Short Article

Preparation, and analysis of 2-hydroxyarchaeol isomeric mixture prior to the determination of stereochemistry

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Abstract

A mixture of 2-hydroxyarchaeol isomers is chemically synthesized from commercially available racemic isophytol. In the gas chromatogram of TMS-derivative of the synthesized material, two peaks of equal intensity are observed, suggesting the occurrence of two stereoisomers at C-3 (hydroxylated carbon in the isoprenoid chain). The ^{13}C NMR spectrum of the synthesized material is slightly different from that of 2-hydroxyarchaeol from microbial origin, which is also consistent with the occurrence of two isomers in the former. By contrast, natural 2-hydroxyarchaeol from microbes and sediments is inferred to consist of a single stereoisomer at C-3.

Key words: methanogen, AMNE, archaea, hydroxyarchaeaol, stereochemistry

1. Introduction

Archaea live in a relatively harsh condition such as marine hydrothermal vents (high temperature, low pH), swamp mud (anaerobic), and salt marsh (high salt concentration). Their living condition may be thought to resemble to the environment of primitive earth. Among them, "methanogens" are obligate anaerobic archaea and live in wetland, marsh, sediment, and in digestive tract of ruminant. They can produce methane from substrates such as H_2/CO_2, formate, acetate, or methyl compounds (methanol, methylamines, dimethyl sulfide) by characteristic CH_3-S-CoM reductase with the aid of F_{430} cofactor (Ferry, 1992).

Instead of the main isoprenoidal diether lipid-core (archaeol (**1**)) commonly observed in archaea especially in the *Euryarchaeota*, the major lipid-core of anaerobic archaea including methanogen and methanotrophs, which can oxidize methane anaerobically, are 2- and 3-hydroxyarchaeol (**2** and **3**).

Sprott and coworkers isolated the novel lipid core 3-hydroxyarchaeol (**3**) from *Methanothrix concilii*, and 2-hydroxyarchaeol (**2**) from *Methanosarcina* species (Sprott et al. 1990). These structural studies only used by ^{13}C and ^{1}H NMR spectroscopy.

Anaerobic oxidation of methane (AOM) is one of the major sinks of methane in marine environment (Zhang et al. 2003). This process involves both anaerobic archaea and sulfate reducing bacteria (SRB). The
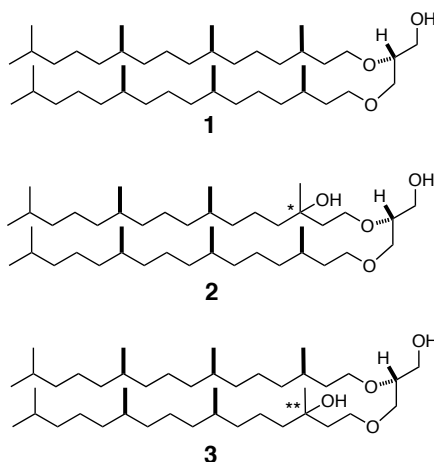

Fig. 1. Structure of isoprenoidal lipid-core of archaea.

archaeal parts of the microorganisms are three distinct anaerobic methanotrophs named AMNE-1, 2 and 3 (Niemann et al. 2006). Methane hydrate is an important resources in near future. A significant part of methane is produced by methanogens under seafloor in the sediment under the AOM layer, which is characteristic low δ^{13}C and δ^2H value of methane in methane hydrate (Whiticar 1999). Classification of AMNE-1, 2, and 3 is determined genetically, but molecular evidence for genetic counterpart is also reported (Blumenberg et al. 2004). The classification of AMNE is important for

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microbial ecology of consortia in AOM, and relation of the activity of AOM and methane hydrate accumulation in the field.

Hydroxyarchaeol is an important biomarker for the existence and the nature of AOM and methane hydrate accumulation. The analyses of hydroxyarchaeol in sediments (and a ruminant source) were accomplished with the TMS ether derivative (Standiniskaia et al. 2005). Thus, the standard sample should be necessary for wider analysis in this field. The supply for these is restricted from the microbial source, and the obligate anaerobic condition is needed for the incubation of methanogen and methanotrophs. If the stereochemical ambiguity of hydroxyl group is affordable for the analytical standard, the supply for the chemical synthesis may be very easy. Thus, the synthesis of hydroxyarchaeol "equivalent" molecules were planned and carried out.

2. Materials and methods

Phytol was purchased from Kanto Kagaku Co. ltd. For the removal of non-polar impurity ($\sim$5%), the purchased material was purified by silica gel chromatography. Chromatographic separations were carried out with Merck Kieselgel 60, 70–230 mesh columns (Merck KGaA, Darmstadt, Germany). Isophytol (racemic) were purchased from Tokyo Kasei Co. ltd. ^{13}C spectra were recorded with a JEOL EX-90 spectrometer (JEOL Ltd., Tokyo, Japan). The center signal of CDCl$_3$ (77.0 ppm) was used as an internal standard. GC-MS spectra were recorded with a Shimadzu QP-5000 spectrometer (Shimadzu Corp., Kyoto, Japan). GC-MS analytical conditions were as follows. The column was an Inert Cap 5 (OV-5 equivalent), 30 m $\times$ 0.25 μm, 0.4 mm df (GL Science, Inc., Torrance, CA, USA). The injection temperature was at 300ºC, GC-MS interface temperature at 260ºC, and column temperature initially at 180ºC, elevated at 3ºC/min to 320ºC, 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 intervals. At this condition, the retention time of chromatograph at TMS derivative of archaeol (1) was 63.5 min.

3. Results and discussion

3.1. Chemical Synthesis of 2-hydroxyarchaeol equivalent

Fig. 2 shows synthetic scheme. Starting from commercially available phytol, phytanyl iodide part 5 was prepared. Preliminary, the C-3 methyl group of 5 was prepared racemic for the easier preparation

from phytol with usual catalytic hydrogenation of 4. Also, hydroxyisoprenoidal part 7 was prepared from commercially available isophytol (hydroxyl group of isophytol is racemic). Compound 5 was condensed with chiral glycerol derivative 8 by a classic etherification manner, and then hydrolyzed to diol 9. Compounds 7 and 9 were made acetal in the presence of acid catalysis to give 10. Compound 10 was reduced with DIBAL (di-isobutylaluminium hydride), 11 (less polar, minor product) and 12 (more polar, major product) were obtained and can be separated with silica gel column chromatography (Eguchi et al. 1997). Deprotection of 12 yielded 2-hydroxyarchaeol equivalent 13.

3.2. ^{13}C NMR spectrum of 2-hydroxyarchaeol equivalent

Fig. 3 shows ^{13}C NMR spectrum of 13. The ^{13}C NMR spectrum of natural 2-hydroxyarchaeol (2) was previously presented by Sprott et al (Sprott et al. 1990). Compared with 2, almost identical spectrum was obtained. However, the peaks for C-4 and C-2' were clearly separated, and the lines for C-2, C-5, and C-17 were broadened. These line broadening and peak separation are observed for the carbons near C-3 (chiral carbon) in the isomer mixture. On the other hands, no peak separation or line broadening is observed for the glycerol carbons. Chemical shifts of carbons around C-3 may be slightly different between the 2 isomers at C-3 (hydroxylated carbon in the isopranyl group),
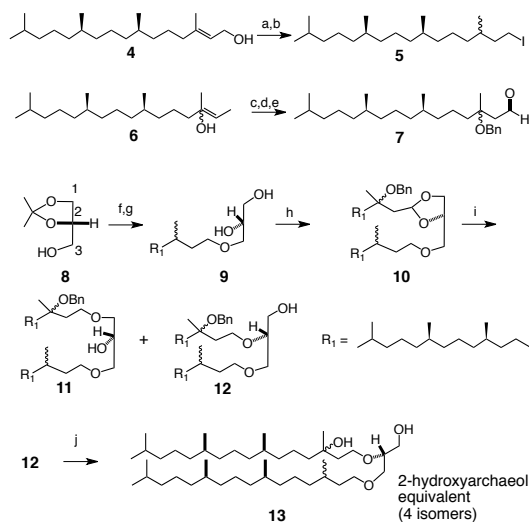

Fig. 2. Synthesis of 2-hydroxyarchaeol equivalent. Reagents and conditions, a: H$_2$/PtO$_2$; b: HI; 89 %, c: BnBr, NaH, nBu$_4$NI; d: BH$_3$-THF, then H$_2$O$_2$; e: DMSO, P$_2$O$_5$; 49 %, f: 5, KOH, Benzene, g: EtOH, HCl; 65 %, h: 7, pTsOH, MgSO$_4$; 67 %, i: DIBAL, 33 % for 11 and 66 % for 12, j: H$_2$/Pd-C, 86 %.

which may identify the stereochemisry of 2-hydroxy-archaeol. Therefore, the ^{13}C NMR spectrum of **13** is consistent with a mixture of (at least) two isomers of equal amount. This result further indicates that the natural 2-hydroxyarchaeol from microbial source consists of a single isomer at C-3.

3.3. Gas chromatographic behavior of 2-hydroxyar-chaeol equivalent.

Gas chromatographic trace and GC-MS spectrum and of di-TMS derivative of **13** is shown in Fig. 4. Same as ^{13}C NMR spectrum, two equal amount of diaste-reomers were separated on the gas chromatogram. The
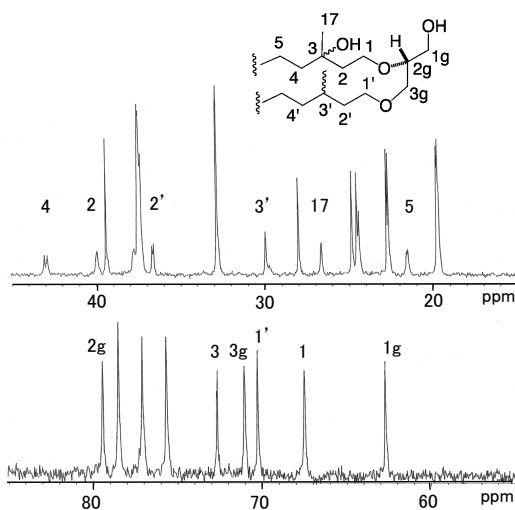

Fig. 3. ^{13}C NMR ($15-45$ ppm (upper) and $55-85$ ppm (lower)) of **13**. Signal assignment was according to Sprott et al.
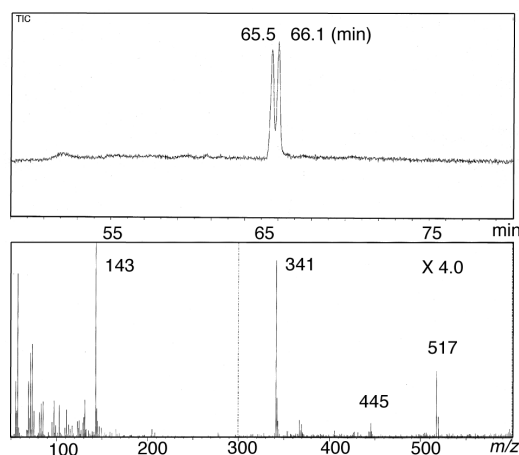

Fig. 4. Gas chromatogram (upper) and mass spectrum(lower) of **13**. Same specra were obtained from 2 peaks of chromatogram.

mass spectra of the two peaks were identical to that of 2-hydroxyarchaeol in the literature (Standinitskaia et al. 2005). 2-Hydroxyarchaeol (**2**) was detected (and quantitated) in sediment from the AOM or methano-genic sites (e. g. Ziegenbalg et al. 2012). These results showed 2-hydroxyarchaeol as a single peak. Several studies showed the coexistence of 3-hydroxyarchaeol (**3**), however, at different concentration.

Synthetic isomeric mixture **13** is not sufficient for the analytical standard for the analysis of **2** because stereochemical difference of the isomeric mixture of hydroxylated carbon in the isopranyl group is large enough to separate the di-TMS derivative of **13** on the gas chromatogram. However, a compound **2** may be a single isomer at the C-3 hydroxyl group from the microbiological source and, the sedimental source.

Kinetics of epimerization of **2** in burial diagenesis remains unclear. It will be possible to study the stability and structural change of **2** in thermal and some acidic, basic conditions by using with **13** as a structural standard alternative for **2**.

4. Summary and conclusion

This report presents the chemical procedures to synthesize a mixture of 2-hydroxyarchaeol isomers. The occurrence of (at least) two isomers is suggested from the ^{13}C NMR spectrum of the synthesized material in comparison with that of 2-hydroxyarchaeol from with microbial source. This possibility is further supported from the gas chromatogram of the TMS derivative showing two peaks of equal intensity, and from their mass spectra being identical to that of ditrimethylsi-lylated 2-hydroxyarchaeol from sediment. It is strongly suggested that 2-hydroxyarchaerol from natural source consists of a single stereoisomer at the hydroxylated carbon in the isoprenoid chain.

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