The structure of sn-2-sestaterpenyl (C25) “extended” archaeol with the comparison of sn-3-sestaterpenyl counterpart in halophilic archaeal lipid core and possibility of novel halophilic archaea

Noriaki Yamauchi *

(Received July 9, 2019; Accepted November 13, 2019)

Abstract

Two potential regioisomers of “extended” C20-C25 archaeol were chemically prepared and mass spectra of their trimethyl silyl ethers were observed. The previously reported biological samples were confirmed as sn-2-sestaterpenyl (C25) isomer, with the longer C25 isoprenoidal chain linked at the center hydroxyl group of the glycerol. The mass spectrum of a sn-3-sestaterpenyl isomer was different. However, the corresponding C20-C25 archaeol extracted from a halite previously reported could be a mixture of the two regioisomers. The result suggests the existence of novel (unrevealed) halophilic archaea inhabiting ancient hypersaline environments or that grow very slowly in halites.

1. Introduction

Halophilic archaea inhabit hypersaline environments such as estuaries, salt lake, and salterns (Kates, 1977, 1993). In addition, they have been isolated from halite and commercial salt (Schubert et al., 2010; Minegishi et al., 2010). Considering their tolerance to relatively severe conditions and environments, including desiccation, cosmic ray radiation, and high pressure (Kotthenmann et al., 2005; Kish et al., 2012; Webb et al., 2013), they are potential organisms that could survive in other planetary environments such as Mars. In contrast to the uniqueness of the thermophilic archaea which are thought to reflect the earth’s prebiotic environment because they can inhabit thermoacidophilic environments, halophilic archaea are intriguing organisms from an ecological standpoint in space environments. They had the tolerance to severe conditions and environment which is sometimes occurred in the other planet than earth. Studies with the aim of identifying halophilic archaea in Mars have been conducted (Fendrihan et al., 2009, 2012; Foster et al., 2010; Oren et al., 2014).

The most characteristic feature in archaea is isoprenoidal lipid bearing glycerol (Kates 1993; Kamekura and Kates 1999). With regard to the isoprenoidal lipid, a major part of the lipid core in halophilic archaea is known as an archaeol (1). Two C20 isoprenoidal lipids are linked with the glycerol, which makes the sn-2,3-diterpenyl (C20-C25) glycerol shown in Fig. 1 (Kates et al., 1967). In addition, in halophilic archaea, one isoprenoidal chain is altered with a C25, sesterterpenyl alcohol, which is sometimes called an “extended” archaeol (C20-C25 archaeol, 2) as shown in Fig. 1 (De Rosa et al., 1982). Some thermophilic archaea may produce C20-C25 archaeol derivatives. However, almost all the identification of 2 is from the halophilic archaea, which are sometimes alkaliphilic halophilic archaea (De Rosa et al., 1982; Tindall et al., 1984; Morita et al., 1998; Xu et al., 1999, 2001; Waïnø et al., 2000; Xin et al., 2000; Hezayen et al., 2002; Castillo et al., 2006; Gutiérrez et al., 2007; Minegishi et al., 2010). In addition, C25-C25 diether lipid cores have been isolated from the lipid cores of halophilic archaea (De Rosa et al., 1983) and hyperthermophilic archaea (Mori et al., 1999).

When the different ether chains are linked with glycerol, the two regioisomers are thought to be C20-C25 archaeol derivatives (2 and 3 in Fig. 1). In an earlier study by De Rosa et al., in an alkaliphilic halophilic archaea’s lipid core, the authors determined that the sesterterpenyl (C25) saturated isoprenoidal unit was linked to the sn-C-2 position of glycerol as shown (2 in Fig. 1) through the isolation of normal archaeol with HPLC (high-performance liquid chromatography) and fragmentation analyses of its acetate derivative (De Rosa et al., 1982). Morita et al. also isolated C20-C25 ar-
chaeol derivatives from halophilic archaea and benzolate using centrifugal partition chromatography, and determined the C-2 linkage of saturated C<sub>25</sub> isoprenoidal portion through mass fragmentation of the benzolate derivative (Morita et al., 1998). Therefore, the biological sample of C<sub>20</sub>-C<sub>25</sub> archaeol from halophilic lipid cell was <i>sn</i>-2-O-sesterterpanyl-3-phytanyl glycerol 2 only.

Teixidor et al. identified archaeol and C<sub>20</sub>-C<sub>25</sub> archaeol from cultured samples of halophilic archaea and organic matter extracted recent halite samples from a solar saltern in Santa Pola, and Tertiary halite samples from Lorca and the Remorinoth Basin in Spain with trimethylsilyl (TMS) derivatives in a short capillary column and capillary GC-MS spectrum (Teixidor et al., 1993).

In addition, HPLC-APCI (atmospheric pressure chemical ionization) analyses and identification methods for archaeol derivatives in environmental samples (Turich and Freeman 2011) and intact polar lipids in cultured lipid samples were developed (Yoshinaga et al., 2011). Recently, Natalicchio et al. reported an archaeal biomarker record of paleoenvironmental change before and after the Messinian Salt Crisis and the potential of the effectiveness of the analysis of C<sub>20</sub>-C<sub>25</sub> archaeol in high salt concentration environments (Natalicchio et. al., 2017).

Dawson et al. also explored the analysis of saturated and unsaturated C<sub>20</sub>-C<sub>25</sub> and C<sub>20</sub>-C<sub>25</sub> archaeol derivatives in several halophilic archaea at different salt concentrations. Some species produced considerable amounts of unsaturated C<sub>20</sub>-C<sub>25</sub> and C<sub>20</sub>-C<sub>25</sub> unsaturated archaeol derivatives, and the relationships between the amount of unsaturated archaeol derivatives and salt concentrations were discussed (Dawson et al., 2012). This results were quite intriguing. The results revealed the relationships between C<sub>20</sub>-C<sub>25</sub> archaeol, unsaturated archaeol derivatives and the diversity of halophilic archaea's lipid core and environmental change.

However, in Teixidor and Dawson's reports, the presented structures of C<sub>20</sub>-C<sub>25</sub> archaeol (and its unsaturated derivatives) is <i>sn</i>-2-O-phytanyl-3-O-sesterterpanyl (and sesterterpenyl) isomers 3. This discrepancy has been sometimes observed in the literature, while the determination of the structure of the biological samples were 2-O-sesterterpanyl isomer 2. In addition, the mass spectra of the TMS ether derivatives of C<sub>20</sub>-C<sub>25</sub> archaeols were different between the two reports. The discrepancy could be due to lack of analytical data on “unnatural” <i>sn</i>-2-O-phytanyl-3-O-sesterterpanyl isomer 3. However, the existence of 3 in environmental samples cannot be denied currently. The two regioisomers cannot be distinguished using the APCI ionization of archaeol derivatives by using the tandem mass spectrometry daughter scan of archaeol (Turich and Freeman, 2011; Yoshinaga et al., 2011).

The author’s investigation of biosynthetic studies and analysis of 1 and 2 were conducted in halophilic archaea cells at different environmental condition. The precise fragmentation analysis of TMS ether derivative of archaeol, C<sub>25</sub>-C<sub>25</sub> archaeol, and the fragmentation of the TMS ether of several unsymmetrical alkyl diethers have previously been elucidated (Yamauchi 2008, 2014). The natural 2 have chiral methyl groups at the C-3<sub>l</sub>, 7<sub>l</sub>, 11<sub>l</sub>, 15<sub>l</sub> at C<sub>25</sub> isoprenoidal and C-3<sub>r</sub>, 7<sub>r</sub>, 11<sub>r</sub> at C<sub>20</sub> isoprenoidal hydrocarbon and ether portion (Fig. 1). The natural 3 have also the chiral methyl groups at C<sub>25</sub> and C<sub>20</sub> hydrocarbon residues. From the point of raw material supply, phytol is inexpensive and available in large quantities. However, the methyl group in at C-3 from phytol with simple catalytic hydrogenation yield racemic compound. Chiral catalytic hydrogenation or other asymmetric synthesis about the C-3<sub>l</sub> of...
C₂₅ (and C₃₅) isoprenoid methyl group is needed to the "complete synthesis of the natural extended archaeol". However, the difference of 1 and 2 in several chemical properties was unknown at this time, and the differences in the stereochemistry of the C-3 methyl group in C₂₅ and C₃₅ isoprenoidal portion may not influenced mass fragmentation behavior. Further, it was expected that the mass spectrum would be different if the length of the ether residue was different from literature (Pancost et al. 2001) and previous studies (Yamauchi 2008, 2014). Then, the target compound of the synthesis was set to 4 and 5 where the corresponding methyl group is a racemate. In the present study, the two regiosymmetric diethers of possible C₂₅-C₃₅ saturated isoprenoidal diethers (4 and 5) were chemically synthesized and the mass fragmentations were observed.

2. Materials and Methods

Infrared spectra were obtained using a PerkinElmer Spectrum One FT-IR spectrometer (PerkinElmer Inc., Waltham, MA, USA). Samples were dissolved in CHCl₃ and measured in a NaCl cell. ¹H-NMR and ¹³C-NMR spectra were recorded on an ECP-400, or ECZ-400 spectrometer (JEOL Ltd., Tokyo, Japan). Tetramethysililane (0 ppm) was used as internal standards for ¹H-NMR spectra recorded in CDCl₃, respectively. ESI-MS was recorded using an ABSciex Mariner spectrometer (SCIEX, Framingham, MA, USA). GC-MS analyses were conducted on a Shimadzu QP-5000 spectrometer (Shimadzu Corp., Kyoto, Japan). Chromatographic separations were carried out over silica gel (Merck Kieselgel 60, 70–230 mesh, Merck KGaA, Darmstadt, Germany).

Chemical synthesis of sn-2-O-sestaterpenyl-3-O-phytanyl glycerol and the sn-3-O-sestaterpenyl counterpart.

Phytol was purchased from Tokyo Kasei Co. Ltd. Phytanol (7) was prepared from phytol with catalytic hydrogenation with PtO₂ in a hydrogen (H₂) atmosphere (Fürstenau et al., 2012).

3,7,11,15-tetramethylhexadecanal (8)

A solution of 5.24 g (17.5 mmol) of (phytanol, 3,7,11,15-tetramethylhexadecanol) in 40 ml of dichloromethane was cooled to room temperature. Dimethyl sulfoxide 3.8 ml (4.1 g, 52.5 mmol, 3 eq. for alcohol) and 14.8 g (52.5 mmol, 3 eq.) of phosphorous pentoxide were then added. The mixture was stirred for 1 hr. at room temperature. The mixture was re-cooled to 0°C in an ice-water bath and 9.7 ml (7.1 g, 70 mmol, 4 eq. for alcohol) of trimethyleneamine and stirred for 1 h at 0°C and 1 h. at room temperature. The mixture was diluted with 50 ml of hexane and 50 ml of water. The product was extracted with hexane (50 ml) two times. The combined organic layer was washed with brine and dried over Na₂SO₄. Filtration and evaporation of the solvent yielded an oily residue. The product was purified using a short silica gel column (hexane:EtOAc (ethyl acetate) 20:1) yielding 4.79 g (88.0 %) of 8 (Fürstenau et al., 2012).

Methyl 5,9,13,17-tetramethyl-2,3-octadecenone (9)

1-(triphenyolphosphanylidene)-2-propanone (6.6 g, 20.9 mmol, 1.3 eq.) of was added to a solution of 4.79 g (16.1 mmol) of 3,7,11,15-tetramethylhexadecanal (8) in 50 ml of toulene and the mixture was stirred for 1 h at 100°C for 12 h. The mixture was then cooled to room temperature and 50 ml of hexane was added and the precipitate filtered. The filtrate was evaporated and the residue was purified on a silica gel column (hexane: EtOAc 20: 1) to yield 4.46 g (82.6 %) of product 9 with E-isomer. The stereoisomers were not purified further and were used in subsequent steps.

¹H-NMR (400 MHz): H 0.83 (6H, d, J = 6.8 Hz), 0.85 (6H, d, J = 6.8 Hz), 0.89 (3H, d, J = 6.8 Hz), 1.05 to 1.28 (m), 1.62 (2H, m), 2.05 (1H, m), 2.21 (1H, m) 2.24 (3H, s), 6.06 (1H, dt, J = 1.2 and 16.0 Hz), 6.77 (1H, dt, J = 7.3 and 16.0 Hz). IR: 2956, 2869, 1667, 1623, 1463, 1364, and 980 cm⁻¹. EI-MS 336 (M⁺), 318, 278, 153, 111, 84, 71, 57, 43. HRMS (ESI, positive, [M+H]⁺) Caled. for C₁₉H₃₄O: 337.3473, Found:337.3487.

Methyl 5,9,13,17-tetramethyloctadecane (10)

5% Pd-C (30 mg) was added to a solution of 4.46 g (13.3 mmol) of methyl 5,9,13,17-tetramethyl-2,3-octadecenone (9) in 40 ml of EtOAc and the mixture stirred in a H₂ atmosphere. The mixture was filtered and evaporated. The residue was purified on a silica gel column (hexane:EtOAc 20:1) to obtain 4.24 g (94.4 %) of product 10.

¹H-NMR (400 MHz): H 0.82 (6H, d, J = 6.2 Hz), 0.84 (6H, d, J = 7.3 Hz), 0.86 (6H, d, J = 6.8 Hz), 1.04 to 1.59 (m), 2.13 (3H, s), 2.39 (2H, t, J = 7.9 Hz).


Ethyl 3,7,11,15,19-pentamethyl-2,3-icosenate (11)

Ethyl diethylphosphonoate (6.6 ml, 11.0 mmol, 3 eq.) was dissolved in 30 ml of tetrahydrofurane and NaH (60 % oil dispersion, 792 mg, 33 mmol, 3 eq.) was added to the solution. The mixture was stirred for
1 h at room temperature. Methanol (1 ml) was then added to quench excess NaH, and 3.80 g (11.0 mmol) of methyl 5,9,13,17-tetramethyloctadecane (10) was added in 20 ml of tetrahydrofuran and stirred for 30 min at room temperature. The mixture was then heated to reflux and stirred for 2 h. The mixture was cooled to room temperature, and 20 ml of water was added. The mixture was extracted with hexane (30 ml) and the water residue was re-extracted with hexane (30 ml). The combined organic layer was washed with brine and dried over Na2SO4. Filtration and evaporation of the solvent yielded an oily residue. The product was purified on a short silica gel column (hexane only to the solvent) to obtain 4.35 g (94.8 %) of product 11.

The combined organic layer was washed with brine and it was stirred for 1 h. Na2SO4 was filtered. Saturated aqueous NaHCO3 and brine, dried, and filtered, and concentrated to dryness. The residue was simply purified with a short silica gel column (hexane:EtOAc 20: 1) to obtain 4.35 g (94.8 %) of product 11.

Purified on a short silica gel column (hexane:EtOAc 20: 1) to obtain 3.80 g (94.8 %) of product 11.

The combined organic layer was washed with brine and dried over Na2SO4. Filtration and evaporation of the solvent yielded an oily residue. The product was purified on a short silica gel column (hexane only to the solvent) to obtain 4.35 g (94.8 %) of product 11.

1H-NMR (400 MHz): H 0.84 (6H, d, J = 6.8 Hz), 0.86 (9H, d, J = 6.8 Hz), 0.92 (3H, broad, OH), 3.68 (2H, m), 3.80 (2H, m).

3H-NMR (400 MHz): H 0.84 (6H, d, J = 6.8 Hz), 0.86 (9H, d, J = 6.8 Hz), 1.00 to 1.69 (m), 1.93 (1H, t, J = 6.0 Hz), 1.97 (1H, t, J = 6.2 Hz), 2.30 (1H, broad, OH), 3.68 (2H, m). 13C-NMR (100 MHz) : 19.68, 19.75, 19.83, 22.71, 22.80, 24.46, 24.56, 24.88, 28.05, 29.60, 29.78, 32.86, 37.37, 34.48, 37.53, 37.58, 39.45, 40.01, 40.10, 61.28. IR: 3623, 3460 (broad), 3019, 2955, 2928, 2869, 1378, 1213, and 1063 cm⁻¹. El-MS (trimethylsilyl derivative) 425 ((M-CH3)⁺), 350((M-(CH3)-SiOH)⁺), 297, 181, 167, 153, 111, 97, 83, 71, 57, 43. Ex. Anal., Caled for C25H53O: C 81.44, H 14.22, Found: C 81.60, H 14.36.

1-(3)- O-3',7',11',15'-tetramethyhexadecylglycerol was prepared according to the method described by Joo et al. (Joo et al., 1968), while 1,2(2,3)-di-O-isopropylidene glycerol was prepared with racemic from glycerol and acetone.

1-(3)- O-3',7',11',15'-Tetramethyhexadecyl-2-O-3',7',11',15'-pentamethylicosanylglycerol (4) was prepared by the oxidation of 3,7,11,15,19-pentamethylicosanol (13) with dimethyl sulfoxide and phosphorus pentoxide similar to the preparation of 7 from 6 described as above. Simple purification with a short silica gel was carried out in the next step. A mixture of 1(3)- O-3',7',11',15'-tetramethyhexadecylglycerol (14) (303 mg, 0.78 mmol), 3,7,11,15,19-pentamethylicosanol (15) (283 mg, 0.76 mmol), p-toluenesulfonic acid (20 mg), and anhydrous Na2SO4 (800 mg) in 4 ml of CH2Cl2 was stirred at room temperature for 3 h. The mixture was diluted with 20 ml chloroform and Na2SO4 was filtered. Saturated aqueous NaHCO3 (20 ml) was then added to the mixture and the organic phase was separated. The aqueous phase was simply purified with chloroform (20 ml). The combined organic layer was washed with brine, dried, and filtered, and concentrated to dryness. The residue was simply purified with a short silica gel column to obtain acetol 16. The acetol (224 mg) was dissolved in 6 ml hexane and a hexane solution of diisobutylaluminum hydride (DIBAL) (1M, in hexane, 6 ml, 6 mmol) was added to the acetol solution at room temperature. The mixture was stirred for 1 h at room temperature. The reaction mixture was quenched by the addition of saturated NH4Cl and the mixture stirred for 10 min. EtOAc and 2M HCl were added. The organic layer was washed with saturated NaHCO3 and brine, dried over Na2SO4, filtered, and concentrated to dryness. The residue was chromatographed over silica gel with hexane-EtOAc (20: 1 to 10: 1) to obtain a less polar product 1(3)- O-3',7',11',15'-tetramethyhexadecyl-3(1)- O-3',7',11',15',19'-pentamethylicosanylglycerol (17) (188 mg, 34.2 %). A more polar product (4) (184 mg, 33.6 %). The titled (desired) product 4 was the more polar one.
The structure of sn-2-sestaterpenyl (C25) extended archaeol with the comparison of sn-3-sestaterpenyl counterpart

17; 'H-NMR (400 MHz, CDCl₃): H 0.84 (12H, d, J = 6.2 Hz), 0.86 (15H, d, J = 6.8 Hz), 0.87 (6H, d, J = 5.2 Hz), 1.04 ~1.62 (m), 2.00 (1H, broad, OH), 3.56 (1H, q, J = 6.0 Hz), 3.66 (1H, dt, J = 6.0 and 9.6 Hz), 3.44 (4H, m), 3.49 (4H, m), and 3.93 (1H, quintet, J = 5.7 Hz). ¹³C-NMR (100 MHz): δ 19.70, 19.78, 19.84, 22.70, 22.80, 24.45, 24.57, 24.88, 28.06, 29.79, 29.97, 32.88, 36.68, 36.75, 37.15, 37.23, 37.38, 37.49, 37.54, 37.58, 39.46, 63.19, 68.74, 70.26, 71.08, 78.35. IR: 3580, 3468, 3010, 2956, 2928, 2869, 1463, 1377, and 1110 cm⁻¹. The structure of sn-2-sestaterpenyl (C25) extended archaeol with the comparison of sn-3-sestaterpenyl counterpart

(4: 1) to give the standard lipid (1) and 2, as a mixture) from archaea.

GC-MS sample preparation and analytical condition

GC-MS spectra were recorded with a Shimadzu QP5000 spectrometer (Shimadzu Corp., Kyoto, Japan). Samples were prepared with the addition of 100 Sl of N, O-bis(trimethylsilyl) trifluoroacetamide with 1 % trimethylchlorosilane to alcohols (~ 0.1 mg). GC-MS analytical conditions were as follows. The column was an Inert Cap 5 (OV-5 equivalent), 30 m × 0.25 mm, 0.4 μm (GL Science Inc., Torrance, CA, USA). The injection temperature was at 300°C, GC-MS interface temperature at 260°C, and column temperature initially at 180°C, elevated at 3°C/min to 320°C, and held at the final temperature for 10 min. The helium carrier gas was at 100 kPa and mass spectra scanned at m/z 40 to 600 at 2 sec interval at 70eV as an ionization voltage and 260°C as ionization temperature. At this condition, the retention time of chromatograph at TMS derivative of these C₁₂-C₁₅ archaeol were 63.5 min. No separation of each isomer was observed at this condition and other (OV-1 or OV-17 equivalent 30 m length) columns.
3. Results and discussion

Synthesis of two regioisomeric isomers of C_{20}-C_{25} archaeol

Numerous research on the synthesis of archaeal ether lipid analogs has been published. However, in the present case, different alkyl chains were needed to connect regiospecifically at the sn-2 and sn-1(3) of the glycerol derivatives. The synthetic routes to the two lipid core are illustrated in Scheme 1. The C_{25} alkyl chain was elongated from a C_{20} isoprenoid phytol. At first, the double bond of phytol was reduced through catalytic hydrogenation. The differences in the stereochemistry of the C-3 methyl group and stereochemistry of glycerol does not influence gas chromatographic and mass fragmentation behavior, which was confirmed with the synthetic preparation of archaeol equivalent 6 using a Kates' procedure (Joo et al., 1968). No gas chromatographic separation behavior or a carbon resonance peak in $^{13}$C NMR spectra was observed. Therefore, stereochemistry of the C-3 methyl group of the C_{20} and the C-7 methyl group C_{25} alkyl chains were racemic. The C_{25} saturated isoprenoidal alcohol was prepared as follows in Scheme 1. Racemic phytanol 7 was converted its aldehyde 8 by oxidation of 7 with dimethylsulfoxide and P_{2}O_{5}. Three carbon elongation to 8 was performed by the Horner-Emmons reaction of 1-(triphenylphosphanyliden)-2-propanone, yielding an unsaturated C_{25} ketone (exclusively E-isomer) 9. The catalytic hydrogenation yields a saturated C_{25} ketone 10. In addition, two carbon elongation was also carried out with the Horner-Emmons reaction of ethyl diethylphosphonoacetate and 10 to obtain an unsaturated C_{25} carboxylate 11 in the E-Z mixture (E:Z = 7:2, unidentified). Catalytic hydrogenation of 11 yielded saturated ester 12. As stated above, the stereochemistry of the methyl group in the C_{25} hydrocarbon not be influenced by the mass fragmentation of the final diether. The ester was finally reduced to alcohol using LiAlH_{4} to obtain a C_{25} alcohol 13.

The linking of different alkyl chains at the C-2 and C-1(3) of the glycerol portion was carried out as follows as shown in Scheme 2; 1) The first alkyl chain at C-1(3) was introduced at the classical ether linkage formation of 1,2-isopropylideneglycerol and alkyl bromide, 2) The second alkyl chain was introduced with the acetal for-
mation of C-1(3) alkylated 1,2-diol and alkyl aldehyde. Reduction of cyclic acetal with DIBAL gave the 1,3-di-
alkylated 2-ol and 1,2(2,3)-dialkylated 3(1)-ol (Eguchi et
al., 1997). Considering the nature of secondary alcohol
and primary alcohol, the two diethers were easily separ-
ated using a silica gel column and the more polar one
was the desired C_{25}, C_{25} archaeol derivative. The reduc-
tion of acetal with DIBAL yielded two compounds at al-
most similar yields (1:1) and a half of a diether with an
undesired compound. However, the regioselective diether
was obtained following a change in the compound at the
first alkylation and introduction of second alkyl chain.
The synthetic two isomers of C_{20}, C_{25} archaeol (4 and
5) were almost identical in the 'H NMR and 'C NMR
spectra. However, mass fragmentation behavior in the
TMS ethers of the two compounds was different, as
described below.

Mass fragmentation analysis of two isomer of “natural” and unnatural C_{20}, C_{25} archaeol

Before the analysis and of the fragmentation of the synthetic C_{20}, C_{25} archaeol isomers, Satouchi et al. ob-
served mass spectra of the TMS ether of 1,2-dialkylglyc-
erols (with a similar alkyl chain), and key fragmenta-
tion of the determination of the length of alkyl chain was
an alcoholic residue with an m/z corresponding to
[R] (R = alkyl chain), [RO-1 + 73], and [R + 131] (Sa-
touchi et al., 1978). Deuterium labeling experiments
with the natural archaeol and C_{20}, C_{25} archaeol mixture
and observation of mass spectra with 1,2-dialkylglyc-
erol which the different alkyl group was linked at C-1
and C-2 concluded in a previous short report (Yamauchi 2014). The critical fragment of the TMS ether
of the 1,2-dialkylglycerol was the three m/z corre-
B745.519 and the structure of the TMS ether of the 1,2-
dialkylglycerol were ([R + 131], [R + 145], [R_{2} + 88], R_{1} = C_{20}H_{41},
R_{2} = C_{20}H_{41}) identical with those predicted (Fig. 2).
However, it was a rather simple spectrum when com-
pared with the diether in the halite in Teixidor's report
(Teixidor et al., 1993). The rather simple mass spectra
with the lipid core obtained from the microbiological
source were also observed in the benzoate of C_{20}, C_{25}
archaeol reported by Morita et al (Morita et al., 1997).

The mass fragmentation behavior of the two syn-
thetic TMS ether of C_{20}, C_{25} archaeol isomers (4 and
5) was observed. Under gas chromatographic conditions,
the two isomers were not distinguishable based on re-
tention time as same as 'H and 'C NMR spectra.

The mass spectrum of the TMS ether of 4 (Fig. 3c)
corresponding to the “natural” isomer 2 with the C_{25}
long chain linked at the O-2 was identical to the spec-
trum from the microbiological sample (Fig. 3b) and
the spectrum described by Dawson et al (2012). The ob-
served fragments were m/z 369, 426, and 482. Also, the
critical fragments of the TMS ether of the 1,2-di-
alkylglycerol are ([R + 131], [R + 145], [R_{2} + 88], R_{1} = C_{20}H_{41},
R_{2} = C_{20}H_{41}) identical with those predicted from the
structure of the diether (Fig. 2). Alkyl chain derived
mass fragment was also determined to be m/z 278.

The mass spectrum of the TMS ether of 5 corre-
sponding to the “unnatural” isomer 3 with the C_{25}
long chain linked at the O-1(3) (Fig. 3d) was also rather simple
spectra compared with the spectra obtained from the
halite sample, but quite different from the “natural”
one. The observed fragments were m/z 348, 412, 439, and
496. The three critical fragments of the TMS ether
of the 1,2-dialkylglycerol are ([R + 131], [R + 145],
[R_{2} + 88], R_{1} = C_{20}H_{41}, R_{2} = C_{20}H_{41}) identical to that of
the prediction (Fig. 3). Alkyl chain derived mass frag-
ment was also determined to be m/z 348. It may be as-
signed by the [C_{20}H_{41}], apparently different from the
mass fragmentation of 4.

The result confirmed the regioisomeric structure of
C_{20}-C_{25} archaeol, for which the ether linkage in the compound at longer chains was at O-2, the center secondary alcoholic residue of glycerol. The result is consistent with that of De Rosa et al. (1982) and the result reported by Morita et al (1997). The present study could minimize any inconsistency.

However, a comparison of Teixidor's spectrum (Teixidor et al., 1993) and the results of the present study are still different. In addition, if my two mass spectra of the TMS ether of natural and "unnatural" C_{20}-C_{25} archaeol were overwrapped within one sheet, the overwrapped "spectrum" was almost similar in appearance to Teixidor's C_{20}-C_{25} archaeol.

Three interpretations could be made from the result. 1) Teixidor's spectrum of C_{20}-C_{25} archaeol from halite was not clear due to the presence of contaminants. 2) Teixidor's sample was an isomeric mixture of C_{20}-C_{25} archaeol isomers (2 and 3) because the presence of the halophilic archaea produced isomeric C_{20}-C_{25} archaeols selectively or two isomeric mixtures non-selectively. 3) Teixidor's sample was an isomeric mixture of C_{20}-C_{25} archaeol isomers converted from an original C_{20}-C_{25} archaeol (2) through biological or nonbiological processes.

Compared with the spectrum obtained from the TMS ether of the C_{20}-C_{25} archaeol in Teixidor's samples, the spectrum of the TMS ether of the C_{20}-C_{25} archaeol is slightly unclear. However, the relative intensities of the base peaks could be observed in the corresponding fragments. Nonbiological selective migration of ether bonds may be difficult considering the chemical nature of ether, and a biological process may be improbable.

However, Teixidor's samples were collected from the Messinian evaporite. The extinct halophilic archaea may have existed in the Messinian age (around 6 Ma). Very small halophilic archaea in halites have also been found. Walsby's "square bacterium" found on the surface of a halite (Walsby 1980) revealed very thin slow growing halophilic archaea (Bolhuis et al 2004; Burns et al., 2004). Currently extinct halophilic archaea might be lived in habitating ancient hypersaline environ-
ments such as Messinian Salt Crisis, and may accumulate the unusual C_{20}-C_{25} archaeol and the usual archaeol in halite, or very slow growing halophilic archaebacteria may inhabit such halites and produce the C_{20}-C_{25} archaeol regioisomer. The C_{20}-C_{25} archaeol from the biological sample was from a well-growing cultured broth. Studies of the structure of the lipid core of slow growing archaea have not been reported and it may be difficult to determine the fine structure.

4. Conclusion

Two regioisomers of “extended” C_{20}-C_{25} archaeol with the longer chain is linked with sn-2 and sn-3 isomer were chemically synthesized for the first time. The mass spectrum of the trimethylsilyl ether derivative of the two regioisomers was obviously different from each other. The result of the fragmentation analysis was consistent with the analysis from literature (Pancost et al. 2001) and previous studies (Yamauchi 2008, 2014). The regiochemistry of the previously determined C_{20}-C_{25} archaeol from microbial samples were confirmed as sn-2-2-sestaterpenyl (C_{25}) isomer. However, the content of C_{20}-C_{25} archaeol fraction extracted from halite was strongly suggested as a two sn-2-C_{25} and sn-3-C_{25} isomer. The result may induce a discovery of new, unrevealed halophilic archaea such as inhabiting in halite (Schubert et al., 2010; Fendrihan et al., 2012) having sn-3-C_{25} isomer as a diether lipid core.

Acknowledgments

400 MHz NMR measurements were conducted as the Centre of Advanced Instrumental Analysis, Kyushu University. Elemental analyses were measured at the Service Centre for the Elementary Analysis of Organic Compounds, Faculty of sciences, Kyushu University. I am grateful to Dr. K. Asahina (National Institute of Advanced Industrial Science and Technology (AIST)) for constructive comments and suggestions helped improve this manuscript.

References


Society, Washington DC.