

Articles

Identification of alkylthiophenes and C₁₈ thiophene carboxylic acids in sediment of an extraordinarily sulfur-rich maar, Lake Katanuma, in northeastern Japan.

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Abstract

A short surface sediment core (40cm) was collected from a strongly acidic (pH=2.2) freshwater lake, Katanuma, Miyagi Prefecture, Japan. Lake Katanuma is a maar, the water of which is rich in sulfate, sulfides and free sulfur and is located in the active Naruko geothermal field. Lipid compounds in the uppermost sediment were extracted and analyzed for organosulfur compounds (OSCs) by gas chromatography (GC) and gas chromatograph-mass spectrometry (GC-MS). The OSCs identified in the lake sediment were C₁₃-C₂₀ 3-methyl- and 3-ethyl-5-alkyl thiophenes, C₃₅ hopanoid thiophenes and C₁₈ thiophene carboxylic acids (TCAs), in addition to C₂₀ isoprenoid thiophenes. In the present work, production of C₁₈ TCAs was examined by a mild laboratory simulation reaction of reagent grade linoleic acid (*cis*, *cis*-9,12-octadecadienoic acid: C_{18:2}) as well as the solvent extracts from the lake sediments, in H₂S saturated water. We confirmed that the C₁₈ TCAs could be in fact produced under the simple experimental system. However, the abundances of the C₁₈ TCAs detected were too small compared with those of possible precursors, C_{18:2} acids, which is ubiquitous in the particulate matter in the Lake Katanuma water, but almost completely disappeared in subsurface sediment. This finding suggests that sulfur plays a role in a very early stage of diagenesis for sequestering chemically reactive lipid molecules by transforming them into complex geopolymers rather than into small OSCs. No apparent variations in concentration of those OSCs were observed downwards the sediment core. The immediate precursors of alkylthiophenes and hopanoid thiophenes remain to be unraveled.

1. Introduction

Significant compositional changes of lipid molecules occur in surface sediments. For instance, unsaturated fatty acids decrease gradually in the surface sediments, which had been ascribed to a selective degradation, because of less stability of unsaturated moieties primarily against microbial attacks in the environments (e.g., Gaskell et al., 1976; Read et al. 1979; Meyers and Ishiwatari, 1995).

Meanwhile, advanced studies on organic sulfur compounds (OSCs: Sinninghe-Damsté and de Leeuw, 1990; Urban et al., 1999; Werne et al., 2004) have demonstrated a crucial role of sulfur (elemental sulfur or sulfides) in the reaction and transformation of labile biological organic molecules. Possible reactions of sulfur include intra- and inter-molecular reactions, the former of which will give small identifiable OSCs, while the latter may result in the formation of a complex high molecular-weight organic matter (Putschew et al., 1996; Rullkötter, 2000). Kohnen *et al.* (1991),

Wakeham et al. (1995) and Adam et al. (2000) applied desulfurization and/or selective cleavage reaction of the S-S bond to sedimentary organic matter, and successfully released a great many OSCs, supporting the occurrence of S-linked moieties in geopolymer. It implies that chemically reactive biological molecules might be sequestered by the reaction of sulfur in the early stage of diagenesis, and mostly incorporated into sedimentary macromolecules, where the original carbon skeletons will be preserved for prolonged diagenesis (Sinninghe-Damsté et al., 1989b; Urban et al., 1999; Rullkötter, 2000; Rosenberg et al., 2018). Apart from those chemical incorporation reactions, catalytic participation of sulfur in the oxidation of alkenes (Said-Ahmad et al., 2013) and hydrogenation of poly-unsaturated carotenoids (Hebting et al., 2006) have also been proposed.

The small extractable OSCs hitherto identified in sediments and oils have increased considerably not only in number but also in molecular formula peculiar to biological lipids (Sinninghe-Damsté and de Leeuw, 1990). Despite those findings and anticipation that the

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sulfurization may occur in the early stage of diagenesis, little information is available on the sulfurization reaction under contemporary environments, but for C₂₀ isoprenoid thiophenes. The C₂₀ isoprenoid thiophenes are widespread in modern, aged sediments and oils (Brassell et al., 1986; Sinninghe-Damsté et al., 1987; 1990; Fukushima and Muto, 1992; Putschew et al., 1996). Previously, we had examined a laboratory simulation reaction of phytol or phytadienes in H₂S saturated water, and found that C₂₀ isoprenoid thiophenes could be produced, though in a very low yield (Fukushima et al., 1992).

Afterwards, plausible sulfur incorporation mechanism into phytol and farnesol has been discussed in various manner (Amrani and Aizenshtat, 2004 and references cited therein). Principally, C₁₅ and C₂₀ isoprenoid alkenols should be of first converted to dienes, aldehydes or ketones (Rowland et al., 1993; Krein and Aizenshtat, 1994; Amrani and Aizenshtat, 2004) and thereafter sulfurized by elemental sulfur or polysulfide with or without catalytic reagents. Rowland et al. (1993) employed trimethylamine to accelerate the reaction of phytanal (and phytadienes) with elemental sulfur. Amrani and Aizenshtat (2004) examined photooxidation (1 week of sun-light) of the substrate alcohols in the presence of a photosensitizer (anthraquinone) prior to sulfurization. Following reaction with ammonium polysulfide yielded a substantial amount of isoprenoid thiophenes. Particularly the C₂₀ isoprenoid thiophenes are so frequently observed OSCs in contemporary marine/estuarine sediments that they may be produced *via* those active oxidized intermediates.

The occurrence of isoprenoid thiophenes more than C₂₀ (Sinninghe-Damsté et al., 1986), highly branched isoprenoid (HBI) thiophenes (Sinninghe-Damsté et al., 1989a) and hopanoid thiophenes (Varisolalaoet al., 1984; Sinninghe-Damsté et al., 1989a; Katsumata and Shimoyama, 2001) had been also reported. The OSCs identified are not limited to those having isoprenoid chains or terpenoid structure. Sinninghe-Damsté et al. (1986) determined series of 2,5-dialkylthiophenes ranging up to C₃₄ carbon atoms in the Messinian marl deposited under hypersaline euxinic conditions. They had straight-chain alkyl substituents.

Furthermore, Russell et al. (2000) identified C₁₈ carboxylic acid isomers containing a thiophene ring in the carbon chain (thiophene carboxylic acids: TCAs) from the Messinian Tripoli Formation taken at Lorca Basin in Spain. The Tripoli Formation contains up to 23.3% organic carbon and up to 7.5% sulfur. The precursors of the C₁₈ TCAs are thought to be C_{18:2} acids, e.g., linoleic acid (octadeca-9,12-dienoic acid) and its isomers common to most organisms.

Researches on OSCs in modern highly reducing and sulfur-rich environments and, simple and mild simula-

tion reactions likely to occur in aqueous conditions (Fukushima et al., 1992), should be still of significance, because it will provide promising evidences for the possible sulfurization of biological molecules, as well as the role of sulfur for organic molecule transformation in biogenic and/or volcanic sulfide-rich environments. In the present study, we tried to identify as many OSCs as possible in the solvent extracts from a modern volcanic sulfur-rich lake sediment. Examined was sediment from an extraordinarily sulfur-rich maar, Lake Katanuma, (Satake, 1980; Matsumoto and Watanuki, 1990). Consequently, series of alkylated thiophenes including some C₁₈ TCA isomers were identified.

In this paper, particular attention was paid on the formation of the C₁₈ TCAs. A mild simulation reaction of reagent grade linoleic acid (C_{18:2}) with H₂S was conducted in water to confirm a sulfurization reaction to give TCAs. The same simulation experiment was conducted against natural lipid samples extracted from surface sediments of two lakes, Katanuma and Haruna, both were known to contain meaningful amounts of C_{18:2} acid. The two simulation reactions evidently proved that C₁₈ TCAs were definitely produced at least by the chemical reaction, though in a very small yield.

2. Experimental

2.1. Profiles of the lake and sediment sample

The Lake Katanuma is a small round lake formed in a maar in Miyagi Prefecture, Japan (Fig.1: surface area: 0.126 km², altitude: 306 m and maximum depth: 20m). The chemical properties of the Katanuma surface water are given in Table 1. The strongly acidic condition (pH ≈ 2.2) is caused by the evolution of fumarole gas and hot spring water in and around the lake (Satake and Saijo, 1978) and it indicates that the lake is situated in an active geothermal field. Lake Katanuma has no apparent inflow or outflow streams and the small catchment is covered by a deciduous forest. Until 1969, sulfur mining had been conducted by dredging the bottom mud. At the present, however, influence of human activities is minimal. Due to the low pH regime, acid-tolerant benthic diatom such as *Pinnularia braunii* var *amphicephala* and an insect *Chironomus* sp. are major organisms viable in this lake (Satake, 1980). Organic geochemical properties of the lake sediment had been reported by Matsumoto and Watanuki (1990).

A short sediment core (Kat-04: length 40cm) was collected in July 2004 at the deepest site (water depth: 20m) by using a 5cm I.D. gravity corer. The core was subdivided immediately on the spot at every 1cm, kept in a cooler box with ice for a few days, until they were

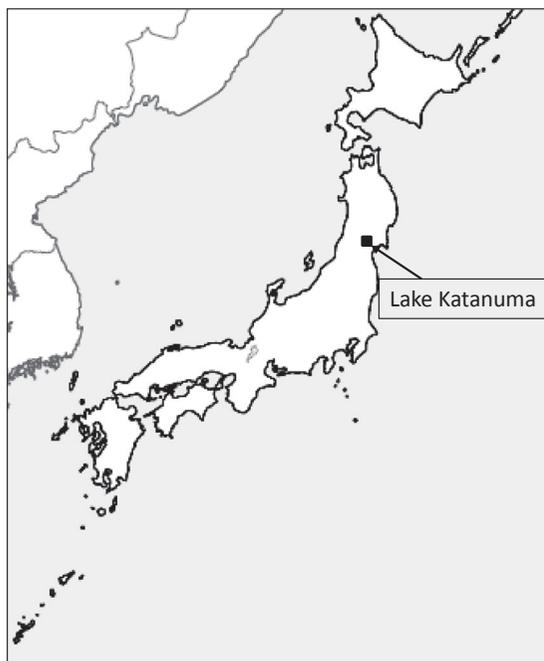


Figure 1. Location of Lake Katanuma.

brought back to laboratory and stored frozen at -20°C . For reference, particulate matter (PM) in surface water (1 m) was collected in 2002, by filtering 40 L of water through a 30cm x 30cm glass fiber filter of nominal pore size $0.6\ \mu\text{m}$ (GB100R, Advantec Ltd., Japan).

The sedimentation rate of Lake Katanuma was reported by Satake and Saijo (1978), who ascribed the black and white-yellow laminae composed of diatom frustules (black layer: summer and winter) and crystalline free sulfur (white layer: spring and fall) to an indication of the seasonally alternate redox condition of the lake bottom. The free-sulfur-rich layer should be formed by oxidation of H_2S during the circulation periods in spring and fall, twice a year. Accordingly, they estimated the sedimentation rate as 4-8 mm/y at the shallower location of 7 m depth. Unfortunately, the laminae were not observed in our sediment sample taken at the deepest site.

It was notified that the temperature of the bottom section (40cm) was high enough to exceed 40°C when the sediment core was collected. It indicates a large geothermal heat flow through the Lake Katanuma sediment.

2.2. Analytical Procedure

2.2.1. Lipid analysis

The freeze-dried sediment (1-2g) was extracted with dichloromethane (DCM)/methanol (MeOH) (3:2, v:v, $30\ \text{ml} \times 3$) in a 50 ml centrifuge tube, using sonification. The suspension was centrifuged, and the supernatant was carefully transferred by a pipette to a round-bottom flask. Solvent was removed by rotary evaporation. The extract was again dissolved in a least amount of the same solvent and then passed through a column packed with small amount of silica gel (see below) and enough amount of activated colloidal copper prepared just before use (Blumer, 1957). The complete removal of free sulfur was to avoid an unintentional sulfurization during the analytical procedure. The free-S removed extract was recovered by eluting with three column volume of DCM/MeOH (3:2, v:v). Thorough removal of free-S was confirmed by adding activated Cu granules (see 2.3. sulfur incorporation experiment section) to the eluates. Then the extract was dried and saponified in a sealed ampoule with 5% water-added 0.5M KOH in MeOH at 100°C for 4 hrs.

After saponification, unsaponified neutral constituents were extracted with *n*-hexane (Hx)/diethylether (DEE) (9:1, v:v) and dried. Fractionation into aliphatic hydrocarbons, aromatic hydrocarbons and polar substances was conducted on a silica gel column chromatography (Marinckrodt Co. Ltd., USA, 100 mesh inactivated by adding 5% of water). The aliphatic hydrocarbons eluted by Hx were further fractionated into saturated and unsaturated compounds, by applying them onto a 10% AgNO_3 impregnated silica gel column. The Ag-impregnated silica gel was prepared by dropwise adding 1 g AgNO_3 dissolved in 0.5 mL distilled water to 10 g dry silica gel, preliminarily baked at 400°C and then kept in an oven set at 100°C . Afterwards the Ag-impregnated silica gel was vigorously shaken for a while to homogenize in an Erlenmeyer flask shaded by aluminum foil and then soaked with Hx. Eluents used for chromatography were Hx for saturated and Hx/DEE (1:1, v:v) for unsaturated hydrocarbons, respectively.

The saponified acid constituents were extracted with Hx/DEE (9:1, v:v), after the aqueous MeOH layer was acidified with conc. HCl to less than $\text{pH}=2$. The carboxyl group was methylesterified with 14% borontrifluoride (BF_3) in MeOH (Wako Pure Chemicals Ltd., Japan) by heating at 100°C for 30 min in a sealed am-

Table 1. Chemical properties of the Lake Katanuma surface water collected in Nov. 2002.

EC $\mu\text{S}\cdot\text{cm}^{-1}$	pH	Na^+ ($\text{mg}\cdot\text{l}^{-1}$)	K^+ ($\text{mg}\cdot\text{l}^{-1}$)	Mg^{2+} ($\text{mg}\cdot\text{l}^{-1}$)	Ca^{2+} ($\text{mg}\cdot\text{l}^{-1}$)	F ⁻ ($\text{mg}\cdot\text{l}^{-1}$)	Cl ⁻ ($\text{mg}\cdot\text{l}^{-1}$)	SO_4^{2-} ($\text{mg}\cdot\text{l}^{-1}$)
2,440	2.22	10.1	2.1	0.6	2.1	0.7	7.8	379.5

poule. The alkanolic acid methyl esters were extracted to Hx/DEE (9:1, v:v) phase, dried and purified on a silica gel column by eluting with benzene.

The lipid constituents in PM were extracted from lyophilized glass fiber filter with DCM/MeOH (3:2, v:v). The filter was placed in a 300 mL tall beaker with the solvent and extraction was repeated three times aided by sonification. Following fractionation and analytical procedures were all the same as those applied to sediment.

Free sulfur concentration in Lake Katanuma sediment was determined gravimetrically by extracting the dried sediment (500 mg) with carbon disulfide (CS₂). The sediment and 3 mL CS₂ were placed in a 10 mL glass centrifuge tube with ground glass stopper. After ultrasonic extraction, the extracts were centrifuged and transferred to a vial. Extraction was repeated twice. The CS₂ solution was carefully dried in a ventilator at room temperature, because CS₂ was extremely flammable. Supposed that the CS₂-soluble substances other than free S should be negligibly small by weight, sulfur concentration was determined by weighing dried crystalline extract.

2.2.2. Analytical Instruments

The CN elemental analysis was performed on a YANACO MT-5 CHN analyzer (Yanagimoto Co. Ltd., Japan). Ionic composition of the Lake Katanuma water was determined by using an ion chromatograph, IC7000E (Yokogawa Analytical Systems Co. Ltd., Japan).

The lipid constituents were analyzed on an HP-5890 Series II gas chromatograph (GC) and an HP-6890 GC coupled with an HP-5973 MSD (GC-MS). The column used for GC was 0.25mm I.D. × 30m fused silica capillary coated with cross-linked 5% phenylmethyl siloxane (DB-5; film thickness 0.25 μm; J & W Co. Ltd.). A DB-5MS (0.25mm I.D. × 30m, film thickness 0.25 μm; J & W Co. Ltd.) column was used for GC-MS analysis.

2.3. Sulfur incorporation experiments

2.3.1. Reaction of linoleic acid in H₂S saturated water

Reagent grade linoleic acid (octadeca-9,12-dienoic acid, C_{18:2}, Wako Pure Chemicals Co. Ltd.) dissolved in benzene (10 μg) was placed in a 10 mL glass ampoule and the solvent was removed in vacuo. Meanwhile, H₂S gas generated by the reaction of iron sulfide with sulfuric acid, was blown into distilled water for more than 30 min to give H₂S saturated water. The H₂S saturated water (5mL) was added to the ampoule and sealed (Fukushima et al., 1992). The ampoule was heated at around 60 °C for 40 hours in an oven (dark) to accelerate the reaction. The reaction product was transferred to a centrifuge tube with 15 mL of water, a

small amount of MeOH and 15 mL of Hx/DEE (9:1, v:v). After shaking and centrifugation, the organic solvent layer was transferred to a round bottom flask. The extraction was repeated twice. Free sulfur generated during the reaction was removed by adding acid- and solvent-washed reagent grade Cu granules to the extracts, until the surface of the Cu granules remained lustrous. The Cu granules were pre-treated just before use, with diluted (4M) hydrochloric acid to activate the metal surface, washed with solvents (MeOH and then DCM) and dried.

The Cu granules and exfoliated black copper sulfide powder were filtered. After removal of solvent, methylation, extraction and purification of the alkanolic acid methyl esters were conducted as described above.

2.3.2. Reaction of lipids extracted from lacustrine sediments in H₂S saturated water

The same sulfurization experiment was conducted against natural sedimentary lipids extracted from Lake Katanuma and a normal freshwater Lake Haruna (Ishiwatari et al., 1980). Prior to the reaction, the extracts were desulfurized by activated Cu (Blumer, 1957) and divided into equal amount of two parts, one of which was analyzed without sulfurization as a control. The reaction products in the aqueous phase were extracted with Hx/DEE (9:1, v:v). The residue on the ampoule wall was washed with DCM. Both extracts and washings were combined, desulfurized, filtrated and saponified in 1 M KOH in MeOH. Analytical procedure of the reaction products was all the same with that used in the section 2-2-1.

3. Results

3.1. Profiles of the Lake Katanuma sediment and sedimentary lipids

Based on the sedimentation rate estimated by Satake and Saijo (1978), the 40cm length of core may be thought to encompass the last several decades. In the past, however, sulfur mining had been conducted in this lake. The lake bottom mud had been dredged until Jan. 1969, i.e., about 30 years before the sampling date. The sulfur mining should have considerably disturbed the sediment, so that we cannot expect that the 40cm sediment core should record the time-course variation, except for surface several centimeters.

The sediment of Lake Katanuma can be characterized by an extraordinary high free sulfur concentration enough to give a yellow crystalline extract. Fig.2 indicates the depth profiles of total organic carbon (TOC) and free sulfur concentrations. TOC was less than 1% and exceeded 1% only in the top surface 0-4cm layers (1.2-2.5%). A high concentration of free S was also

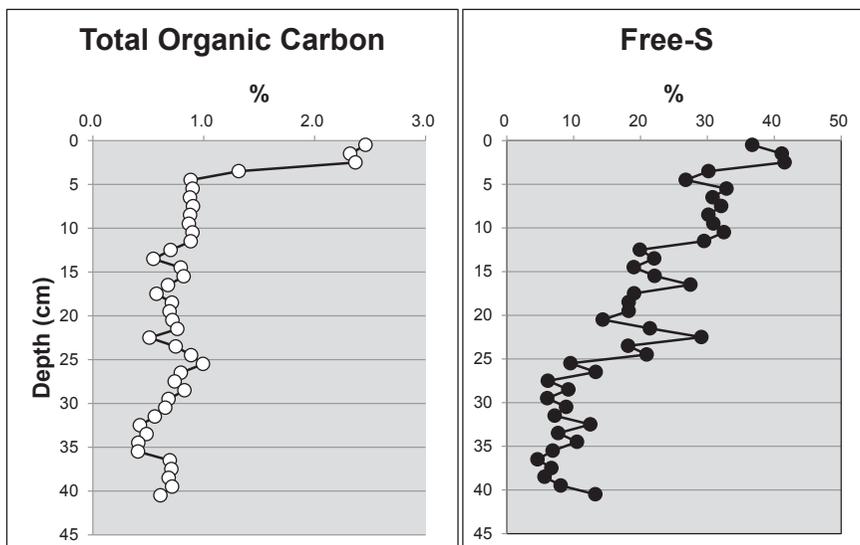


Figure 2. Total organic carbon (TOC) and free sulfur concentrations in the sediment core of Lake Katanuma collected in July 2004.

found in the top 0-3cm, where it amounted to 35-40% by weight. Free S gradually decreased downwards to the range of 5-10%. Water contents were 67-71% in the surface 0-3cm and decreased downwards to 36-60%, indicating that the sediment was principally composed of coarse pyroclastic material. Relatively high TOC, free sulfur and water contents in the surface 0-4cm layers may well indicate that they should be fresh sediment deposited after termination of the sulfur mining in 1969. Thus, the sedimentation rate of the surface sediment was estimated to be around 1 mm y^{-1} at the deepest site of the lake.

Hydrocarbons and alkanolic acids in the Lake Katanuma sediment had been described by Matsumoto and Watanuki (1990). Here we show only qualitatively the vertical variation of alkanolic acids in the surface 0-4cm sediment layers. Fig.3 shows that the particulate matter contained large amounts of C_{16} and C_{18} unsaturated acids, whereas those unsaturated fatty acids decreased significantly and almost completely disappeared in the sediment layers deeper than 3cm.

3.2. OSCs in the Lake Katanuma sediment

The chromatographic profile of the aliphatic hydrocarbons varied little throughout the sediment core. Major peaks were *n*-alkanes. However, detailed inspection of the mass spectra of the small peaks suggested the occurrence of a series of alkylthiophenes. Then the aliphatic hydrocarbons were further chromatographed on a $AgNO_3$ impregnated silica gel column to give unsaturated or low-molecular weight aromatic hydrocarbons coeluting with aliphatic hydrocarbons.

Figs.4a and 4b show m/z 111 and m/z 125 mass frag-

mentograms diagnostic to methyl (MATs) and ethyl alkylthiophenes (EATs), together with the representative mass spectra of C_{13} species found in the surface sediment section (0-1cm). As shown in the figures, occurrence of series of peaks from C_{13} to C_{20} MATs and EATs, as well as C_{20} isoprenoid thiophenes was apparent. They are tentatively assigned as 2-methyl- and 2-ethyl-5-alkyl thiophenes, considering their simple mass spectra exclusively composed of two ions (M^+ and m/z 111 or m/z 125: Sinninghe-Damsté et al., 1986). It may be possible that the compounds assigned as 2-ethyl-5-alkylthiophenes here should be dimethyl-alkylthiophenes. However, supposed that those alkyl thiophenes may be produced by addition of sulfur at the terminal position of the long-chain lipid substrates (alkadienes, aldehydes, ketones and so-on) as is the case of C_{20} isoprenoid thiophenes, the substrates have to contain methyl side chain neighboring the functional group. Without detection of those methyl branched alkyl compounds, it would be appropriate to assign them as EATs. Despite low concentrations compared with the C_{20} isoprenoid thiophenes, the MAT and EAT homologues were present in every sediment section of the core. They ranged from C_{13} to C_{20} , showing a similar carbon-number distribution to each other and the odd-even preference is not apparent except for C_{13} to C_{15} . The terminal thiophenes (presumed to have a diagnostic ion at m/z 97 or m/z 98) or propyl to pentyl alkylated thiophenes (m/z 139, m/z 153 and m/z 167), were, if present, negligibly small, the profile of which was obviously different from those reported by Sinninghe-Damsté et al. (1986) for the Messinian sediment.

Notified are two peaks in the high-boiling point re-

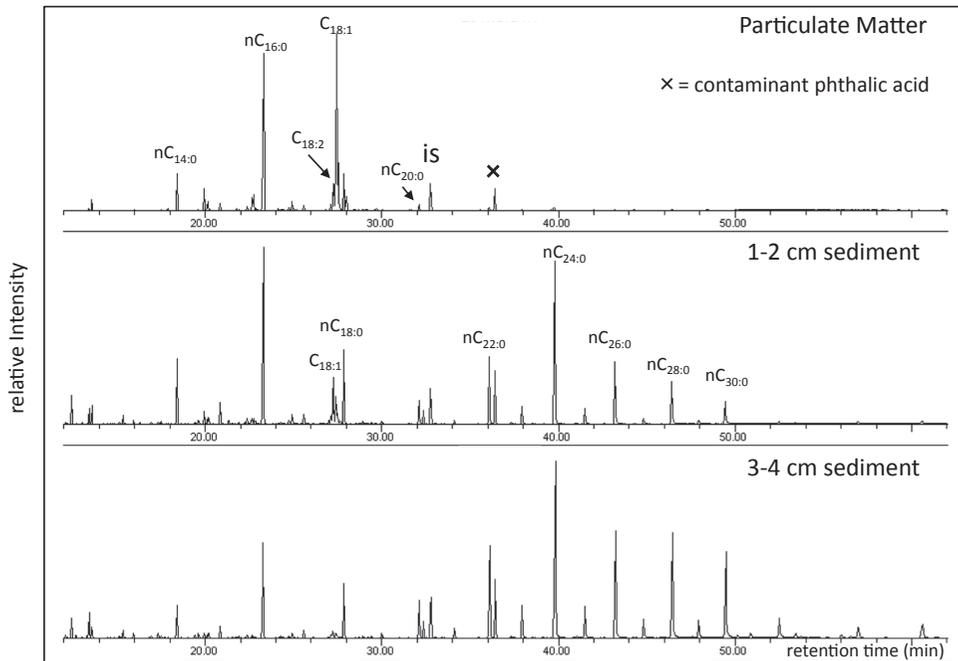


Figure 3. Vertical variation of fatty acids in the sediment. Unsaturated C₁₆ and C₁₈ acids mostly disappeared below the 3-4cm layers.

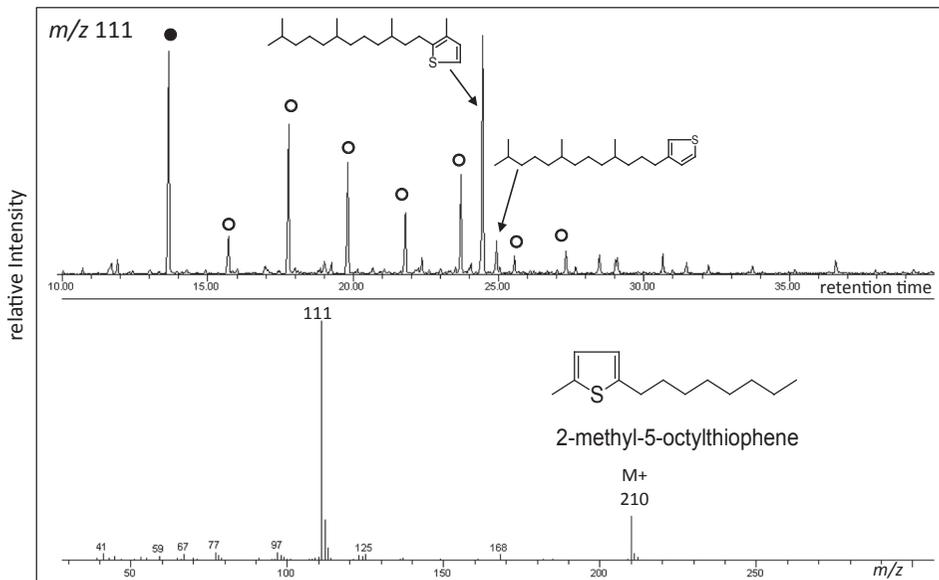


Figure 4a. Mass fragmentogram (m/z 111) of unsaturated aliphatic hydrocarbons from the uppermost sediment (0-1cm) and a representative mass spectrum (●) identified as 2-methyl-5-octyl-thiophene. Occurrence of C₁₃-C₂₀ methyl-alkylthiophene congeners (MATs) (open circled) are shown.

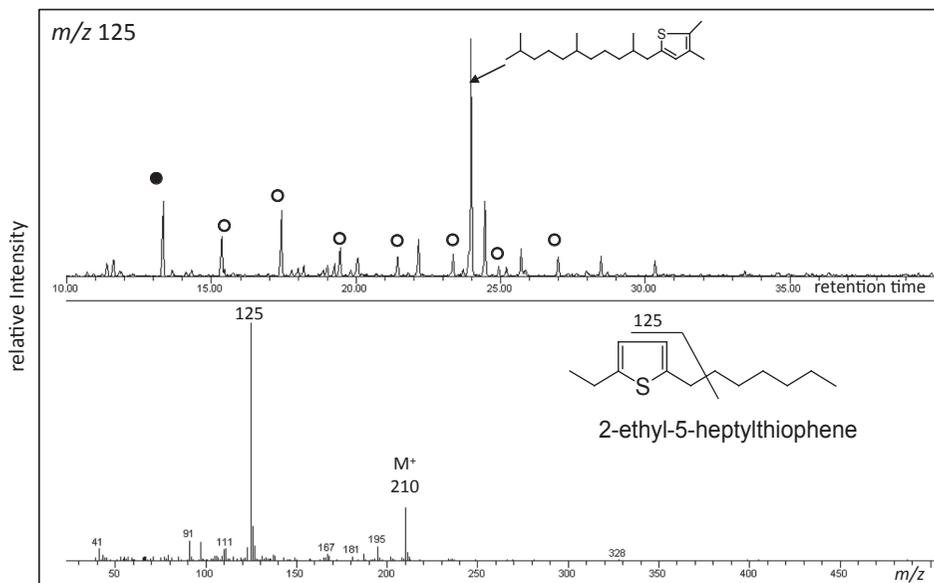


Figure 4b. Mass fragmentogram (m/z 125) of unsaturated aliphatic hydrocarbons from the uppermost sediment (0-1cm) and a representative mass spectrum (●) identified as 2-ethyl-5-heptyl thiophene. Occurrence of C_{13} - C_{20} ethyl-alkylthiophene congeners (EATs) (open circled) are shown.

gion of the m/z 111 and m/z 97 mass fragmentograms. The two peaks were observed in every sediment section. However, they were too small to give definite mass spectra in the uppermost sediment. Hence Figs. 5a and 5b show their mass spectra recorded for the extracts from 7-8cm layer. The mass spectra of these two compounds had an M^+ ion at m/z 508 and they were identified as 31-(2-thiophenyl)-17 β (H), 21 β (H)-homo-hopane (Valisolalao et al., 1984) and its isomer 30-[2-(5-methyl-thiophenyl)]-17 β (H), 21 β (H)-hopane (Sinninghe-Damsté et al., 1989b; 1990).

Trace amount of OSCs were also detected in the alkanolic acid fraction. Fig. 6 shows partial m/z 324 (molecular ion of C_{18} thiophene carboxylic acid methyl ester: $C_{19}H_{32}O_2S$) mass fragmentogram of the alkanolic acid fraction extracted from the 0-1cm layer. The mass spectra of the small broad peaks eluting just after the internal standard (n - $C_{24}D_{30}$) indicate the occurrence of at least five isomers identifiable as C_{18} TCAs (Russel et al., 2000). The most abundant isomers are those having pentyl (C_5) and hexyl (C_6) alkyl substituents in the thiophene ring. The typical mass spectrum of C_6 -TCA is shown in Fig. 6. Referring to diagnostic fragment ions, m/z 139, m/z 153 and m/z 195, others are identified as propyl (C_3), butyl (C_4), and heptyl (C_7) alkylated TCAs.

3.3. Model reaction of hydrogen sulfide with unsaturated fatty acid: linoleic acid ($C_{18:2}$) and lipids extracted from lacustrine surface sediments

Fig. 7 shows the partial m/z 324 mass fragmento-

gram of the alkanolic acids extracted from the reaction products of linoleic acid in H_2S -saturated distilled water. Minute amounts of two isomers were identified as C_5 and C_6 TCAs. The yield of TCAs was small. Significant decrease and isomerization of linoleic acid occurred during the sulfurization experiment and gave at least three additional $C_{18:2}$ acids. The mass spectra of those isomers and linoleic acid were similar to each other, so that it was not known whether the isomerization indicates double bond migration or stereoisomerism.

The sedimentary lipids extracted from the surface layer (0-1cm) of Lakes Katanuma and Haruna were reacted with H_2S under the same experimental conditions (Figs. 8a and 8b). In the control (unreacted) samples, trace amounts of C_{18} TCAs were detected from Lake Katanuma but none from Lake Haruna sedimentary lipids. In the case of the Lake Katanuma sedimentary lipids, the amounts of TCAs, particularly of C_5 and C_6 isomers, evidently increased after sulfurization experiment (Fig. 8a). Meanwhile, with regard to the Lake Haruna lipids (Fig. 8b), the de novo formation of TCAs was obvious. In the reactions of the lacustrine lipids, decrease and isomerization of linoleic acid were also observed.

In the hydrocarbon fraction of the sulfurization products from the lake lipids, however, neither alkyl thiophenes (MATs and EATs) nor C_{35} hopanoid thiophenes were detected.

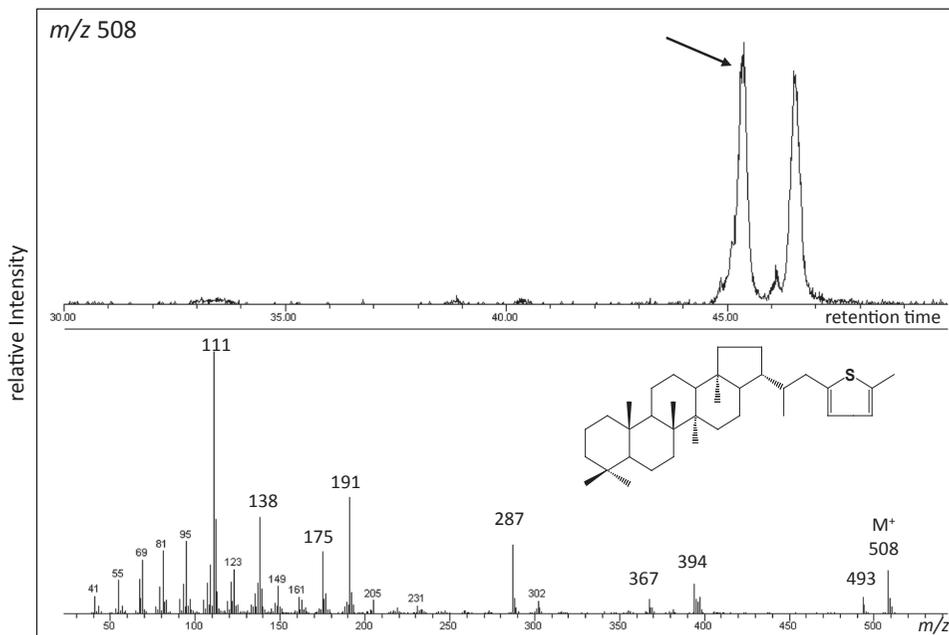


Figure 5a. Mass fragmentogram (m/z 508) and the mass spectrum of the former C₃₅ hopanoid thiophene.

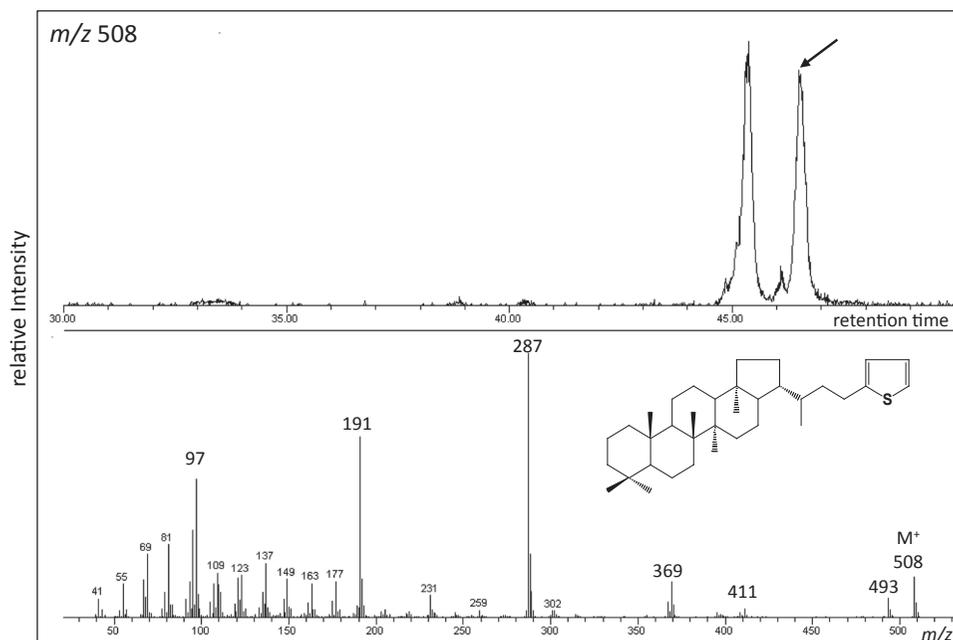


Figure 5b. Mass fragmentogram (m/z 508) and the mass spectrum of the latter C₃₅ hopanoid thiophene.

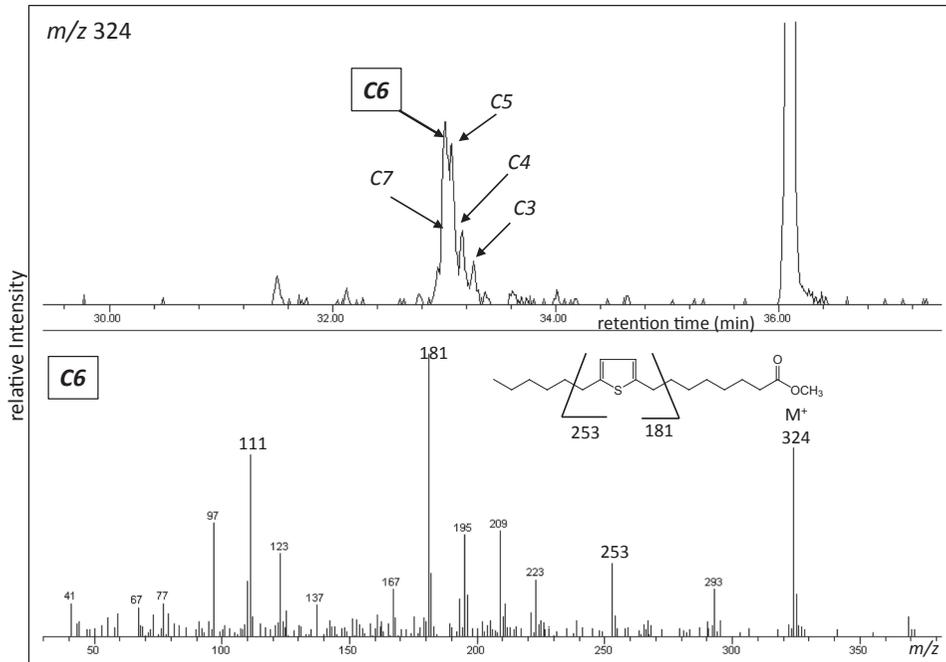


Figure 6. Mass fragmentogram (m/z 324: molecular ion of C_{18} TCAs) of fatty acid methyl esters and a representative mass spectrum identified as C6 TCA (2-hexyl-5-octyl-thiophenyl) carboxylic acid (3-4cm). Five isomers with propyl (C3) to heptyl (C7) chain were identified.

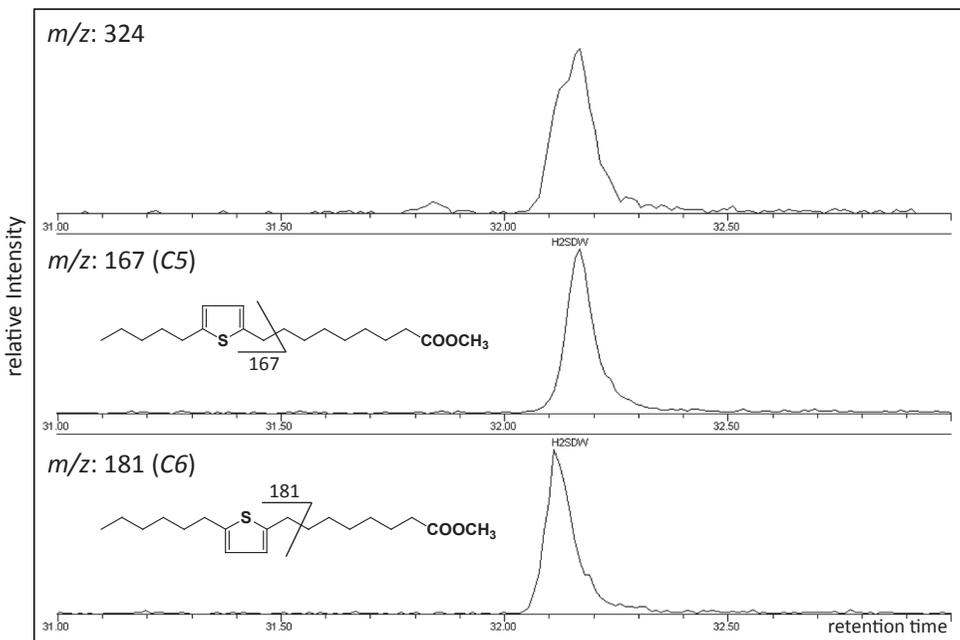


Figure 7. Mass fragmentogram (m/z 324) of fatty acid methyl esters produced by the reaction of linoleic acid and H_2S in water. Two peaks assignable to C5 and C6 TCAs were observed.

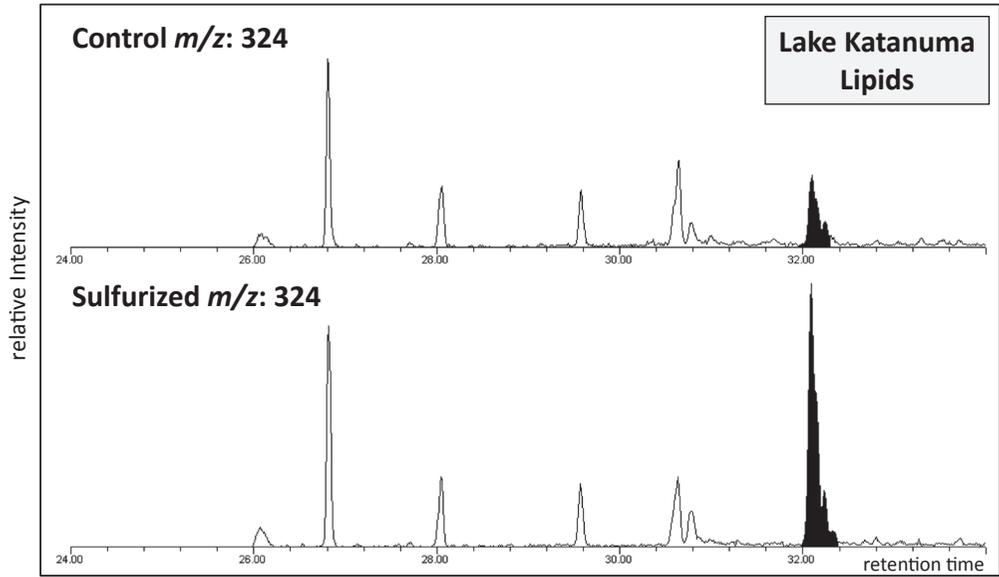


Figure 8a. Sulfurization of lipids extracted from Lake Katanuma obviously caused increment of C_{18} TCA. (up: control; bottom: sulfurized)

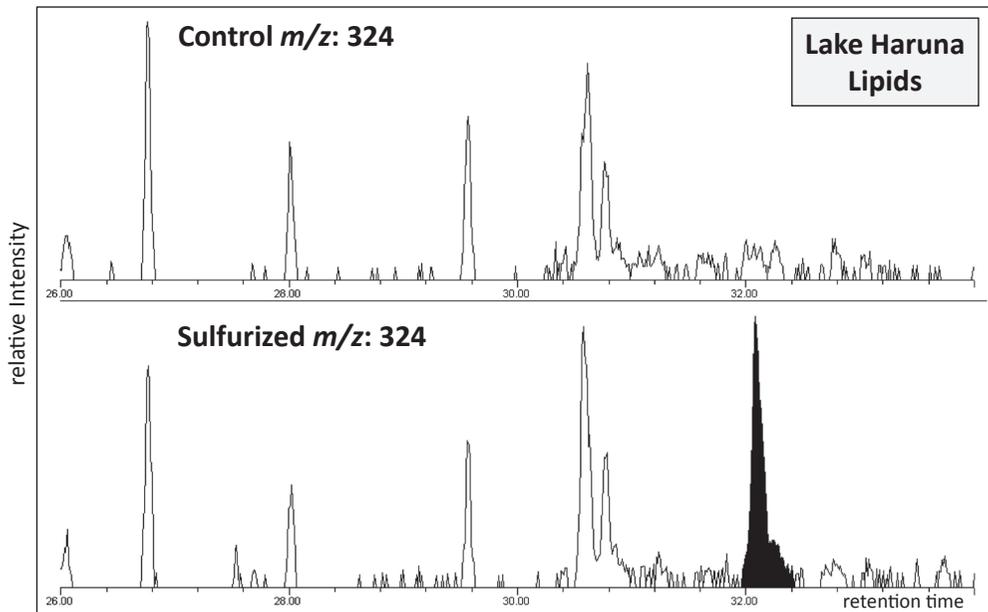


Figure 8b. Sulfurization of lipids extracted from Lake Haruna anew produced C_{18} TCA. (up: control; bottom: sulfurized).

4. Discussion

Our previous study (Fukushima and Muto, 1992) demonstrated that the C₂₀ isoprenoid thiophenes are ubiquitous in lacustrine and coastal marine sediments in Japan, either rich in biogenic sulfides or volcanic origin of sulfides. In a highly reducing meromictic lake sediment, C₁₅ isoprenoid thiophenes probably derived from farnesol were also detected. Following study revealed that the C₂₀ isoprenoid thiophenes can be produced chemically by the reaction of H₂S with phytol in water (Fukushima et al., 1992). In the similar manner, it is likely that the OSCs identified in this work should have been produced in the aquatic environment under the presence of active sulfur species within a few decades after deposition, considering that the sedimentation rate is around 1.0 mm yr⁻¹.

Due to strongly acidic condition (pH= 2.2) of the lake water, *in-situ* biological productivity in Lake Katanuma is thought to be low. It can be seen by the *n*-fatty acid distribution in sediment, where long-chain saturated fatty acids predominated, mostly derived from terrestrial plants. Nevertheless, a few acid-tolerant benthic diatoms forming diatomaceous ooze in the periphery of the lake and some insects may supply authigenic compounds to the lake water. It reflects on the alkanolic acid composition of the particulate matter and top surface sediment, where C₁₆–C₁₈ unsaturated fatty acids are present in meaningful concentrations (Fig.3). In the subsurface sediments beneath 2cm, however, those unsaturated compounds decreased considerably. The *iso* and *anteiso* C₁₅, C₁₇ fatty acids are also scarce, indicating that the heterotrophic bacterial activity is also limited in this lake. The significant decrease of unsaturated fatty acids in surface sediment and production of OSCs found in Lake Katanuma sediment should be brought about by the chemical reactions with sulfur.

In our experiments, however, neither detailed description of the reaction mechanism nor effective synthesis of the OSCs was not intended. The purpose of our simulation experiments was only to provide a possibility of the sulfur incorporation to give small OSCs in contemporary environment, even if the yield may be trace. Thus, no peculiar treatments such as photolysis and addition of catalytic reagents were included in our simulation experiments.

The simulation experiments to produce C₁₈ TCAs was conducted in the same manner as described previously for C₂₀ isoprenoid thiophenes (Fukushima et al., 1992). Reaction of linoleic acid (octadeca-9,12-dien-1-oic acid) in H₂S saturated distilled water actually gave a small but meaningful amount of C₁₈ TCAs under the simple reaction condition. The coalesced peak

found in the mass fragmentogram of *m/z* 324 (M⁺) was composed of C₅ (C5) and C₆ (C6) alkylated C₁₈ TCAs as shown in Fig.7. The sulfurization experiment of the sedimentary lipids from Lakes Haruna and Katanuma also showed that the TCAs were produced in this experimental condition. The C₁₈ TCAs other than C5 and C6 TCAs found in Lake Katanuma sediment, *i.e.*, C3, C4 and C7 TCAs should have been produced by sulfurization of the corresponding C_{18:2} fatty acid isomers. Occurrence of some C_{18:2} acid isomers was known by tracing M⁺ (*m/z* 294) mass fragmentogram and inspection of individual peaks in the particulate matter from Lake Katanuma and in the lipids extracted from the two lake surface sediments. On sulfurization reaction, relative proportions of those isomers increased. Nevertheless, it was not yet apparent whether or not the isomerization should indicate migration of double bonds to provide other C₁₈ TCAs than C5 and C6 or not. Major products of the simulation experiments were always C5 and C6 TCAs. Possible formation reactions of the C5 and C6 C₁₈ TCAs from linoleic acid are proposed in Fig.9.

The sources of C₃₅ hopanoid thiophenes may be C₃₅ bacteriohopanetetrol (Varisolalao et al., 1984), though the source hopanoid alcohols could not be analyzed by derivatization to TMS ethers employed in this work. However, the C₃₅ hopanoid thiophenes were not detectable in the reaction products of H₂S with the lipids extracted from the lake sediments. Presumably it suggests that their formation may require much more time (>several days), higher (>60 °C) temperature and/or substrates incorporated within the unextracted geopolymer.

On the other hand, the source organic molecules of MATs and EATs are not known. Monounsaturated C₁₇ and C₁₉ alkenes were present in the particulate matter and uppermost sediment section of Lake Katanuma. If they are the sources, however, the distributions of MATs and EATs having a maximum at C₁₃ and ranging up to C₂₀ (Figs. 4a and 4b) could not be explained. It may suggest a uniqueness of Lake Katanuma situated in active geothermal field. Thermal degradation or cracking of straight-chain substances in subsurface sediment may provide alkadienes, which are facile to incorporate sulfur. However, questions remain why the carbon numbers are limited up to C₂₀. Further works are required to decipher the source-OSCs relationships.

The gas chromatograph-flame photometric detector (GC-FPD) is a powerful tool to detect minute amounts of OSCs (Sinninghe-Damsté et al., 1986; 1987; Fukushima and Muto, 1992; Fukushima et al., 1992; Amrani and Aizenshtat, 2004). Unless FPD is available, however, detection and identification of OSCs (in this case, thiophenes) could be conducted fruitfully by tracing *m/z* 97 (or 98), *m/z* 111 and *m/z* 125 ions on GC-MS.

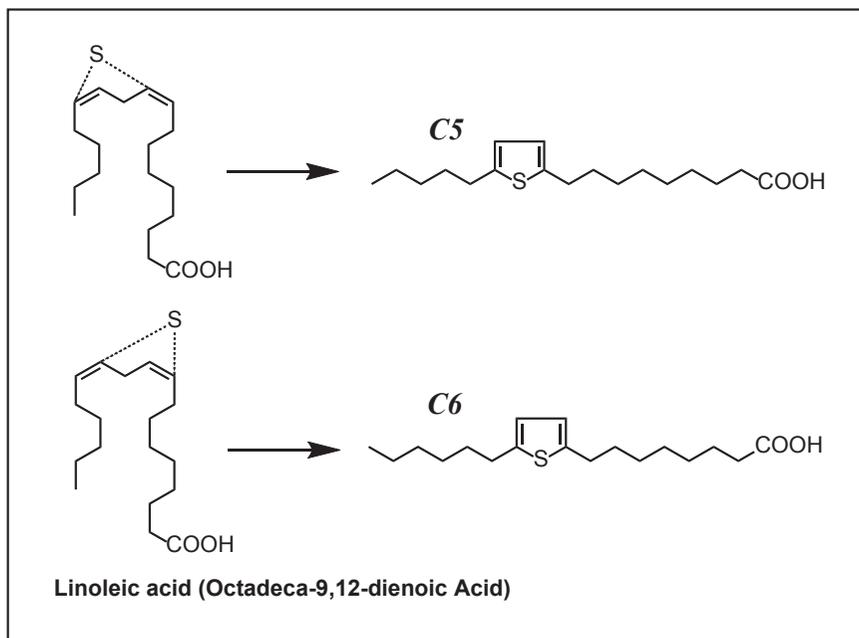


Figure 9. Possible sulfurization pathway of linoleic acid to give C5 and C6 TCAs.

5. Conclusion

Trace but several OSCs were identified in the sulfur-rich Lake Katanuma sediment. They were C₁₃–C₂₀ *n*-alkyl thiophenes, C₂₀ isoprenoid thiophenes, C₃₅ hopanoid thiophenes and C₁₈ thiophen carboxylic acids. OSCs other than C₂₀ isoprenoid thiophenes were of first identified in modern environment.

The formation of several OSCs in the Lake Katanuma surface sediment should be facilitated by high sulfur concentration as well as by a large heat flow supplied from beneath the lake bottom. Considering that sulfur readily reacts with C=C double bonds, particularly dienes having structures facile to give thiophene ring (Sinninghe-Damsté et al., 1989), the precursors of the TCAs are definitely octadecadienoic acids (for instance, linoleic acid: *cis, cis*-9,12-octadecadienoic acid) common to most organisms and its isomerization products in the environment. Laboratory experiment of linoleic acid and the solvent extracts (lipids) from lacustrine sediments in H₂S-saturated water, substantiate the pathway as a likely process.

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