

Petroleum Sulfur Biomarkers Analyzed by Comprehensive Two-Dimensional Gas Chromatography Sulfur-Specific Detection and Mass Spectrometry

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ABSTRACT: An atypical distribution of steroids occurred in oilsand and oil samples in the Jinxian Sag, Bohai Bay Basin, northern China. It is featured by usually abundant C₂₁–C₂₆ short-chain steranes that are derived from the cleavage of the weak C–S bonds at the C-17 and C-20 positions. Sulfur-containing petroleum biomarkers were further analyzed in these sulfur-rich heavy oils by comprehensive two-dimensional gas chromatography (GC × GC) using sulfur-specific detection to locate and confirm sulfur species and GC × GC time-of-flight mass spectrometry (TOF MS) to identify previously unknown sulfur-containing biomarkers. Preliminary GC–MS analysis revealed a series of compounds yielding characteristic fragment ions consistent with thiophenic substructures. For confirmation, GC × GC–sulfur chemiluminescence detection (SCD) was used to confirm the sulfur moiety. The molecular structures of sulfur biomarkers were proposed as unsaturated isoprenoid thiophenes based on fragmentation patterns observed in their 70 eV electron-impact ionization (EI) mass spectra. There were constant neutral losses of 71 and 111 Da from the molecular ions in the 70 eV EI mass spectra throughout a whole series. The endeavor of sulfur-containing biomarker characterization may provide some clues of paleotransformation of biological precursors and geological products in oil/kerosen under thermal stress during burial and resolve some missing links between the biosphere and the geosphere.

INTRODUCTION

When exploratory wells of Zhao-7 and Zhao-9 were drilled in Jinxian Sag of Bohai Bay Basin, northern China (see the map in Figure 1), extremely high concentrations (as high as 92%) of hydrogen sulfide in the reservoir gas and sulfur contents as high as 14.7% in the associated crude oils were discovered.^{1,2} Limited amounts of the oil samples were collected before the wells were sealed for safety concerns.

In a previous characterization of the Zhao-7 and Zhao-9 oils by routine gas chromatography–mass spectrometry (GC–MS) analysis, a series of short-chain steroids and their regular carbon numbered analogues, including lanostanes (C_{24–25} and C₃₀), regular steranes (C_{27–30}), 4-methyl steranes (C_{22–23} and C_{27–30}), 4,4-dimethyl steranes (C_{22–24} and C_{28–30}), short-chain steranes (C_{23–26}), abundant pregnanes (C_{21–22}), and androstanes (C_{19–20}), together with S-containing steroids (20-thienylpregnanes and thienylandrostanes) were detected and identified.¹ In addition, C_{22–27} monounsaturated sterenes were also detected in relatively high concentrations in an immature oilsand sample in the vicinity of this region.² The prevalence of these short-chain steroid analogues, i.e., C₂₁–C₂₆ > C₂₇–C₃₀, in oil-related samples of this region was believed to relate to the natural sulfurization process, which played a significant role in the formation of these short-chain steroids. High concentrations of benzothiophenes, dibenzothiophenes, and benzonaphthothiophenes are dominant in the aromatic fractions.^{1–5} Sulfur-containing steroids were also found in these sulfur-rich heavy

Table 1. List of Molecular and Characteristic Fragment Ions of the Alkenylthiophene Series

peak number	(M – 111) ⁺	(M – 71) ⁺	M ⁺	molecular formula	R =
<i>a</i>	97	137	208	C ₁₃ H ₂₀ S	<i>b</i>
1	111	151	222	C ₁₄ H ₂₂ S	H
2	125	165	236	C ₁₅ H ₂₄ S	CH ₃
3	139	179	250	C ₁₆ H ₂₆ S	C ₂ H ₅
4	153	193	264	C ₁₇ H ₂₈ S	C ₃ H ₇
5	167	207	278	C ₁₈ H ₃₀ S	C ₄ H ₉
6	181	221	292	C ₁₉ H ₃₂ S	C ₅ H ₁₁
7	195	235	306	C ₂₀ H ₃₄ S	C ₆ H ₁₃
<i>a</i>	209	249	320	C ₂₁ H ₃₆ S	C ₇ H ₁₅

^aFound in GC × GC MS, with no spectrum shown. ^bNo methyl group on the thiophene ring as others shown in structure A.

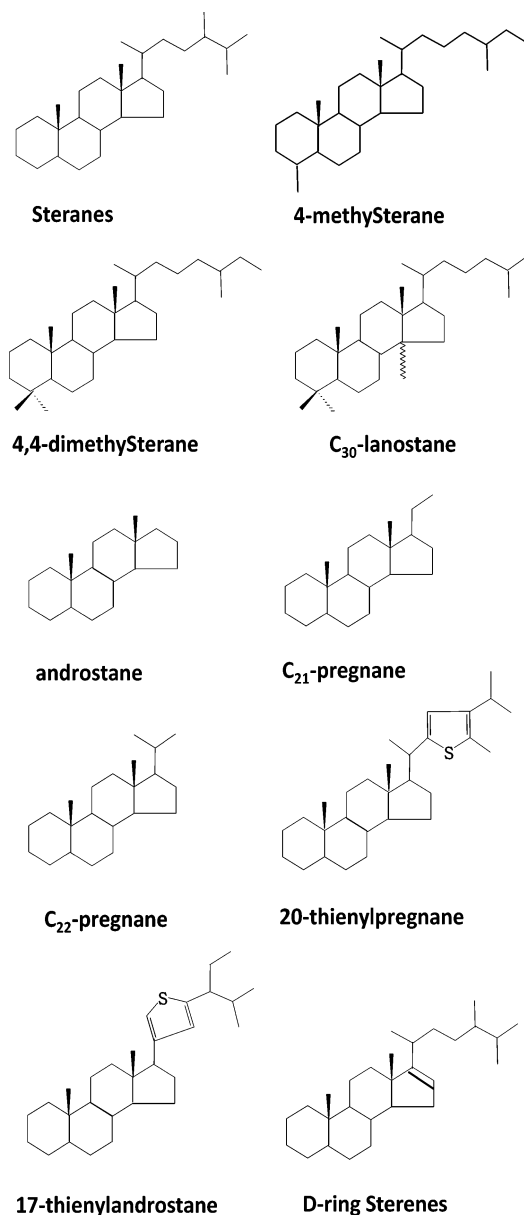
oils.⁴ Cleavage of weak C–S bonds at the C-17 and C-20 positions of the sulfurized steroids and lanostane sulfides in the reservoir microbial biodegradation process was therefore proposed to explain the formation of the short-chain steroids.¹ Apparently, more

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discoveries specific for sulfur-containing steroids or isoprenoids were still needed.



Although sulfur is essential to life, being a constituent of proteins, enzymes, and cofactors, the accumulated amount of sulfur in the living systems converted during sedimentary burial cannot account for the abundance of sulfur compounds in the petroleum systems. Therefore, the sulfur organics in petroleum are apparently derived from inorganic sulfur incorporation with decayed bioorganic molecules.⁵ The identification of sulfur compounds could provide some clues of paleo-transformation of bioorganics into geoorganics in oil or kerogen.

Preliminary GC–MS analysis of the Jinxian Sag samples using a quadrupole mass spectrometer indicated the possible presence of previously unknown thiophenic compounds. However, the low-resolution 70 eV electron-impact ionization (EI) mass spectra of thiophenic compounds are similar to those of aromatic hydrocarbons. Without the use of high-resolution mass spectrometry, a sulfur-specific detector can not only confirm the sulfur moiety in the molecules but also provide the graphic distribution of sulfur components.

Comprehensive two-dimensional gas chromatography (GC × GC) provides an additional dimension to separate overlapping components in the one-dimensional GC analysis, thus providing

cleaner mass spectra for compound identification.⁶ With the aid of sulfur-specific detection, identification can be focused on the species that contain sulfur.⁷

The Jinxian Sag oils contain excessive amounts of sulfur compounds provide us with a rare opportunity to look into novel sulfur-containing species that can be potential biomarkers in sufficient concentrations for mass spectral analysis. In this study, GC × GC using sulfur-specific chemoluminescence detection (SCD) together with GC × GC time-of-flight (TOF) MS was used to obtain the distribution and identification of previously unknown sulfur-containing compounds.

EXPERIMENTAL SECTION

Material. The sulfur-rich oils were obtained from Zhao-7 (2280 m, Es₄–Ek₁) and Zhao-9 (2360.6–2390.0 m, Es₄–Ek₁) wells of Jinxian Sag in Bohai Bay Basin of northern China.¹ The sulfur content of the oils in the region can be as high as 14.7%, with an associated gas containing H₂S as high as 92%. The oil is derived from Eocene formation as carbonates and evaporitic deposits in a hypersaline lacustrine environment.

The crude oil sample was subjected to saturates, aromatics, resin, and asphaltene (SARA) fractionation by the Chinese Standard Analytical Method for Petroleum and Natural Gas Industry: SY/T 5119-2008.⁸ The saturate fraction was collected for current analysis.

Instrumentation. GC × GC (Leco Corp.) equipped with a flame ionization detector (FID) and an Agilent SCD was used. The comparison of SCD and FID traces confirms and locates sulfur-containing compounds within the complex overall compound distribution to aid the GC × GC MS identification focused on sulfur species. Leco Pegasus 4D GC × GC TOF MS was used for identifying the sulfur compounds. Both instruments had split/splitless injectors and cryogenic modulators (liquid N₂). The first dimension GC column was a nonpolar 100% dimethyl polysiloxane column (BP-1, 60 m length, 0.25 mm inner diameter, and 0.25 μm film thickness, SGE, Inc.). The retention time of the component on this column is linked to its boiling point. The second dimension was a moderately polar 50:50 methyl/phenyl silicone column (BPX50, 1.4 m length, 0.25 mm inner diameter, and 0.1 μm film thickness, SGE, Inc.) to separate components based on polarity or aromaticity. The GC oven for the first-dimension column was held at 40 °C for 0.5 min, then programmed at 1 °C/min to 340 °C, and maintained at 340 °C for 20 min. The temperature offsets for the secondary oven and the modulator were 10 and 15 °C, respectively, higher than the first-dimension column oven. The modulation time was 12 s, and the hot pulse time was 3 s. In the GC × GC TOF MS experiment, a 70 eV electron beam was used for EI with a scan range from *m/z* 40 to 550 during modulation. The GC × GC MS data were processed with Leco's ChromaTOF (version 4.45).

RESULTS AND DISCUSSION

Preliminary GC–MS analyses of Zhao-7 and Zhao-9 saturate fractions show similarity between the two oils. Hence, only the Zhao-7 saturate fraction was selected for the GC × GC analysis. A FID was used for determining the overall compound distribution of the sample, as shown in Figure 2a. The GC × GC SCD contour plot, shown in Figure 2b, is compared to the GC × GC FID contour plot to locate (sort out) sulfur compounds among all compounds shown in Figure 2a.

The GC × GC TOF MS reconstructed ion chromatogram (RIC) is shown in Figure 3, which is equivalent to the FID contour plot. On the basis of the GC × GC SCD results, we divided the sulfur distribution into two regions. Figure 4 is an expansion of region 1 in Figure 3. The locations of sulfur components in Figure 4 were determined by comparing FID and SCD results. A peak number is given for each identifiable sulfur component, with the corresponding 70 eV EI mass spectra displayed in Figure 5.

The mass spectra of peaks 1–7 exhibit consistent fragmentation patterns. In addition to the presence of the molecular ion, there are significant (*M* – 111)⁺ and (*M* – 71)⁺ ions in these spectra. The mass units of molecular ions indicate a homologous

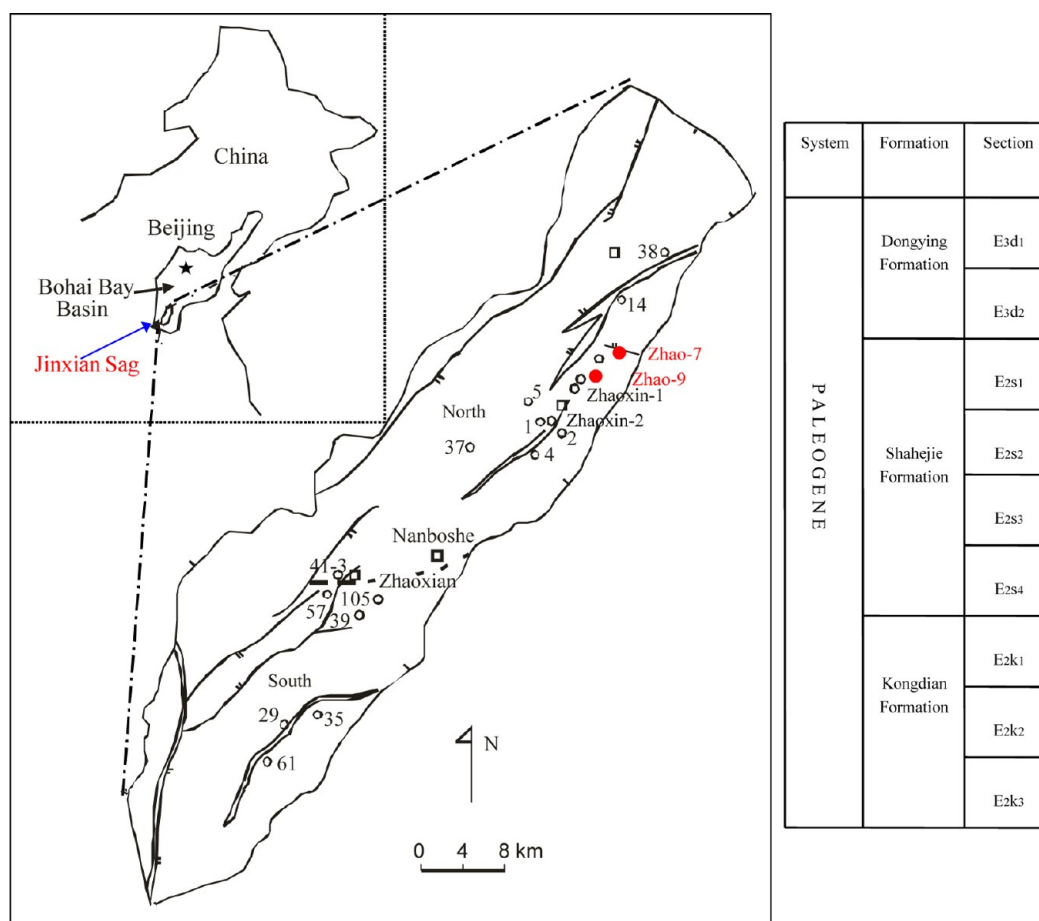


Figure 1. Locations of Zhao-7 and Zhao-9 wells in Jinxian Sag of Bohai Bay Basin, northern China, with some geologic setting information.

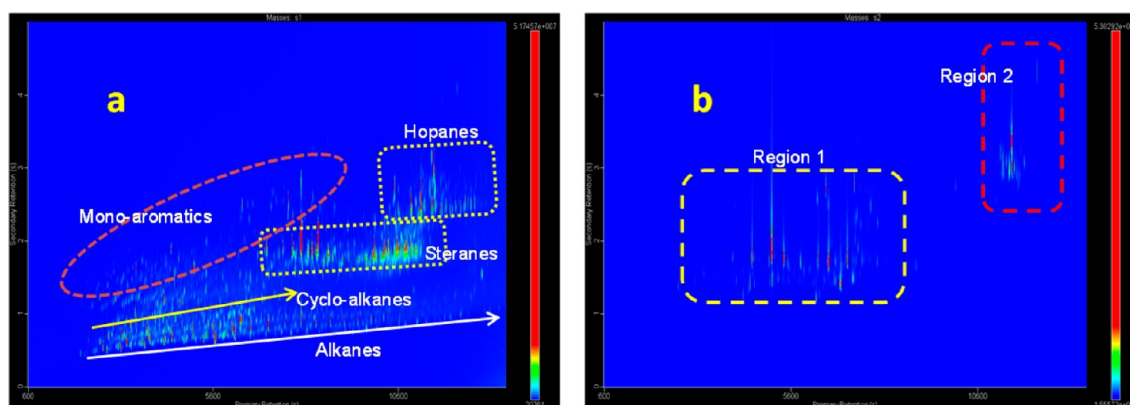
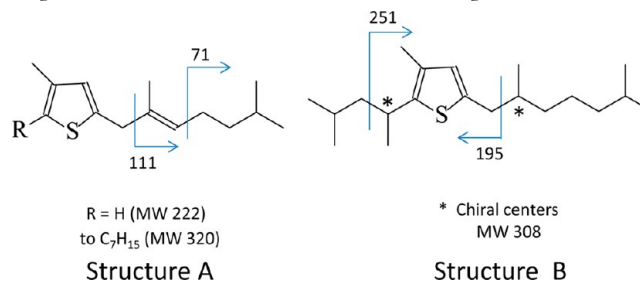


Figure 2. (a) GC \times GC FID and (b) GC \times GC SCD contour plots of the Zhao-7 oil saturate fraction.

series of compounds with increasing carbon numbers. The major ions of peaks 1–7 are listed in Table 1, including two additional components of molecular weights at 208 and 320 Da, which were also found in low abundances (with no spectra shown). The fragment ions of the m/z 97 series (97, 111, ..., 209) indicate a methylthiophene core in each of the molecules, with consecutive addition of a methylene group (i.e., a homologous series). The loss of 111 mass units is equivalent to a loss of a C_8H_{15} group from the molecular ion, accompanied by a loss of 71 mass units of a C_5H_{11} group from the molecular ion. These two fragmentation pathways are unlikely from a high strain three-membered ring structure in the side chain or a cyclopentano ring condensed to

the thiophene ring. Hence, it is determined the 111 Da loss is due to an octenyl loss, with a postulated structure derived from the isoprenoid skeleton shown as structure A for peaks 1–7.



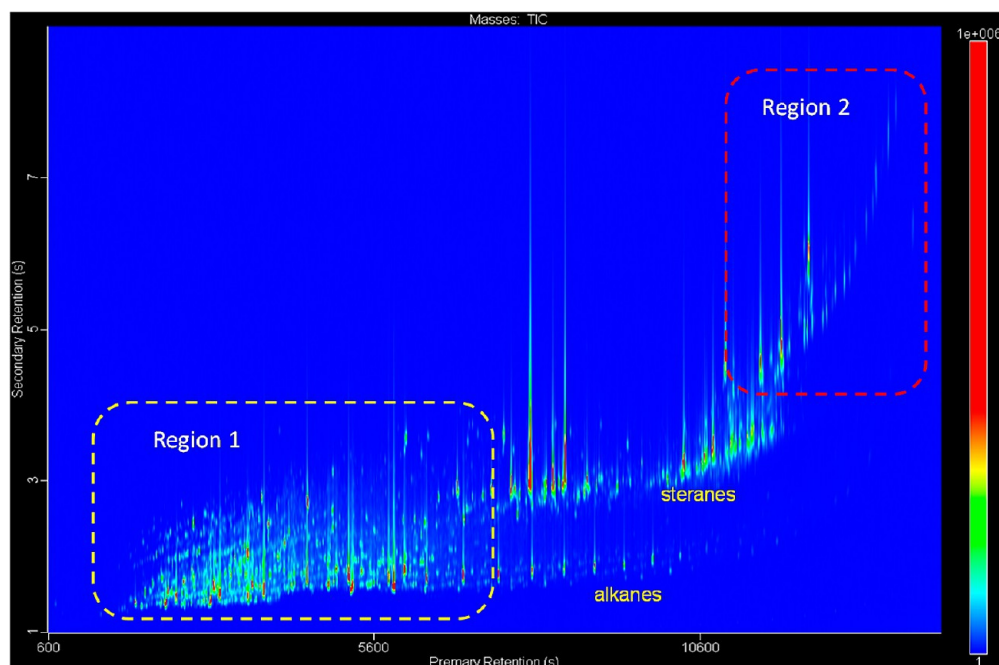


Figure 3. RIC of the Zhao-7 oil saturate fraction from GC \times GC TOF MS analysis.

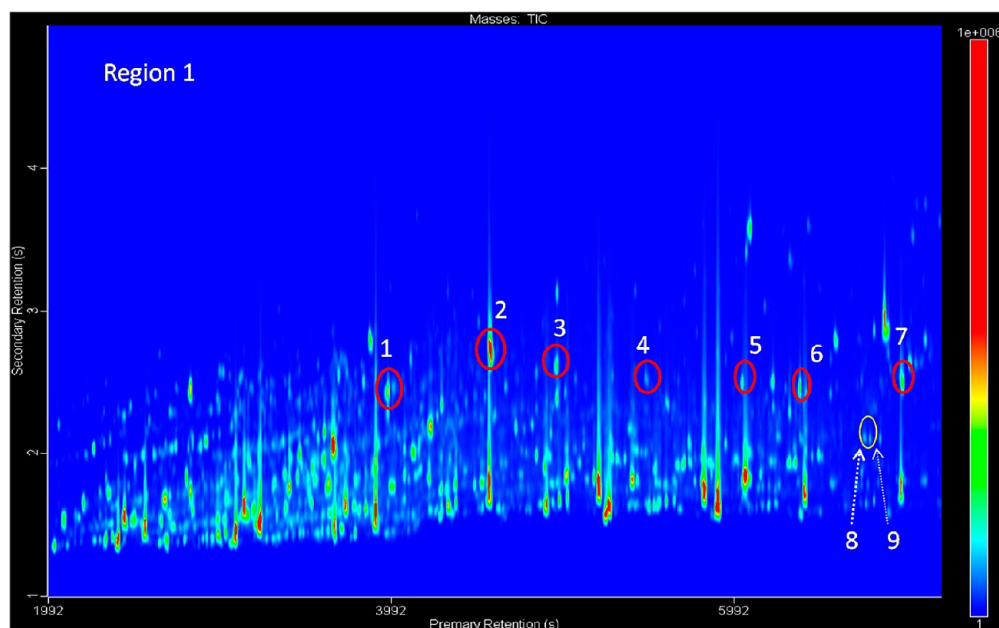
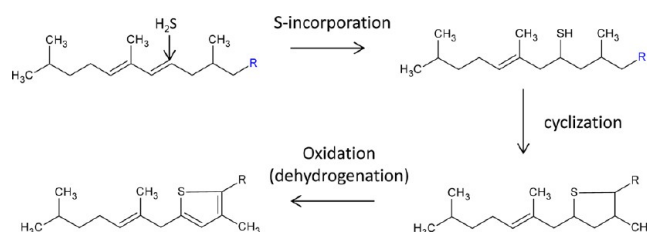


Figure 4. Expansion of region 1 in Figure 3

The isoprenoid thiophenes reported in the literature contain highly branched saturated substituents with carbon numbers of C_{20} , C_{24} , C_{25} , C_{26} , C_{30} , and C_{35} .^{9–15} However, the C_{13} – C_{27} sulfur-containing isoprenoids detected in our oil samples yield different 70 eV EI mass spectra than those reported in the literature because of the presence of unsaturated substituents.

It is very rare that petroleum contains olefinic components or substituents. However, a class of unsaturated isoprenoids, C_{22} – C_{27} monounsaturated sterenes, was found in an immature oil sand sample in the vicinity of this region.² The usual unsaturated isoprenoid thiophenes could be formed by cyclization of thiols that were formed through sulfur incorporation (elemental S, H_2S , SO_4^{2-} , etc.)⁵ into the isoprenoid side

chain followed by oxidation (dehydrogenation) of the cyclic sulfides (thioethers), resulting in the formation of thiophenes with an unsaturated isoprenoid side-chain structure. A proposed mechanism of the formation of olefinic thiophenes is shown below:



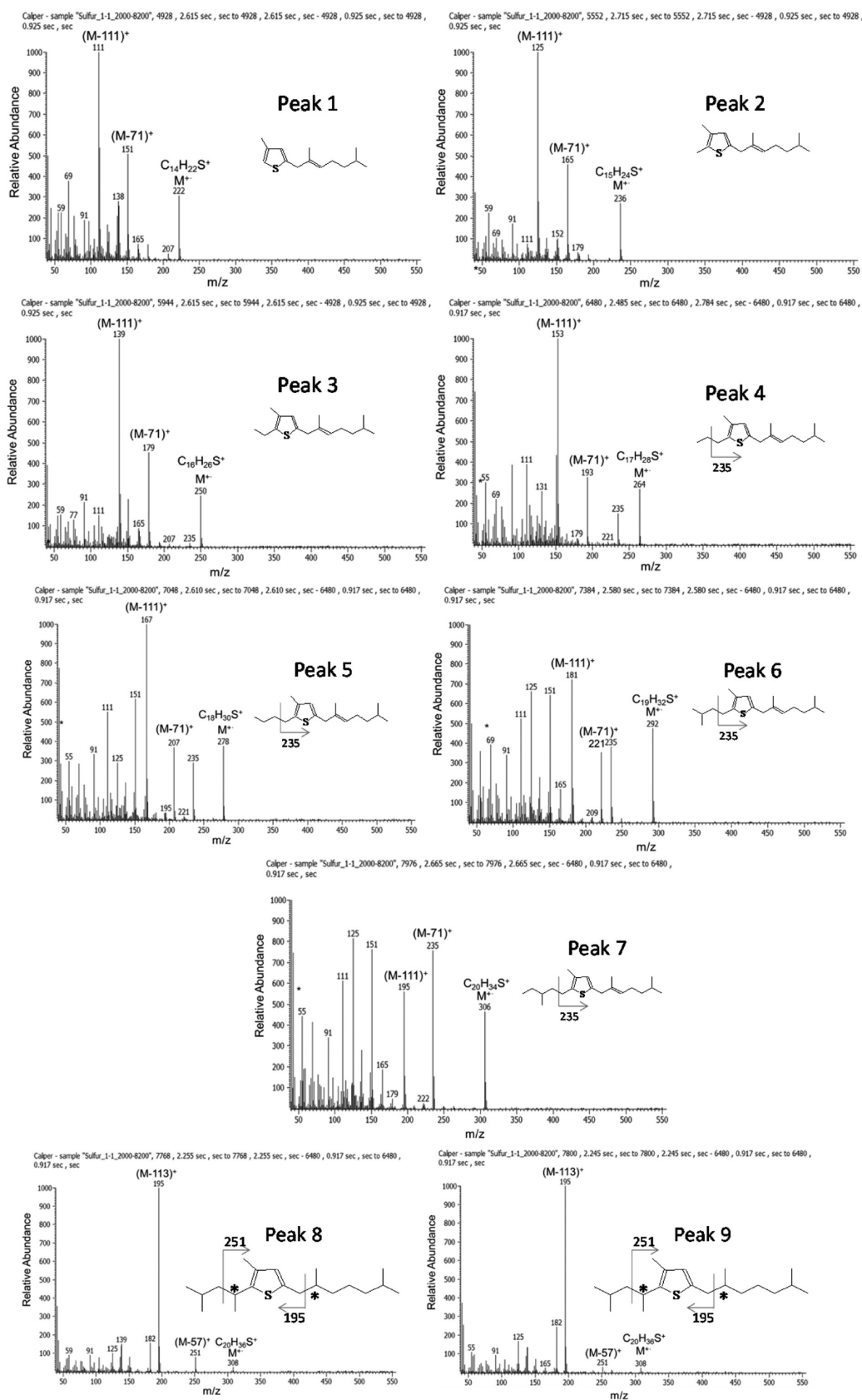


Figure 5. EI mass spectra (70 eV) of peaks 1–9 in Figure 3

The molecular weights of peaks 8 and 9 shown in Figure 5 are 308 Da, two mass units higher than peak 7. Their postulated structures as saturated substituent analogues of peak

7 are shown as structure B (having chiral centers for two optical isomers). Their isomers were reported by Sinnighe Damste et al.⁹ with different 70 eV EI mass

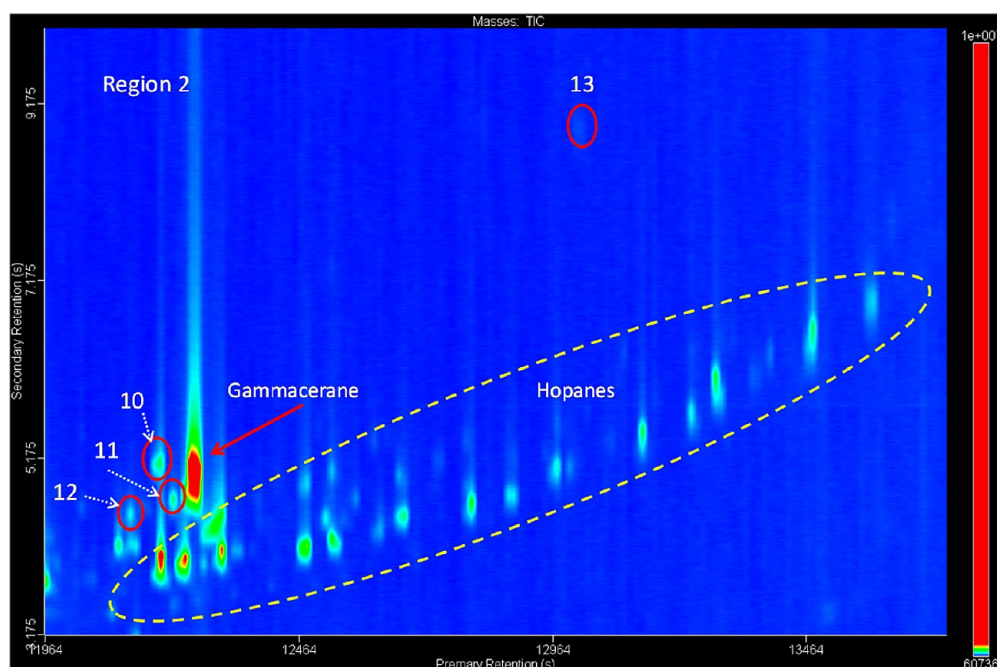


Figure 6. Expansion of region 2 of Figure 3

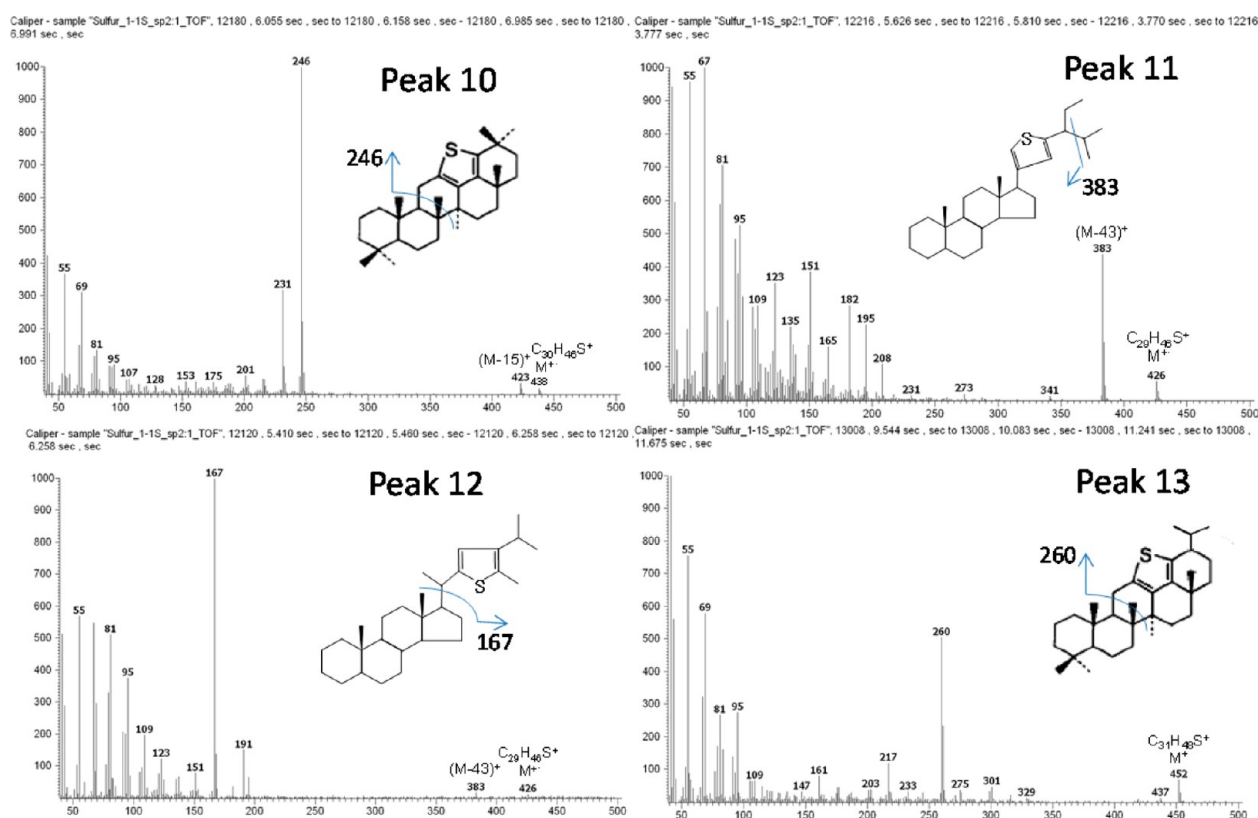


Figure 7. EI mass spectra (70 eV) of peaks 10–12 shown in Figure 6

spectral fragmentation patterns because of the difference in thiophene substituents.

Figure 6 is an expansion of region 2 of Figure 2, with the mass spectra of four sulfur-containing components shown in Figure 7.

The spectrum of peak 10 is consistent with a synthetic compound of 12,19-epithioleane-12,18-diene [$C_{30}H_{46}S$; molecular weight (MW), 438] reported by Poinsot et al.¹⁶ that was also

found in the evaporitic sediment of the Camargue Basin in France. Peaks 11 and 12 yield identical spectra as reported by Lu et al.,⁴ which are identified as thienylandrosterane and 21-thienylpregnane,^{17,18} respectively. Peak 13 is a homologue of peak 10 with an additional carbon, $C_{31}H_{48}S$. The proposed structure is based on the fragmentation of m/z 260 \rightarrow m/z 217 (an isopropyl loss), as compared to the fragmentation of m/z 246 \rightarrow m/z 231 (a methyl loss) in the peak 10 spectrum.

CONCLUSION

Novel sulfur-containing biomarkers were tentatively identified by GC \times GC coupled with SCD and TOF MS. The plausible structures were postulated from mass spectral fragmentation patterns and derived from known reaction chemistry. Further confirmation would require extensive synthetic work or elaborate separation (isolation) of the trace amounts of target species and then analyzed by other analytical techniques for structure identification/confirmation, such as nuclear magnetic resonance spectroscopy (NMR). However, the endeavor of sulfur-containing biomarker characterization, although relatively rare in the literature compared to hydrocarbon biomarkers, may provide some clues of paleotransformation of biological precursors and oil products and resolve some missing links between the biosphere and the geosphere. The low abundance of sulfur in the biological systems cannot account for the much more abundant sulfur species in crude oils. Sulfur apparently comes from inorganic sources forming sulfur organics through sulfur incorporation with decayed biomolecules.

In this report, the presence of novel short-chain unsaturated isoprenoid thiophenes was possibly due to extensively bacteria sulfate reduction (BSR) during reservoir biodegradation, evidenced by an extraordinarily high concentration of H_2S in gas and sulfur organics in liquid. It can also be an indication of immature oil.

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Notes

The authors declare no competing financial interest.

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