relative to kerogen, bitumen and to the rest of the fractions, by an average of ~3‰ (Table. 4S). The $\delta^{13}C$ values of individual BTs show large variation, ~8‰ (Fig. 3e and 3f; Table 4S), while the $\delta^{34}S$ values of BTs varied by up to 15‰ compared with the kerogen (Shawar et al., 2020).

4. Discussion

4.1 The origin and formation pathways of alkylthiophenes in immature sediments

4.1.1 Alkylthiophenes are derived from multiple precursors and environments

Isoprenoid based ATs, such as iC_{20} thiophenes show a moderate change in their $\delta^{13}C$ values relative to the normal (linear) ATs, throughout the studied section (Fig. 2e and 2f). In addition, isoprenoid ATs are generally ^{13}C and ^{34}S depleted relative to normal ATs, down to ~5‰ and ~12‰ respectively (as is shown for $iC_{20,}$ nC_{19} and nC_{18} thiophenes in Fig. 4 a and b, and in more detail in Tables. 2S and 4S). This can be attributed to the synthesis of isoprenoid and normal lipids through different biosynthetic pathways (Chikaraishi et al., 2004). Moreover, the difference in $\delta^{13}C$ values of normal and isoprenoid ATs may suggest that isoprenoid and normal ATs are generated by different organisms that occur in different parts of the water column and thus experience different environmental conditions. Subsequently, normal and isoprenoid lipids might have different functionalities and thus undergo different sulfurization pathways that govern the variation in their S isotope composition.

Isoprenoid ATs have similar $\delta^{13}C$ values to those of steranes (e.g., C_{27} sterane; blue shaded area in Fig. 5a; Tables 1 and 4S), while $\delta^{13}C$ values of normal ATs overlap with the range of hopanes (e.g., C_{31} hopane; blue shaded area in Fig. 5b; Tables 1 and 4S). These observations further support previous suggestions that the carbon skeleton of isoprenoid ATs mainly comes from photosynthetic organisms (or heterotrophs that feed on phototrophs) that represent the upper part of the water column environment (Sinninghe Damsté et al., 1986; Kohnen et al., 1990b; Grice and Brocks, 2011). Normal ATs may originate mainly from prokaryotic lipids that represent the lower part of the water column environment (Grice and Brocks, 2011).

Interestingly, there are differences of up to 2‰ and 8‰ in C and S isotope composition, respectively, of different isomers for the same AT (Figs. 6a and b; Tables. 2S and 4S). This

consistent enrichment in ^{13}C and ^{34}S in *most* of the methylated ATs (m/z 111) relative to the non-methylated (m/z 97), may point for different precursors and/or functionality with different $\delta^{13}C$ values. This precursor/functionality difference might also change their reactivity and thus their sulfurization order which in turn may affect their $\delta^{34}S$ values.

4.1.2 Formation processes of Alkylthiophenes

The formation processes of both isoprenoid and normal ATs as summarized in Fig. 7 are: I) direct formation of ATs, without the need of thermal alteration, by S incorporation into unsaturated functionalized organic compounds (path I in Fig.7), II) thermal alteration through either aromatization of alkylthiolanes that formed in early diagenesis or thermal decomposition of S cross linked macromolecules (path II in Fig.7).

I) Do Alkylthiophenes form directly at low temperature in recent sediments?

Alkylthiophenes were observed in recent sediments, and immature rocks (Brassell et al., 1986; Barbe et al., 1990; Kenig and Huc, 1990; Grimalt et al., 1991; Hartgers et al., 1997; Zhang et al., 2011). Consequently, several studies have suggested that ATs formed directly (Path I in Fig. 7) as a result of S incorporation into unsaturated functionalized organic compounds at low temperatures (Sinninghe Damsté et al., 1987; 1989; Fukushima et al., 1992; Adam et al., 1993).

Alongside the diagenetic formation pathways discussed above, it has been noted that ATs can be formed artificially in the injector of the GC as rearrangement of precursors with S functionalities, including S cross-linked oligomers or polymers (Krein, 1993; Krein and Aizenshtat, 1994). This may result in erroneous identification of alkylthiophenes in sulfur rich samples. To avoid this, a chemical cleavage of S-S and C-S bonds and their subsequent

methylation (e.g. MeLi/MeI treatment) is needed prior to GC analysis (Kohnen et al., 1991; Krein and Aizenshtat, 1994).

Laboratory experiments have shown that *direct* formation of ATs requires specific conditions, which are, in most cases, irrelevant to normal marine conditions, such as acidic/very basic catalysis, use of organic solvents and often mild heating (LaLonde et al., 1987; de Graaf et al., 1992; Fukushima et al., 1992; Krein and Aizenshtat, 1993; Rowland et al., 1993). The main sulfurized product from laboratory experiments executed under marine-relevant conditions are S cross-linked macromolecules, or thiol and sulfide monomers, rather than ATs (Krein and Aizenshtat, 1994; Schouten et al., 1994; Krein and Aizenshtat, 1995; Amrani et al., 2006). ATs were generated only after mild heating of such "kerogen like" S cross-linked macromolecules (Krein and Aizenshtat, 1994; Schouten et al., 1994; Amrani et al., 2006). This suggests that the dominant formation process of ATs in marine sediments is macromolecular formation rather than direct formation. This may suggest that ATs are generated after several diagenetic processes and mild thermochemical alteration of the sulfurized lipids or precursors (path II in Fig.7). We cannot however neglect the possibility of direct formation of alkylthiophenes from specific (e.g., multifunctional) structures and/or environmental conditions.

II) Do alkylthiophenes in immature sediments indicate that thermochemical alterations started during the diagenetic stage?

The Ghareb Formation samples used in this study are considered to be thermally immature with a T_{max} range of 402-422° C (Table. 1S) (Shawar et al., 2018; 2020). However, our samples are rich in S (kerogen type IIS), which can facilitate and catalyze early thermochemical alterations of sedimentary OM (Baskin and Peters, 1992). Baskin and

Peters (1992) have shown that thermal maturity parameters, such as T_{max}, are negatively correlated with the S/C ratio of the kerogen. As the S content in OM increases, thermal alteration of OM starts at milder thermal stress. Further evidence is found in a recent study conducted by Siedenberg et al., (2018) on immature kerogen type IIS samples from the Jordanian Ghareb Formation, with T_{max} range (397-423^{o}C) similar to that of our studied samples (402-422^{o}C; Shawar et al., 2018). Siedenberg et al. (2018) showed an increase in $\delta^{34}S$ values of bulk phases with increasing thermal maturation (represented by T_{max} values). This was attributed to the release of ^{34}S depleted H_2S during early thermal decay of organic sulfur and reincorporation of this S to form a catagenetic pyrite which is ^{34}S-enriched relative to the primary pyrite (Siedenberg et al., 2018). Koopmans et al. (1996b and 1998) have shown that ATs are formed/released from kerogen as a result of mild artificial thermal maturation of sulfur-rich rocks. Moreover, Krein and Aizenshtat (1995) and Amrani et al. (2006) showed that ATs were produced after heating of S cross-linked synthetic macromolecules at 160-200 ° C for 48 hr. These parameters are equivalent to Easy R_o of up to 0.32% and T_{max} of 415° C according to a simplified kinetic model (Sweeney and Burnham, 1990; Jarvie et al. 2001). These calculated thermal maturation values represent thermally immature levels (Peters and Cassa, 1994). The fact that laboratory experiments showed that ATs could be formed as a result of mild thermal stress (low calculated T_{max} ~415° C) may substantiate that in S rich immature sedimentary rocks thermochemical processes for ATs formation can start already during the diagenesis stage. This in turn might explain the existence of ATs, generated mainly as a result of mild thermochemical alteration of S cross-linked macromolecules, in immature sedimentary rocks from the Ghareb Formation.

Further support for the mild thermochemical formation of ATs in the Ghareb Formation is provided by the S isotope values of these compounds. When ATs are formed during advanced thermochemical maturation the variation in their $\delta^{34}S$ values decreases to <5‰, as a result of S isotope homogenization (Amrani et al., 2006; Ellis et al., 2017; Rosenberg et al., 2017). However, Shawar et al. (2020) reported large variation, ~15‰, in S isotope composition of individual ATs extracted from the Ghareb Formation. Moreover, they have shown that there is no correlation in the *variation* in $\delta^{34}S$ values of different OSCs and T_{max} (Shawar et al., 2020). These observations are consistent with the studied samples having not experienced *advanced* thermal alterations, hence the thermal alteration needed for AT formation is very mild. Moreover, it seems that these mild thermochemical alterations facilitate AT formation with only minor effects on the original S isotope composition of their precursors (e.g., macromolecular fraction). Therefore, the $\delta^{34}S$ and $\delta^{13}C$ variations of ATs in Fig. 4, Tables 2S and 4S, represents differences in the C and S isotope composition of their original precursors or the kerogen they were generated from, rather than thermal alterations.

The general trend of ^{13}C in specific ATs (aromatic compounds) with the increase of T_{max} of the studied samples provides another insight to this early and mild thermochemical alteration pathway of their formation (Fig. 8). Thermal alteration studies, under laboratory stimulation and natural conditions, have shown that the produced organic compounds become ^{13}C-enriched with increasing thermal alteration, relative to their immature specific fraction (Galimov and Simoneit, 1982; Sofer, 1984; Clayton and Bjorøy, 1994). Detailed studies have reported that at initial stages of thermal alteration the organic compounds become depleted in ^{13}C relative to their immature specific fraction and relative to the

kerogen they were generated from (Hill et al., 2003; Tocque et al., 2005). Different fractions respond differently to the increase in thermal alteration (Galimov, 2006). In addition, it was mentioned that the change in $\delta^{13}C$ values of organic compounds from different fractions/families with thermal maturation is affected by other factors such as the kerogen type and OM origin (Lorant et al., 1998; Hill et al., 2003). As the thermal maturation increases, the $\delta^{13}C$ of the generated compounds (e.g., saturates) homogenize while the $\delta^{13}C$ values of kerogen do not change significantly (Lewan, 1983; Clayton and Bjorøy, 1994; Whiticar, 1996). This may suggest that the difference between $\delta^{13}C$ values of the released compounds and the kerogen should decrease with increasing T_{max}. This phenomenon can be observed in the studied system as presented in Fig. 9 a and b. The difference between $\delta^{13}C$ values of organic compounds and their kerogen normalizes the effect of the OM origin on the $\delta^{13}C$ values of the organic compounds. Thus, the general trend of ^{13}C depletion in saturated and AT fractions with increasing T_{max} results mainly from thermochemical alterations (Fig. 9a and b). This trend is not observed in the BT fraction and this could be attributed to the increased thermal stability of BTs compared to saturates and ATs (Fig. 9c). Thus, diagenetic thermochemical alteration is not enough to homogenize their $\delta^{13}C$ values.

In terms of S isotopes, it has been shown that the thermal decomposition of S cross-linked macromolecular fractions (kerogen, bitumen etc) at early thermal maturation is associated with relatively small S isotope fractionation (Amrani et al., 2005). The released ATs have similar $\delta^{34}S$ relative to the source kerogen (Rosenberg et al., 2017). Therefore, it is likely that the ATs in the Ghareb Formation were generated *mainly* by early thermochemical alteration of S cross linked macromolecules and possibly, also, through aromatization of

alkylthiolanes that formed during early diagenesis (Path II in Fig. 7) (Schmid et al., 1987; Sinninghe Damsté et al., 1987; Payzant et al., 1989; Krein, 1993; Krein and Aizenshtat, 1994; Koopmans et al., 1996b; 1998). Such early thermal maturation can occur as long as the precursors of the ATs contain the appropriate functional groups (e.g., double bonds) and abundant reduced S. Therefore, ATs can be formed in high concentrations even during the diagenesis stage within immature sedimentary rocks.

4.2 The origin and formation pathways of BTs in immature sedimentary rocks

A homologue series of benzothiophenes (BTs), C_0-C_5 BTs, were identified in our core samples from the Shefela Basin (Fig. 5b in Shawar et al., 2020). Moreover, BTs were the most abundant OSC group in the bitumen (Fig. 3c-d; Table. 3S). Unlike ATs, they are not found in recent sediments and rarely in immature rocks (Perakis 1986; Sinninghe Damsté et al., 1989a; Shawar et al., 2020). The origin of these compounds in oils and sedimentary rocks is debated but it is suggested they are formed by thermal alteration processes (Aizenshtat et al., 1995). Indeed, BTs are used as thermal maturation tracers (Radke, 1988; Sinninghe Damsté et al., 1988; Sinninghe Damsté and de Leeuw, 1990; Chakhmakhchev and Suzuki, 1995). Therefore, their high abundance in an immature source rock is not well understood and may suggest that thermochemical processes needed for their formation can occur already in the diagenesis stage. Another possibility is that there are some other, unknown processes that may form BTs with no thermochemical processes (i.e. “direct formation”).

In this section we consider three possible hydrocarbon sources and five possible formation pathways for BTs, as illustrated in Fig. 10 and described below:

I) Isoprenoid quinones (biosynthetic BTs): defunctionalization of this biological molecule will lead to the formation of BT (path I in Fig. 10).

II) Alkylthiophenes can form BTs according to the following pathways:

a) High temperature cyclization and aromatization of saturated alkylthiophenes (Schmid et al., 1987; Eglinton et al., 1990; Sinninghe Damsté et al., 1990) (path IIa in Fig. 10).

b) Cyclization of polyunsaturated ATs under mild thermal stress (Aizenshtat et al., 1995) (path IIb in Fig. 10).

III) Aromatic compounds (such as terrestrial material or aromatic carotenoids; namely isorenieratene) or alkylcyclohexanes (such as non-aromatic carotenoids; namely α-carotene, β-carotene and γ-carotene) can form BTs according to the following pathways:

a) High temperature S incorporation and cyclization of aromatic nonfunctionalized compounds (Pryor, 1962; Przewocki et al., 1984) (path IIIa in Fig. 10).

b) S incorporation into polyunsaturated/ functionalized aromatic compounds or into alkylcyclohexanes and subsequent aromatization under mild thermal stress (path IIIb in Fig. 10).

4.2.1. Benzothiophenes generated mainly from carotenoids and/or terrestrial compounds

The $\delta^{13}C$ values of BTs show a wide variation, up to ~8‰, which suggests multiple sources (Fig. 3e and 3f). One of the possible sources for BT in organic rich sediments is isoprenoid quinones (such as caldariellaquinone) that already have BT in their structure (Path I in Fig. 10). It was reported that there is a range of archaea that biosynthesize isoprenoid quinones

as a constituent of their plasma membrane (De Rosa et al., 1977; Collins and Langworthy, 1983; Nowicka and Kruk, 2010). These compounds participate in electron transfer during chemosynthetic oxidation of S compounds and aerobic respiration (De Rosa et al., 1977). Defunctionalization of isoprenoid quinones (removing the carbonyl groups) and cleavage of their side chain, during diagenesis, yields alkylated benzothiophenes (path I in Fig. 10) (De Rosa et al., 1977; Collins and Langworthy, 1983).

Although this pathway does not require thermal alteration, there are several arguments against this possibility. First, these compounds (S-containing isoprenoid quinones such as caldariellaquinone) occur mainly in archaea that lives in harsh conditions (acidophilic and thermophilic archaea) (Collins and Langworthy, 1983; Lübben, 1995). It was reported that the optimal conditions for their growth is pH of 1-3.5 and/or high temperatures of 70-95° C (Collins and Langworthy, 1983). However, there is no evidence that the studied samples were deposited under such harsh conditions (Almogi-Labin et al., 1993; Eshet et al., 1994; Eshet and Almogi-Labin, 1996; Ashckenazi-Polivoda et al., 2011; Meilijson et al., 2014; 2015). Second, since BTs generated from quinones are biosynthetic, they should contain biogenic S (assimilatory S) with similar $\delta^{34}S$ to that of the marine sulfate at that time, ~ 17-20‰, (Claypool et al., 1980; Amrani, 2014) and thus isotopically heavier than the observed $\delta^{34}S$ values of BTs from the studied section (-11‰ to 7‰; Fig. 3a and 3b). Therefore, the BTs in the Ghareb samples are mostly the result of secondary sulfurization during early diagenesis and there is no support for a significant contribution of biosynthetic BTs in the studied samples.

Other possible precursors for BTs in sediments are lipids that underwent sulfurization to firstly form ATs, and then subsequent cyclization and aromatization to form BTs (Schmid

et al., 1987; Eglinton et al., 1990;) (path IIa in Fig. 10). In specific cases when the AT side chain is unsaturated and there is appropriate space between these double bonds, BTs will form as a result of side chain cyclization, without the need of further aromatization (Schmid et al., 1987; Eglinton et al., 1990) (path IIb in Fig. 10). BTs generated in this pathway should have $\delta^{13}C$ values similar to ATs since cyclization/ aromatization is associated with no or small depletion in ^{13}C (up to 1.2‰) (Sofer, 1984; Freeman, 1991; Simoneit et al., 1993; Freeman et al., 1994). However, $\delta^{13}C$ values of BTs, in general, are heavier than those of the ATs by up to 8‰ at 308m and an average of ~3‰ (Table. 4S). This in turn suggests that cyclization and aromatization of ATs does not seem to be the *major* route for BTs formation in our case. However, it is possible that BTs derived predominantly from specific biological precursors which are different from those leading to ATs. BTs and ATs that are derived from different lipid precursors will have different C isotope signatures. Thus, the possibility of BT formation from ATs (especially unsaturated ATs; path IIb in Fig. 10) cannot be completely excluded.

One of the most likely hydrocarbon source for BTs, in the studied section, seem to be naturally occurring (or biosynthetic) aromatic compounds. In such cases the addition of S into the side chain of the aromatic compounds or alkyl cyclohexane can facilitate cyclization and aromatization to yield BTs (Path IIIa and b in Fig. 10).

Here we propose two possible sources for the aromatic/ alkyl cyclohexane compounds that formed BTs in the Ghareb Formation: lignin (terrestrial source) and carotenoids (marine source). Evidence for terrestrial contribution into the Ghareb Formation samples was found in $\delta^{13}C$ values of isoprenoids and in the existence of some terrestrial biomarkers. The $\delta^{13}C$ values of Pr and Ph show differ by up to 3‰ throughout the studied section (Fig. 1a). This

suggests that they generated from different sources, most likely that there was a terrestrial contribution for Pr (Freeman et al., 1990; Van Kaam-Peters et al., 1998). In addition, the general trend of $\delta^{13}C$ values of normal alkanes increases with the carbon number (Table. 1). This in turn further support a terrestrial contribution into the OM extracted from the studied samples (Murray et al., 1994).

Recent studies suggested that methyltrimethyltridecylchromane (MTTCs), biomarkers that have been identified in our samples (Fig. 1b), might be related to terrigenous or higher plant input (Tulipani et al., 2015). Other terrestrial biomarkers, such as perylene, were also detected in the Ghareb samples (Fig. 1c). However, the perylene concentration is very low, up to 1.2 μg/gr TOC, relative to the BTs concentration of up to 60 μg/gr TOC (Figs. 1c and 4c). This may be because our samples are immature and most of these biomarkers are still linked to kerogen and polar fractions (Grice et al., 2009). These biomarkers are evidence for terrestrial input during the deposition of the Ghareb Formation and therefore the relevance of path IIIb (Fig. 10) for the formation of BTs in our samples. Organic compounds from terrestrial origin are believed to be ^{13}C enriched by ~3-5‰, relative to those from marine origin (Galimov, 2006; Grice et al., 2007). This enrichment is consistent with the enrichment in ^{13}C of BTs, ~3‰ in average, relative to the rest of the organic compounds of the Ghareb samples (Fig. 11; Tables 1 and 4S).

Another possible precursor from which BTs can be generated are aromatic carotenoids. Carotenoids vary in their C isotope composition according to their synthetic pathways (Grice and Brocks, 2011; Holman and Grice, 2018). For example, it was reported that β-isorenieratane and β-carotane (the dominant carotenoid forms; ~-26‰), are ^{13}C-depleted relative to isorenieratene produced by chlorobi (~15‰) (Koopmans et al., 1996a; Peters et

al., 2005). In general, chlorobi-derived carotenoids are ^{13}C-enriched relative to the rest of marine biomass because of their photosynthetic pathway that takes place via the reverse TCA cycle (Quandt et al., 1977; Sirevag et al., 1977; Summons and Powell, 1986; 1987; Grice et al., 1996; Koopmans et al., 1996a; Grice et al., 1997).

Thus, if BTs are generated from aromatic and non-aromatic (chlorobi-derived) carotenoids, they should have heavier $\delta^{13}C$ values relative to the rest of marine-sourced OM. Indeed, the BTs averaged $\delta^{13}C$ values is ~-28‰ while the rest of the OM is ~-33‰ which support this hypothesis (Tables 1 and 4S). Previous studies have shown that carotenoids can diagenetically transform to a wide range of products. Carotenoids transformation processes include cleavage, double bond oxidation, migration, aromatization, sulfurization, and polymerization (Sinninghe Damsté et al., 1993; Van Kaam-Peters and Sinninghe Damsté, 1997; Hebting et al., 2006; French et al., 2015). Sulfurization during early diagenesis could result in the formation of a thiophenic ring in carotenoids (Koopmans et al. 1996a, Van Kaam-Peters and Sinninghe Damsté, 1997). Subsequent cyclization of the unsaturated side chain may lead to BT formation similar to path IIb in Fig. 10. In non-aromatic carotenoids, such as β-carotenes, the formation of a thiophenic ring and subsequent aromatization of the cyclohexane may generate BTs. Subsequent cleavage of C-C bonds of the sulfurized and unsaturated carotenoid is expected to occur relatively easily, (Ishiwatari et al., 1990), and generate alkylated BTs even under low temperatures (path IIIb in Fig. 10).

In summary, since our studied samples of the Ghareb Formation are marine sedimentary rocks, it is logical to assume that the major precursor for BTs formation is marine derive (carotenoids) rather than terrestrial material. The abundance of the appropriate precursor(s)

for BTs formation is an important factor governing their occurrence in immature sedimentary rocks.

4.2.2 Benzothiophene formation pathways

The sulfurization of biosynthetic aromatic and alkyl cyclohexane compounds (e.g. lignin, aromatic and non-aromatic carotenoids) can occur by two different pathways, depending on whether they contain functional groups (path III a and b in Fig. 10).

The first pathway, which requires high temperature, is S reincorporation and cyclization in alkyl aromatic compounds (Pryor, 1962; Przewocki et al., 1984) (path IIIa in Fig. 10). The S, incorporated into nonfunctionalized aromatic compounds, is released from thermal decomposition of macromolecular fractions (Przewocki et al., 1984). It was reported that a major product of this process is S-containing polymers rather than free BTs, and BTs will be obtained after heating to elevated temperatures that facilitate the S incorporation into *nonfunctionalized* aromatic compounds (Toland et al., 1958; Toland, 1961; Pryor, 1962; Przewocki et al., 1984). However, such high temperatures are not characteristic of natural sediments and geochemically plausible reactions must occur at temperature below 200° C (de Roo and Hodgson, 1978). In term of S isotope composition, Shawar et al. (2020) have shown that BTs extracted from the studied samples varied in their $\delta^{34}S$ values by up to 15‰ (Fig. 3a and 3b). This variation is significantly larger than the 2‰ reported in $\delta^{34}S$ values of BTs generated by artificial thermal maturation, of immature rock samples from the same section as the studied samples (Rosenberg et al., 2017). Similarly, BTs in natural oils that originated from the Ghareb Formation (e.g. Tamrur, Emuna) also varied by less than 2‰ (Rosenberg et al., 2017). Thermal alteration of OSCs rapidly homogenizes their

S isotope composition (Armani et al., 2006). Thus, the large S isotope variation in our samples excludes the possibility that the studied samples have exposed to high temperature or to advanced thermal alteration processes (Shawar et al., 2020).

The second pathway we proposed is S reincorporation and cyclization and /or aromatization in functionalized aromatic and alkyl cyclohexane compounds (path IIIb in Fig. 10). The presence of functional groups facilitates S incorporation and thus the temperature needed for BT formation will decrease. Laboratory experiments have shown that BTs could be formed from mild thermal stress, 200° C for one week (or Easy R_o of 0.35% which is equivalent to T_{max} of 417° C), of a thermally labile synthetic S-S cross-linked polymer (Amrani et al., 2006). It is also important to mention that this polymer was *unsaturated* which facilitates cyclization reactions, and thus moderate thermochemical processes during the diagenesis stage, as mentioned previously (*section 4.2)*. The fact that laboratory experiments by Amrani et al. (2006) showed that BTs could form at low calculated T_{max}, ~417° C (similar to T_{max} of the studied samples), might explain the existence of BTs in the immature studied sedimentary rocks from the Ghareb Formation when unsaturated lipids are available. This in turn is consistent with our suggestion that mild thermochemical alteration may occurred during diagenesis in the immature sedimentary rocks from the Ghareb Formation. However, in a similar study we did on one sample from the Ghareb Formation from another depositional Basin, Rotem Quarry, we could not detect BTs in the bitumen. A similar phenomenon was observed in immature kerogen type IIS from the Monterey Formation, Naples Beach (Shawar et al., 2020). This is despite the fact that these samples (Monterey Formation and Rotem Quarry) have comparable geochemical parameters (TOC, TS and T_{max}; Shawar et al., 2020). A possible

explanation is the hydrogen index (HI) in the Ghareb Formation, Shefela Basin (an average of 760 mgHC/gTOC; Shawar et al., 2018) that is lower than in the Rotem Quarry (~836 mgHC/gTOC; Amrani et al., 2005). Thus, the Shefela basin samples are more unsaturated relative to the Rotem Quarry sample, which facilitates early and mild thermochemical alteration (*see section 4.2*). Similarly, the production index (PI) in the Ghareb Formation, Shefela Basin (~0.02; Shawar et al., 2018) is larger than in Rotem Quarry (~0.01) and thus there are more OSCs released from the kerogen to the bitumen of the Shefela Basin samples. This further support the notion that the sedimentary OM from the Shefela Basin, unlike the Rotem Quarry, already started a mild thermochemical alteration. This early thermochemical alteration facilitated the formation of BTs, probably from the cleavage of aromatic lipids or S cross-linked compounds with unsaturated side chains, and their subsequent cyclization and aromatization.

5. Conclusions

In this study we investigated an immature organic and sulfur rich rock from the Ghareb Formation, Shefela Basin, Israel. The novel combination of C and S isotope composition analysis of individual OSCs improved our understanding of OSC formation processes and environmental conditions. The main conclusions from this study are:

1. The consistent enrichment in ^{13}C and ^{34}S in most of the methylated ATs (m/z 111) relative to the non-methylated (m/z 97), may point for different precursors and/or functionality which affect their $\delta^{13}C$ values and their distribution. This might also change their reactivity and thus their sulfurization order and associated $\delta^{34}S$ values.

2. The general trend of depletion in ^{13}C of ATs with the increase in T_{max} values suggest that the ATs formed after a set of diagenetic processes and subsequent thermal decomposition of sulfur cross-linked polymer (kerogen) rather than direct formation. The large variation in $\delta^{34}S$ values of individual ATs (~15‰) suggest that the thermal alteration needed for this process is modest and can occur during diagenesis, and this is in agreement with previous studies based on laboratory experiments.
3. Unusually high concentrations of BTs were found in the immature source rock of the Shefela Basin. Their ^{13}C enriched and variable values are likely to be formed from multiple organic compound precursors that are enriched in ^{13}C. The most significant contributions to BTs in our study are the ^{13}C-enriched carotenoids and terrestrial material that contain an aromatic/ cyclohexane group and unsaturation and/or functional groups in the alkyl chain. The sulfurization of this alkyl chain and subsequent ring closure can form BTs under mild thermal maturation. Therefore, their occurrence in immature sedimentary rocks might point to the beginning of thermochemical alteration during the diagenetic stage.
4. The formation of both ATs and BTs in immature sedimentary rocks results from the combination of i) the availability of the appropriate precursors with unsaturation/ functional groups, ii) the occurrence of di-/poly-sulfides in the sedimentary organic matter, which facilitates the third factor governing the formation of ATs and BTs, iii) early thermal alteration of the sedimentary OM. We suggest that diagenetic (early) thermochemical alterations, needed for formation of ATs and BTs, mainly changes the structural ratios within the OM but not the S

isotope composition, unlike catagenetic thermochemical alterations which homogenize S isotopes between all structures.

5. The distribution of ATs and BTs, and their associated S isotope compositions, have shown a potential to be used as a proxy for *diagenetic* thermochemical alteration. Further studies on immature sedimentary rocks from different depositional condition are needed to develop this proxy and to confirm its validity.

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Table and Figure captions

Table 1 The $\delta^{13}C$ values (‰) of normal alkanes, isoprenoids, hopanes and steranes of the saturated fraction extracted from the Ghareb Formation samples.

Fig.1 Depth profiles for **a)** pristane to phytane ratio (Pr/Ph) and their $\delta^{13}C$ values (‰), **b)** methyltrimethyltridecylchromane ratio (MTTC ratio), **c)** perylene concentration (μg/g TOC) and **d)** isorenieratane concentration (μg/g TOC) in the Ghareb Formation samples.

Fig. **2** Depth profiles for **a and b)** $\delta^{34}S$ values (‰), **c and d)** concentrations (μg/gTOC), and **e and f)** $\delta^{13}C$ values (‰) of isoprenoid and normal alkylthiophenes, respectively, extracted from the Ghareb Formation samples. The x in C_x stands for carbon number of

alkylthiophene, i and n for isoprenoid and normal respectively, T for thiophene, and HT for hopane thiophene. The numbers for the base peak (m/z) of different isomers. Figures **a-d** are modified after Shawar et al. (2020).

Fig. 3 Depth profiles for **a and b)** $\delta^{34}S$ values (‰), **c and d)** concentrations (µg/gTOC), and **e and f)** $\delta^{13}C$ values (‰) of C_1-C_3 and C_4-C_5 benzothiophenic compounds (BT), respectively, extracted from the Ghareb Formation samples. The x in C_x stands for carbon number of the BT's side chain, and the numbers for different isomers. Figures **a-d** are modified after Shawar et al. (2020).

Fig. 4 Cross plot of $\delta^{13}C$ values (‰) and $\delta^{34}S$ values (‰) of **a)** isoprenoid C_{20} thiophenes (iC_{20}thiophene) and normal C_{18} alkylthiophenes (nC_{18} thiophene), **b)** isoprenoid C_{20} thiophenes (iC_{20}thiophene) and normal C_{19} alkylthiophenes (nC_{19} thiophene) extracted from the Ghareb Formation samples. For simplicity only two thiophenes are presented in each sub-figure. For the complete data see Tables 2S and 4S.

Fig. 5 $\delta^{13}C$ values (‰) depth profiles for **a)** C_{27} sterane, methylated iC_{20} thiophene (iC_{20}T-111) and non-methylated iC_{20} thiophene (iC_{20}T-97), **b)** C_{31} hopane, normal alkylthiophenes (methylated C_{19} thiophene (C_{19}T-111), non-methylated C_{20} thiophene (C_{20}T-97) and methylated C_{21} thiophene (C_{21}T-111)) extracted from the Ghareb Formation samples. Blue shaded area represents the range of $\delta^{13}C$ averaged values (‰) for **a)** steranes and **b)** hopanes extracted from the Ghareb Formation samples.

Fig. 6 Cross plot of **a)** $\delta^{13}C$ values (‰) of methylated and non-methylated isoprenoid C_{20} thiophenes ($iC_{20}T$-111 and $iC_{20}T$-97), methylated and non-methylated normal C_{18} thiophenes ($nC_{18}T$-111 and $nC_{18}T$-97) **b)** $\delta^{34}S$ values (‰) of methylated and non-methylated isoprenoid C_{20} thiophenes ($iC_{20}T$-111 and $iC_{20}T$-97), methylated and non-methylated normal C_{18} thiophenes ($nC_{18}T$-111 and $nC_{18}T$-97), methylated and non-methylated C_{35} hopane thiophenes ($C_{35}HT$-111 and $C_{35}HT$-97) extracted from the Ghareb Formation samples.

Fig. 7 Possible formation pathways for alkylthiophene in the sediments.

Fig. 8. Cross plot of T_{max} (□C) versus $\delta^{13}C$ values (‰) of alkylthiophenes extracted from the Ghareb Formation samples. The x in C_x stands for carbon number of the alkylthiophenes (ATs), and the number 111 for the base peak (m/z) of ATs different isomers. The shaded area shows the general trend of depletion in ^{13}C of the ATs with the increase in T_{max}.

Fig. 9 Cross plot of T_{max} (º C) versus the difference in $\delta^{13}C$ values (‰) between **a)** saturated compounds and kerogen, **b)** alkylthiophenes (ATs) and kerogen and **c)** benzothiophenes (BTs) and kerogen from the Ghareb Formation samples. The x in C_x stands for carbon number of the organic compound, and the numbers 111 and 97 for the base peak (m/z) of ATs different isomers. n stands for normal and T for thiophene. The arrows show the decrease in the difference between $\delta^{13}C$ values of the organic compounds from different families and the kerogen with the increase in T_{max}. BTs do not show a similar

trend, and this may show that the BTs are generated at later stages of thermochemical alteration.

Fig. 10 Possible formation pathways for benzothiophenes in the sediment. Path **I** represents diagenetic alteration of isoprenoid quinones, path **IIa** high temperature cyclization and aromatization of saturated alkylthiophenes, path **IIb** cyclization of polyunsaturated alkylthiophenes under mild thermal stress, path **IIIa** high temperature S reincorporation and cyclization of aromatic non- functionalized compounds, path **IIIb** S incorporation into polyunsaturated/ functionalized aromatic compounds (from carotenoids and terrestrial matter) under mild thermal stress.

Fig. 11 Cross plot of $\delta^{13}C$ averaged values (‰) of all the benzothiophenes (BTs) versus averaged $\delta^{13}C$ values (‰) of all the saturated compounds, in blue, and all the alkylthiophenes (ATs), in yellow, extracted from the Ghareb Formation samples.

Supplementary material:

Table 1S The bulk parameters (T_{max} (° C), total organic carbon (TOC (wt%)), total sulfur (TS (wt%)), the S content (wt% of TS) and $\delta^{34}S$ values (‰) of kerogen and pyrite, and $\delta^{13}C$ (‰) of kerogen and bitumen) of the core samples from the Ghareb Formation (Shawar et al., 2018).

Table 2S $\delta^{34}S$ values (‰) of alkyl thiophenic (ATs), and benzothiophenic (BTs) compounds extracted from the Ghareb Formation samples. The compound names of ATs include the ion mass (m/z) in order to distinguish between different isomers. The numbers in BT names represent different isomers and not their ion mass since they have the same

ion mass. The retention time of the compounds is reported from the GC-MC-ICPMS analyses (Shawar et al., 2020).

Table 3S Concentration (µg/gTOC) of alkyl thiophenic (ATs) and benzothiophenic (BTs) compounds extracted from the Ghareb Formation samples. The compound names of ATs include the ion mass (m/z) in order to distinguish between different isomers. The numbers in BT names represent different isomers and not their ion mass since they have the same ion mass. The retention time of the compounds is reported from the GC-MS analyses (Shawar et al., 2020).

Table 4S $\delta^{13}C$ values (‰) of alkyl thiophenic (ATs) and benzothiophenic (BTs) compounds extracted from the Ghareb Formation samples. The compound name of ATs includes the ion mass (m/z) in order to distinguish between different isomers. The numbers in BT names represent different isomers and not their ion mass since they have the same ion mass.

Figure

Fig.1

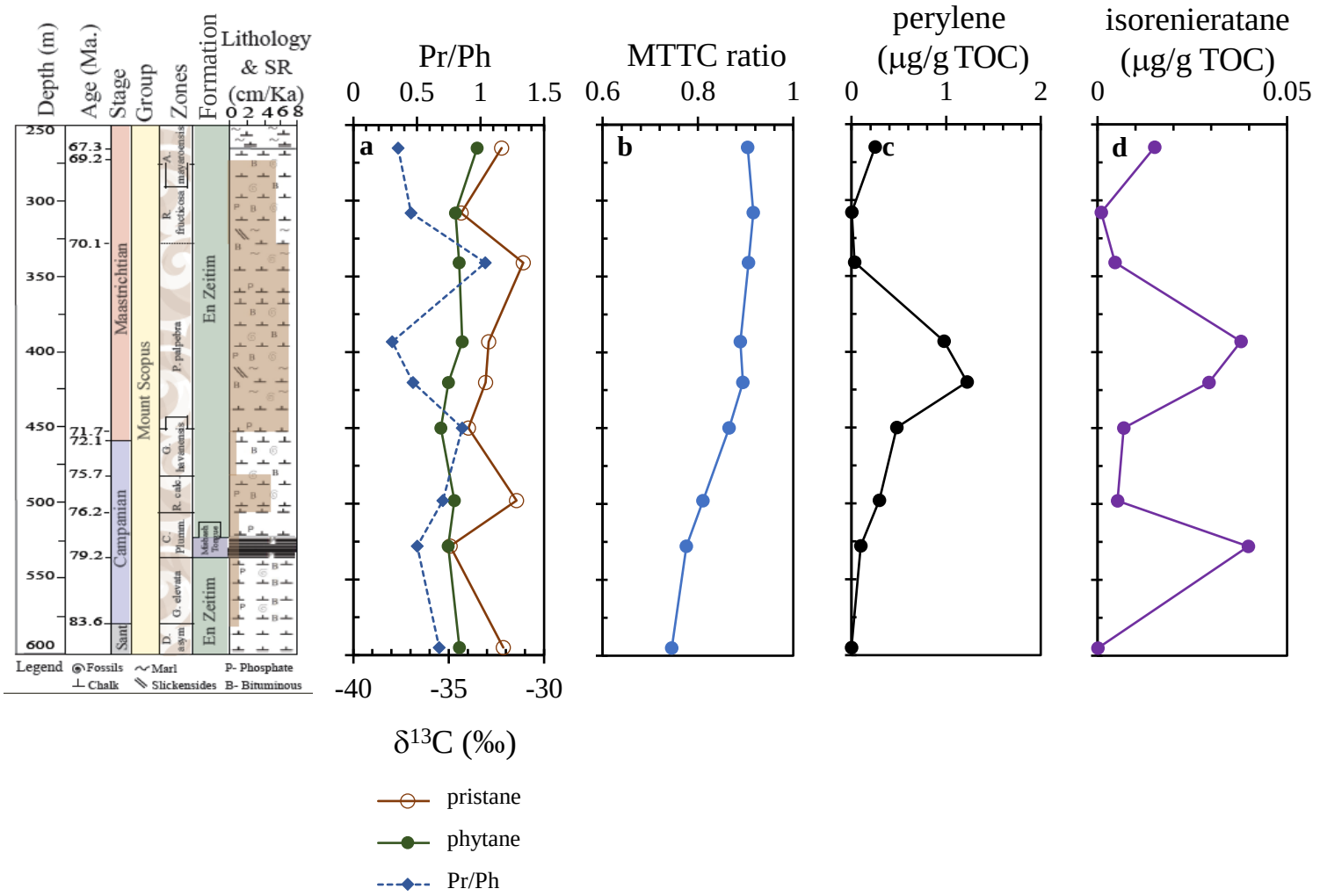

Depth (m)
Age (Ma.)
Stage
Group
Zones
Formation
Lithology & SR (cm/Ka)
0 2 4 6 8
250
300
350
400
450
500
550
600
67.3
69.2
70.1
71.7
72.1
75.7
76.2
79.2
83.6
Maastrichtian
Campanian
Sant
Mount Scopus
En Zeitim
En Zeitim
A. mayaroensis
R. fructicosa
P. palpebra
G. lajvaensis
R. calc.
C. Plumm.
G. elevata
D. asym
Legend
Fossils
Chalk
Marl
Slickensides
P- Phosphate
B- Bituminous
Pr/Ph
0 0.5 1 1.5
a
MTTC ratio
0.6 0.8 1
b
perylene (µg/g TOC)
0 1 2
c
isorenieratane (µg/g TOC)
0 0.05
d
-40 -35 -30
δ13C (‰)
pristane
phytane
Pr/Ph

Fig.2

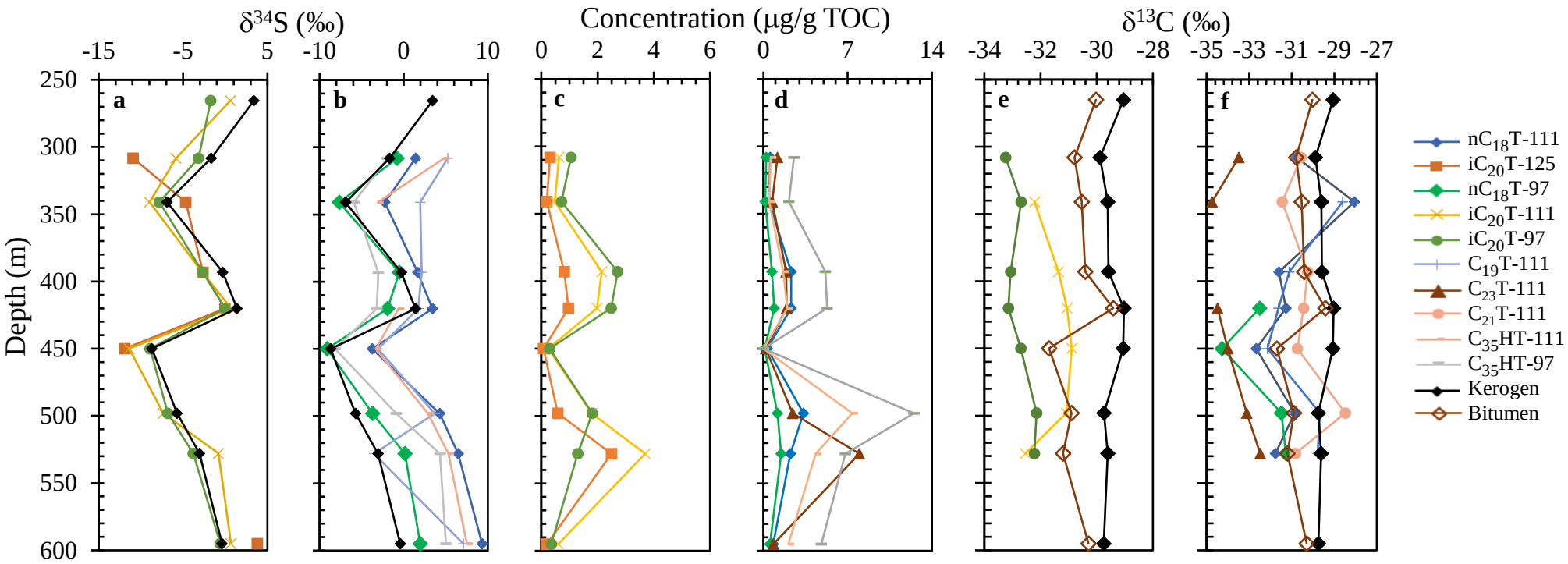

δ34S (‰)
Concentration (μg/g TOC)
δ13C (‰)
-15 -5 5
-10 0 10
0 2 4 6
0 7 14
-34 -32 -30 -28
-35 -33 -31 -29 -27
Depth (m)
250 300 350 400 450 500 550 600
a
b
c
d
e
f
nC18T-111
iC20T-125
nC18T-97
iC20T-111
iC20T-97
C19T-111
C23T-111
C21T-111
C35HT-111
C35HT-97
Kerogen
Bitumen

Fig.3

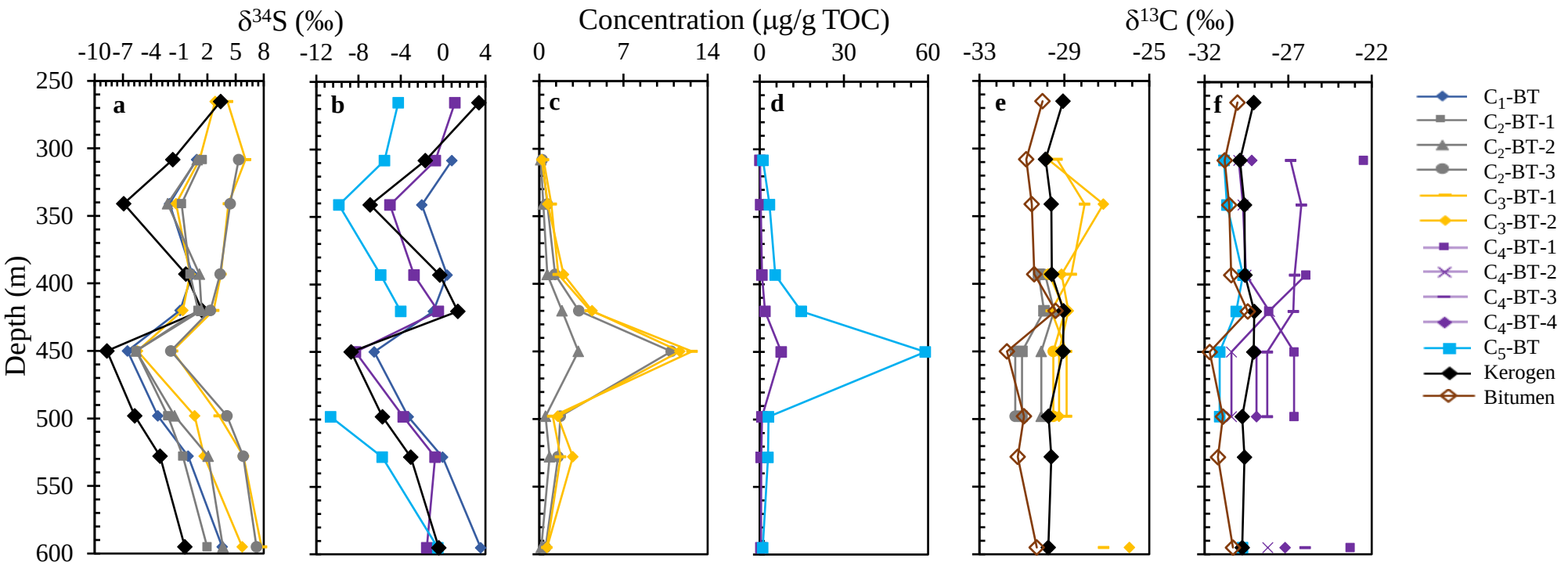

δ34S (‰)
-10 -7 -4 -1 2 5 8
-12 -8 -4 0 4
Concentration (μg/g TOC)
0 7 14
0 30 60
δ13C (‰)
-33 -29 -25
-32 -27 -22
Depth (m)
250 300 350 400 450 500 550 600
a
b
c
d
e
f
C1-BT
C2-BT-1
C2-BT-2
C2-BT-3
C3-BT-1
C3-BT-2
C4-BT-1
C4-BT-2
C4-BT-3
C4-BT-4
C5-BT
Kerogen
Bitumen

Fig.4

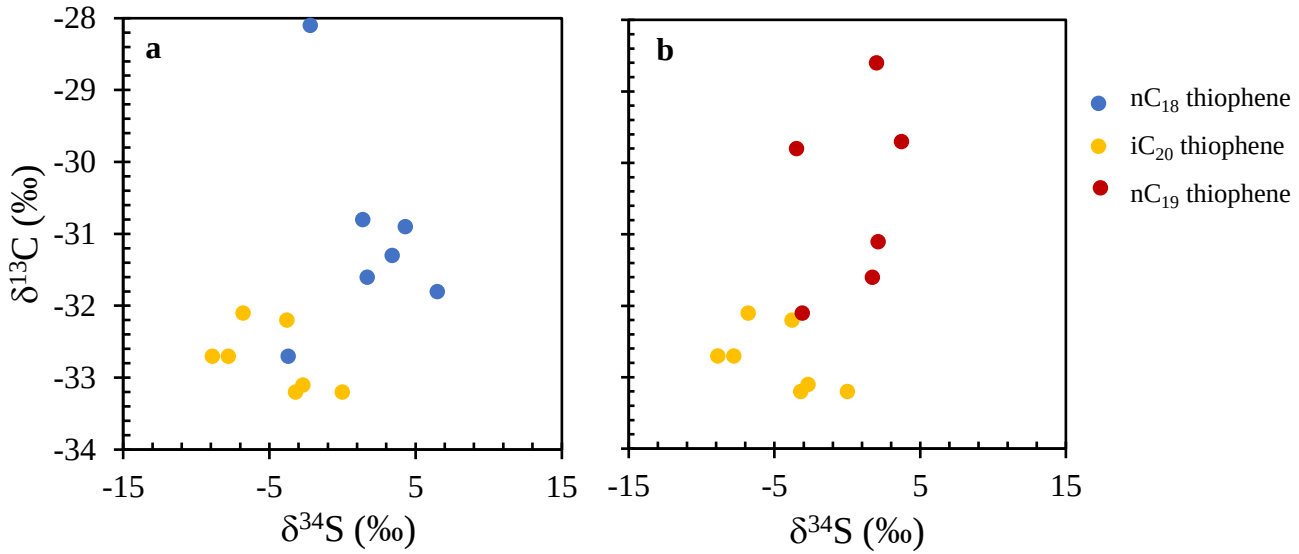

a
b
-28
-29
-30
-31
-32
-33
-34
δ13C (‰)
-15
-5
5
15
δ34S (‰)
nC18 thiophene
iC20 thiophene
nC19 thiophene

Fig.5

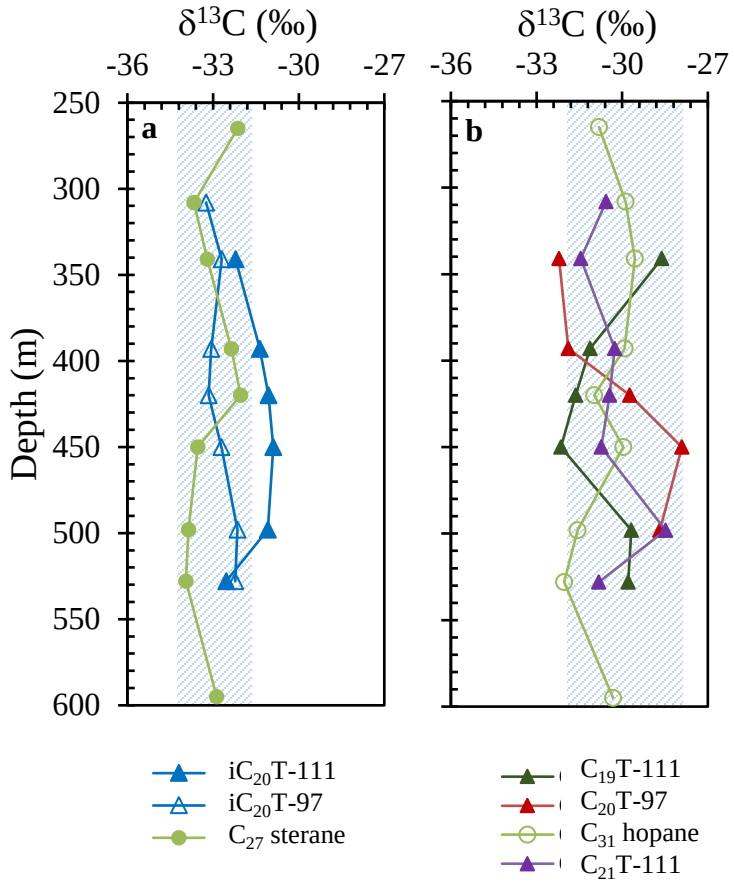

δ13C (‰)
-36
-33
-30
-27
δ13C (‰)
-36
-33
-30
-27
250
300
350
400
450
500
550
600
Depth (m)
a
b
iC20T-111
iC20T-97
C27 sterane
C19T-111
C20T-97
C31 hopane
C21T-111

Fig.6

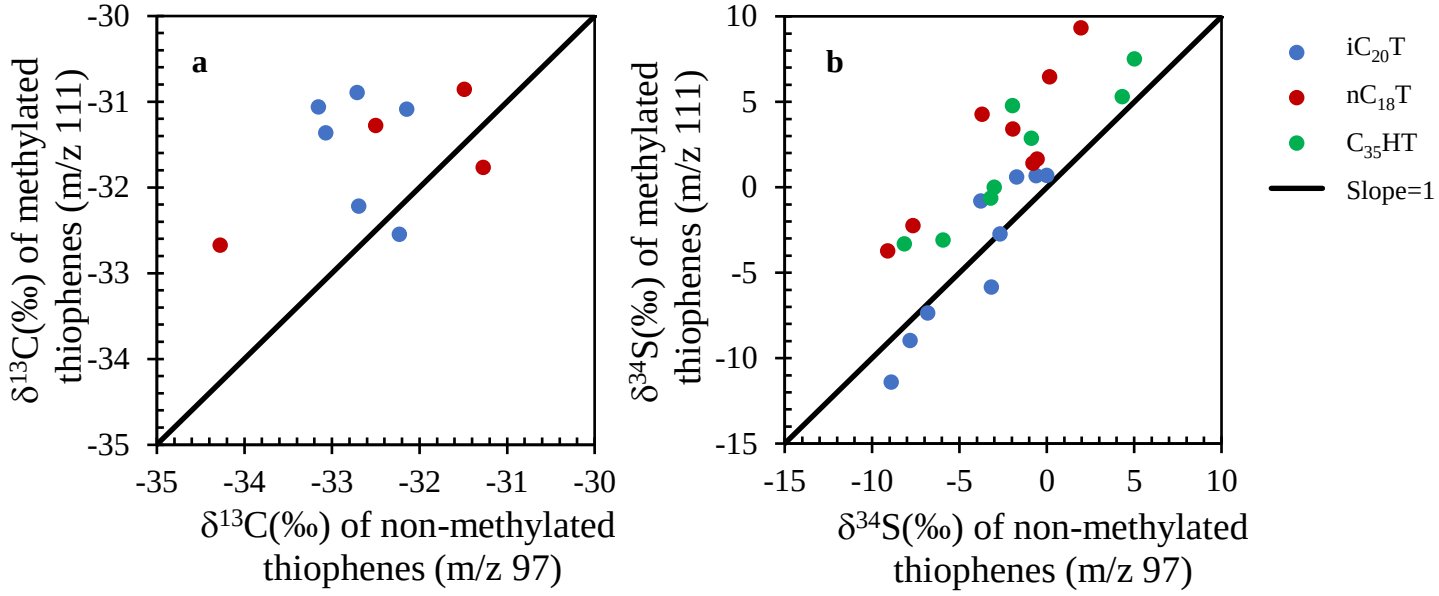

a
b
δ13C(‰) of methylated thiophenes (m/z 111)
δ13C(‰) of non-methylated thiophenes (m/z 97)
δ34S(‰) of methylated thiophenes (m/z 111)
δ34S(‰) of non-methylated thiophenes (m/z 97)
-30
-31
-32
-33
-34
-35
10
5
0
-5
-10
-15
iC20T
nC18T
C35HT
Slope=1

Fig.7

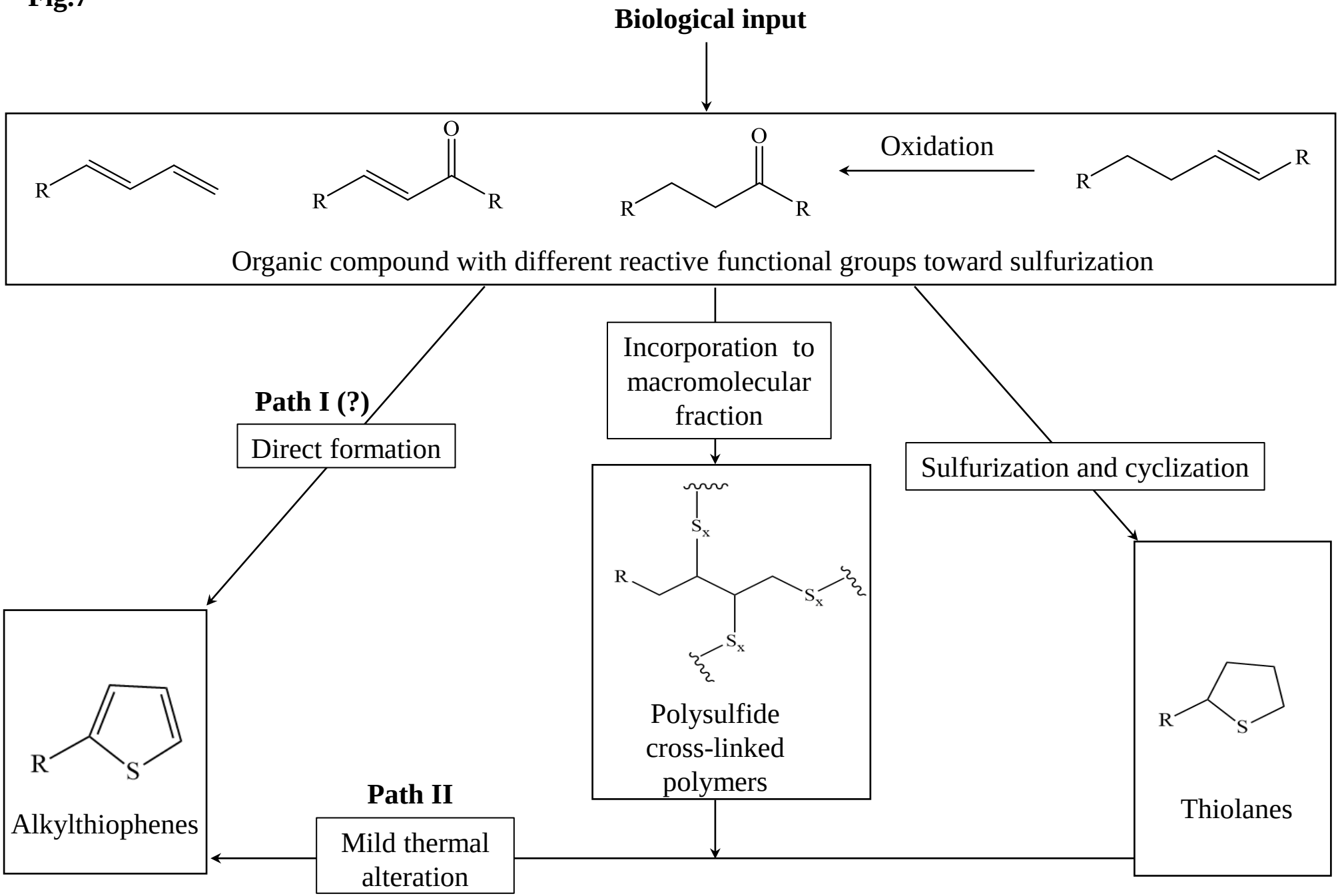

Biological input
Oxidation
Organic compound with different reactive functional groups toward sulfurization
Path I (?)
Direct formation
Incorporation to macromolecular fraction
Sulfurization and cyclization
Alkylthiophenes
Polysulfide cross-linked polymers
Thiolanes
Path II
Mild thermal alteration

Fig.8

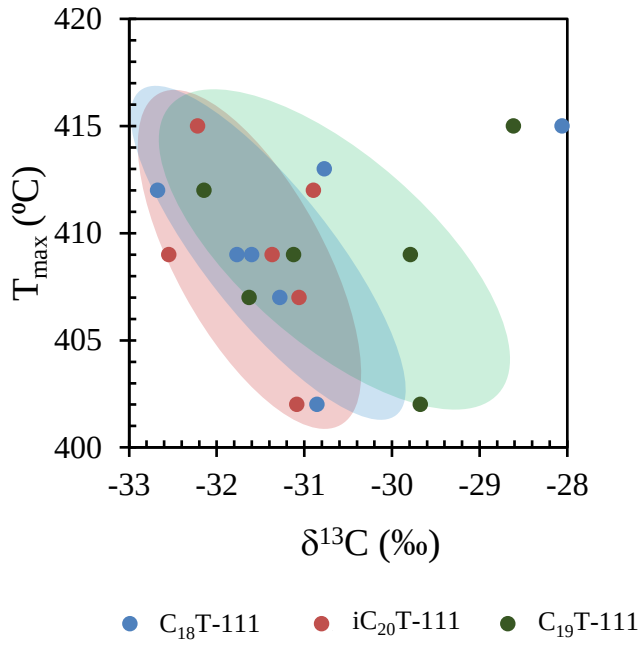

420
415
410
405
400
T_{max} (ºC)
-33
-32
-31
-30
-29
-28
$\delta^{13}C$ (‰)
C_{18}T-111
iC_{20}T-111
C_{19}T-111

Fig.9

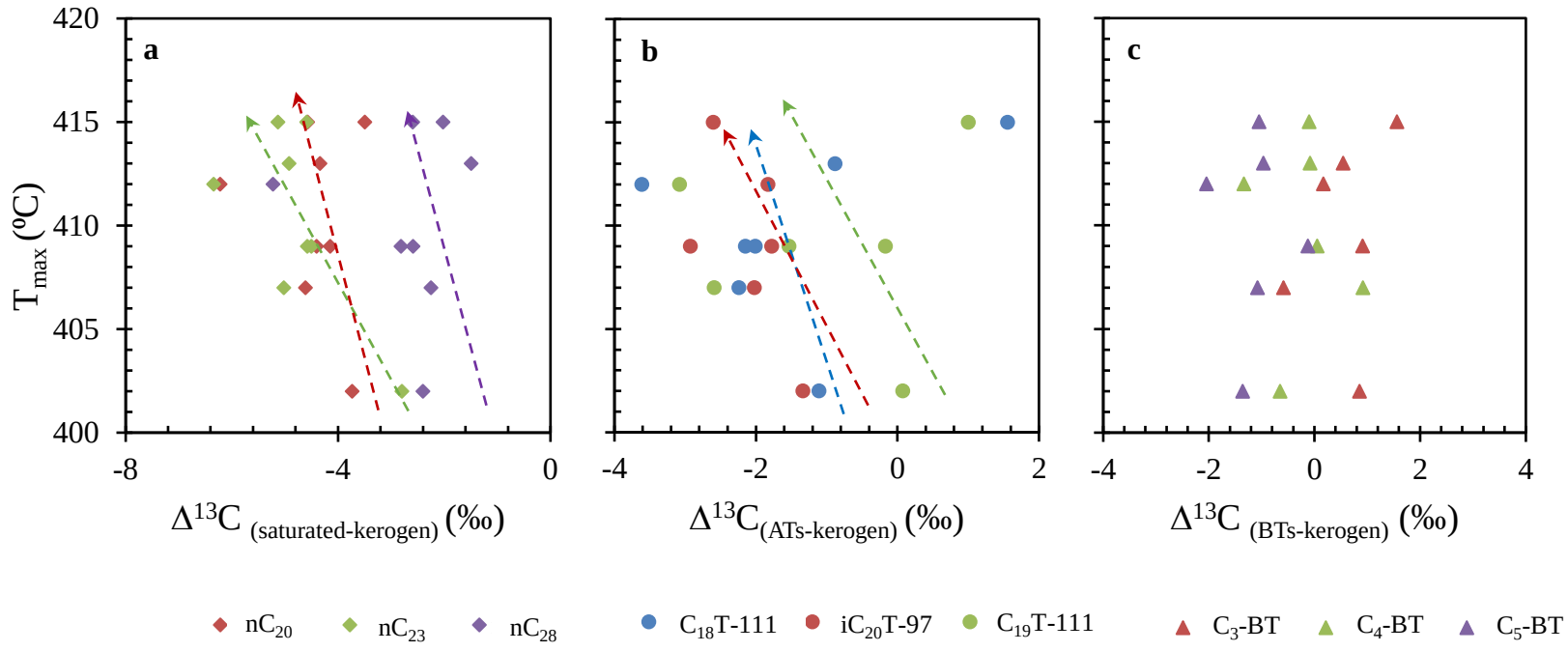

a
b
c
420
415
410
405
400
T_{max} (ºC)
-8
-4
0
-4
-2
0
2
-4
-2
0
2
4
$\Delta^{13}C_{(saturated\text{-}kerogen)}$ (‰)
$\Delta^{13}C_{(ATs\text{-}kerogen)}$ (‰)
$\Delta^{13}C_{(BTs\text{-}kerogen)}$ (‰)
nC_{20}
nC_{23}
nC_{28}
$C_{18}T$-111
$iC_{20}T$-97
$C_{19}T$-111
C_3-BT
C_4-BT
C_5-BT

Fig.10

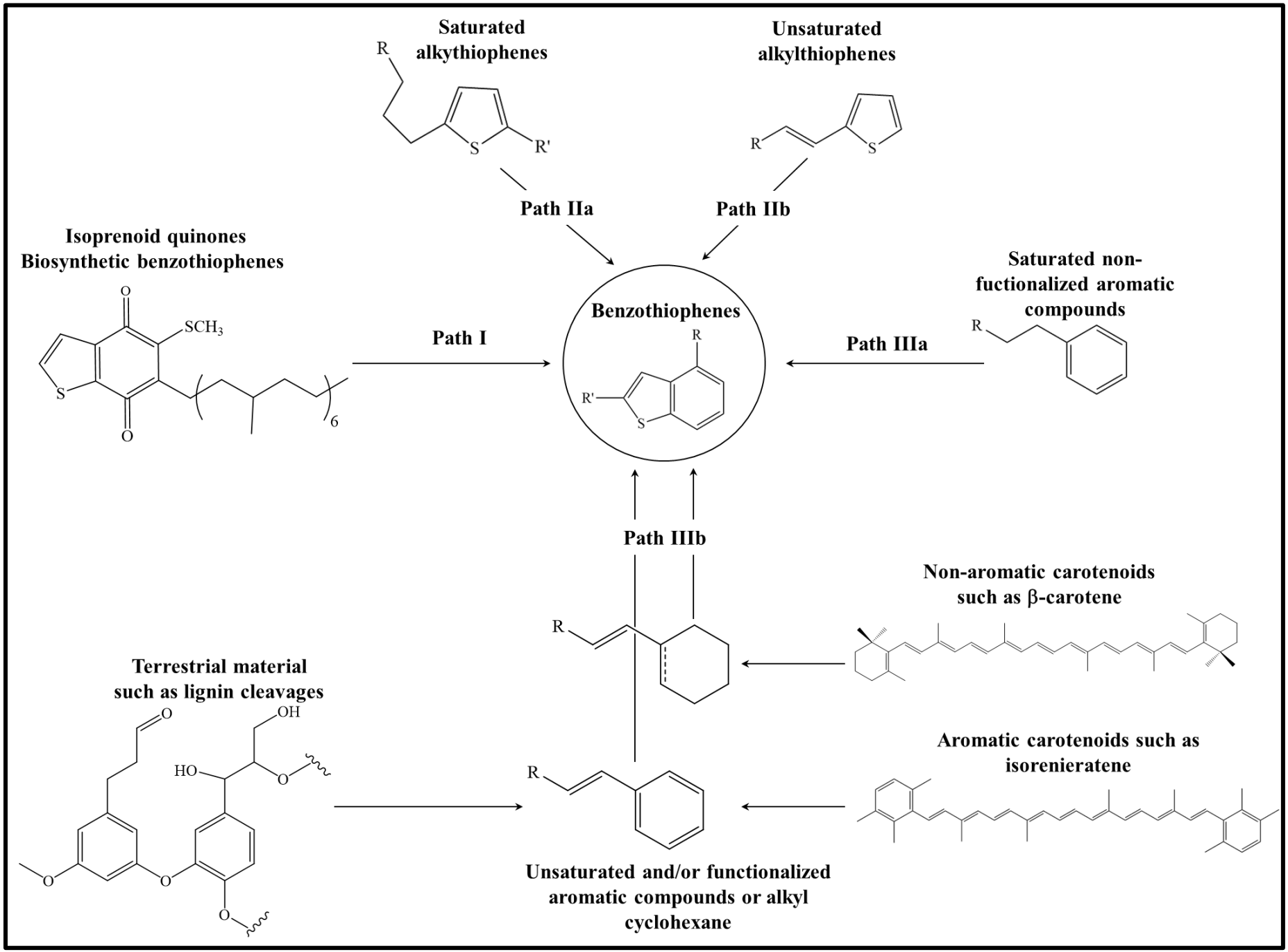
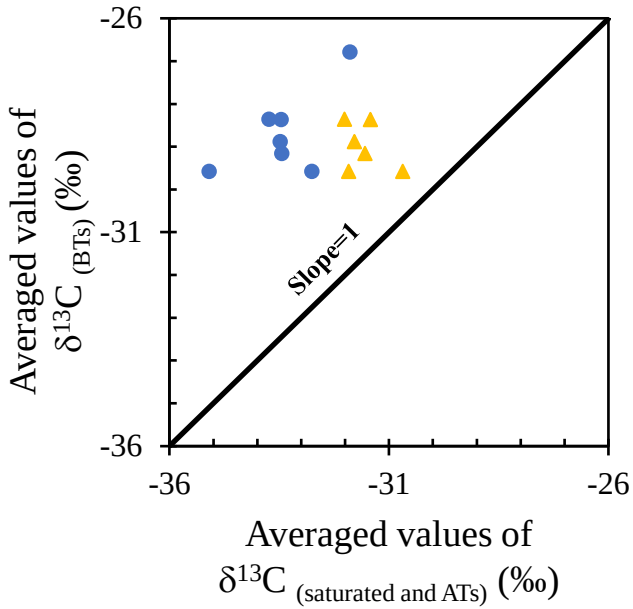

Saturated alkythiophenes
Unsaturated alkylthiophenes
Path IIa
Path IIb
Isoprenoid quinones Biosynthetic benzothiophenes
Benzothiophenes
Saturated non-fuctionalized aromatic compounds
Path I
Path IIIa
Path IIIb
Non-aromatic carotenoids such as β-carotene
Terrestrial material such as lignin cleavages
Aromatic carotenoids such as isorenieratene
Unsaturated and/or functionalized aromatic compounds or alkyl cyclohexane

Fig.11


-26
-31
-36
-36
-31
-26
Slope=1
Averaged values of
δ13C (BTs) (‰)
Averaged values of
δ13C (saturated and ATs) (‰)

Table

Table 1: The $\delta^{13}C$ values (‰) of normal alkanes, isoprenoids, hopanes and steranes of the saturated fraction extracted from the Ghareb Formation samples.

	Depth (m)								
Compound name	265	308	341	393	420	450	498	528	595
Normal alkanes									
[a]nC_{13}	-	-	-	-34.3±0.1	-34.7±0.1	-	-	-	-
nC_{14}	-	-30.0±0.1	-	-33.7±0.1	-34.1±0.1	-34.8±0.1	-32.3±0.2	-30.9±0.0	-
nC_{15}	-30.1±0.1	-34.8±0.1	-35.1±0.1	-34.2±0.1	-34.5±0.1	-36.3±0.0	-32.8±0.0	-34.8±0.1	-32.4±0.2
nC_{16}	-32.5±0.1	-34.1±0.1	-34.4±0.1	-33.8±0.0	-34.5±0.1	-36.2±0.1	-33.4±0.1	-34.5±0.2	-32.1±0.2
nC_{17}	-33.2±0.2	-34.4±0.1	-34.9±0.2	-34.5±0.1	-35.1±0.1	-36.6±0.1	-33.8±0.1	-35.2±0.0	-32.5±0.1
nC_{18}	-32.5±0.2	-34.4±0.1	-34.6±0.0	-33.7±0.0	-34.4±0.1	-35.9±0.1	-34.3±0.0	-34.6±0.1	-32.6±0.1
nC_{19}	-32.9±0.3	-34.5±0.1	-34.2±0.2	-34.0±0.1	-34.2±0.1	-35.7±0.1	-33±0.2	-34.1±0.1	-32.4±0.0
nC_{20}	-32.6±0.1	-34.2±0.1	-34.2±0.0	-33.7±0.2	-33.6±0.0	-35.3±0.1	-33.5±0.1	-34.0±0.0	-32.9±0.2
nC_{21}	-33.6±0.1	-35.2±0.1	-34.8±0.2	-34.5±0.2	-34.1±0.1	-35.7±0.1	-33.9±0.2	-34.1±0.1	-32.2±0.0
nC_{22}	-32.9±0.2	-34.7±0.1	-34.1±0.1	-34.4±0.0	-33.8±0.1	-35.3±0.1	-33.2±0.1	-34.3±0.1	-31.8±0.1
nC_{23}	-33.6±0.1	-34.8±0.1	-34.7±0.0	-34.2±0.0	-34.1±0.0	-35.4±0.0	-32.5±0.1	-34.1±0.1	-31.9±0.0
nC_{24}	-32.4±0.1	-34.0±0.1	-33.8±0.2	-33.8±0.1	-33.7±0.1	-35.5±0.0	-32.7±0.1	-33.5±0.1	-31.4±0.1
nC_{25}	-32.1±0.1	-32.6±0.1	-32.6±0.2	-32.0±0.1	-32.3±0.0	-34.0±0.1	-31.1±0.1	-	-31.5±0.1
nC_{26}	-31.8±0.1	-32.0±0.1	-31.9±0.1	-32.2±0.0	-32.9±0.1	-34.9±0.1	-31.9±0.2	-31.9±0.1	-30.4±0.1
nC_{27}	-32.4±0.2	-32.3±0.1	-32.4±0.1	-31.8±0.1	-32.7±0.1	-34.5±0.1	-31.8±0.0	-32±0.1	-30.8±0.0
nC_{28}	-31.7±0.2	-31.4±0.2	-31.6±0.1	-32.4±0.1	-31.3±0.3	-34.3±0.1	-32.1±0.0	-32.2±0.1	-31.2±0.3
nC_{29}	-31.8±0.2	-32.4±0.2	-32.5±0.1	-33.1±0.1	-32.1±0.0	-34.1±0.1	-31.2±0.1	-30.6±0.2	-30.3±0.1
Isoprenoids									
pristane	-32.2±0.2	-34.3±0.2	-31.1±0.1	-32.9±0.2	-33.1±0.1	-34.0±0.2	-31.4±0.1	-34.9±0.1	-32.1±0.1
phytane	-33.5±0.1	-34.6±0.1	-34.4±0.0	-34.3±0.0	-35.0±0.1	-35.4±0.1	-34.7±0.1	-35.0±0.1	-34.4±0.1
Hopanes									
17α-trisnorhopane	-31.1±0.2	-	-	-	-	-	-30.9±0.1	-	-28.8±0.1
17α-norhopane	-30.0±0.2	-	-	-	-	-	-30.4±0.2	-	-30.1±0.0
[b]C_{31} hopane-1	-30.8±0.4	-29.9±0.1	-29.6±0.2	-29.9±0.3	-31.0±0.3	-30.0±0.2	-31.6±0.2	-32.0±0.3	-30.3±0.1
C_{31} hopane-2	-28.6±0.3	-28.4±0.2	-28.2±0.2	-27.8±0.1	-27.9±0.3	-27.5±0.1	-28.9±0.1	-	-27.8±0.2
C_{31} hopane-3	-	-	-	-	-	-	-	-	-30.7±0.1
Steranes									
C_{27} sterane-1	-32.2±0.2	-33.2±0.1	-32.6±0.2	-31.8±0.2	-31.8±0.2	-33.4±0.2	-32.3±0.1	-33.6±0.2	-31.1±0.1
C_{27} sterane-2	-32.1±0.1	-33.7±0.2	-33.2±0.1	-32.4±0.2	-32.0±0.2	-33.5±0.1	-33.9±0.1	-34.0±0.1	-32.9±0.2
C_{28} sterane-1	-31.1±0.3	-	-	-	-	-	-	-	-
C_{28} sterane-2	-32.4±0.2	-33.9±0.2	-33.9±0.3	-33.2±0.2	-30.5±0.3	-34.6±0.1	-27.2±0.1	-32.3±0.1	-
C_{29} sterane	-29.7±0.1	-31.8±0.2	-32.8±0.2	-32.1±0.1	-32.7±0.3	-33.7±0.1	-	-	-

[a]n= normal.
[b]T_m stands for C_{27} 17α- hopane.
[c]The numbers in hopanes and steranes names represent different isomers.