Geochemical Journal, Vol. 42, pp. 421 to 427, 2008

Acyclic hydrocarbons and ketones in cold-seep carbonates from central Hokkaido, northern Japan

SHIGENORI OGIHARA*

Department of Earth and Planetary Science, Graduate School of Science, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

(*Received March 30, 2007; Accepted May 8, 2008*)

Limestones associated with fossil chemosynthetic biological communities are found in Tappu Kanajirizawa and Teshionakagawa Abeshinaigawa, central Hokkaido, northern Japan. They were studied to ascertain their lipid distributions and $\delta^{13}C$ values of lipid in order to investigate molecular records of archaea and bacteria associated with the anaerobic oxidation of methane (AOM). The limestones contain the tail-to-tail linked irregular isoprenoid hydrocarbons, 2,6,11,15-tetramethylhexadecane (crocetane), and its C_{25}-homologue 2,6,10,15,19-pentamethylicosane (PMI). Furthermore, C_{30}-homologue 2,6,10,15,19,23-hexamethyltetracosane (squalane) was detected. The abundance of these compounds indicates a pronounced role of particular archaea in the cold-seep. The $\delta^{13}C$ values of PMI and crocetane were depleted, ranging from –129‰ to –116‰PDB. New biomarkers found were C_{13} and C_{18} isoprenoid ketones, which could potentially be from anaerobic methanotrophic archaea. Small amounts of C_{14} and C_{19} isoprenoid ketones were also detected. The C_{13}, C_{14} and C_{18} isoprenoid ketones also had extremely low $\delta^{13}C$ values ranging from –115‰ to –104‰. $\delta^{13}C$ values suggest that the origin of the isoprenoid ketones are lipids of anaerobic methanotrophioc archaea, but the diagenetic pathway leading to them is uncertain. This study shows that the limestones are cold seep carbonates and that isoprenoid ketones are potentially useful markers for anaerobic methanotrophic archaea.

Keywords: anaerobic oxidation of methane, biomarker, cold-seep carbonate, fossil chemosynthetic community, isoprenoid ketones

INTRODUCTION

Recent molecular and isotopic studies have reported the importance of methanogenic archaea and sulfate reducing bacteria (SRB) in an anaerobic methane consuming consortium (Elvert *et al*., 1999; Hinrichs *et al*., 1999; Thiel *et al*., 1999). These studies supported the hypotheses that methane is converted to carbon dioxide and hydrogen by methanogens operating in reverse metabolism with SRB. The specific feature is that anaerobic methanotrophic archaea (ANME) in anaerobic environments are novel methanogenic archaea with their reverse reaction (Hinrichs *et al*., 1999). These anaerobic methanotrophic archaea were distantly related to the order *Methanosarcinales* and *Methanomicrobiales* (Hinrichs *et al*., 1999). A combination of fluorescence *in situ* hybridization and secondary ion mass spectroscopy revealed that ANME cells are highly depleted in ^{13}C, and therefore most probably assimilate isotopically light methane (Orphan *et al*., 2002). Genomic evidence indicates that ANME harbor nearly all the genes necessary for methanogenesis (Meyerdierks *et al*., 2005).

Anaerobic oxidation of methane (AOM) produces an increase in the alkalinity of carbonates and favors the precipitation of authigenic carbonates (Aloisi *et al*., 2002). The association of methane seepage on the sea floor and oxidation of methane with carbonate precipitation was established on the basis of carbonate ^{13}C depletion. Thiel *et al*. (2001) and Peckmann *et al*. (1999) indicated that cold-seep carbonates contain biomarkers suggesting the presence of AOM, which plays an important role in cold-seep carbonate precipitation. The most important diagnostic lipid compound for AOM is ^{13}C depleted 2,6,10,15,19-pentamethylicosane (PMI) and probably also 2,6,11,15-tetramethylhexadecane (crocetane) (Peckmann *et al*., 1999; Elvert *et al*., 1999).

The present study was conducted from the AOM environment to increase our understanding of the origin of other organic compound groups such as acyclic isoprenoid ketones, with carbon numbers 13, 14, 18 and 19. There are some reports about the distribution of the C_{13} and C_{18} isoprenoid ketones in sediments (Simoneit, 1979; Ikan *et al*., 1973), water column (Rontani *et al*., 1991), and coals (Tuo and Li, 2005). Previously, little attention has been paid to their potential as biomarkers for AOM.

*E-mail address: ogi@eps.s.u-tokyo.ac.jp

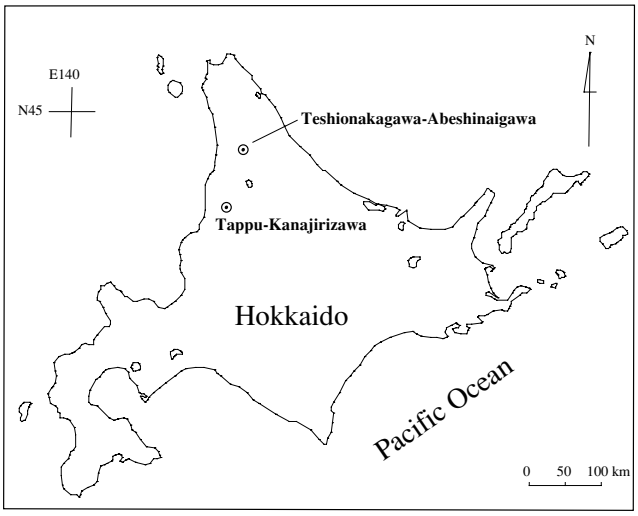

Fig. 1. Map showing the locality of TK (Tappu Kanajirizawa) and TA (Teshionakagawa Abesinaigawa) limestones.

SAMPLES

Two limestone samples collected from Tappu Kanajirizawa and Teshionakagawa Abeshinaigawa were used. Locations are shown in Fig. 1.

Tappu Kanajirizawa limestone (TK limestone)

The Upper Cretaceous sequences in the Tappu area, northwestern Hokkaido, are considerably thicker than in other areas in Hokkaido. The main part of the Cretaceous sequences, which are referred to as the Upper Yezo Group, consists of clayey and silty mudstones (Tanabe *et al*., 1977). A thin muddy limestone layer, containing a chemosynthetic community, is inserted into the clayey mudstone in the middle part of the sequence. Kanie *et al*. (1996) found *Thracia yezoensis* and *Miltha* sp. in the limestone layer and argued that these fossils corresponded to a recent chemosynthetic community dominated by the family of Lucinidae. The recent chemosynthetic communities are reported from the Gulf of Mexico (Neurauter and Roberts, 1994), Oregon subduction zone (Kulm *et al*., 1986) and Kattegat, off the Danish coast (Jensen *et al*., 1992).

This muddy limestone layer is around 4–5 m thick and has a radius of about 500 m. Chimney-shaped nodules, with a diameter between 5–10 cm and length between 15 and 25 cm, can be found above the limestone layer. The matrix of this limestone lens is micritic and characterized by scattered pellets cemented by fine calcite crystals. Prominent bioturbation was also observed in the limestone layer, and the cementation of sparry calcite occurred along with bioturbation. The fossils of worm tubes and calcite-cemented foraminifera could be partially observed in the limestone layer. Inoceramus fossils and ammonites were found in the mudstone above and below the limestone layer, indicating that the age of the limestone layer is Lower Cenomanian (Tanabe *et al*., 1977). Samples were collected from the bottom to top at 50 cm intervals. All samples were used for carbonate carbon and oxygen isotope analysis, and a sample for biomarker analysis was used from the micritic part at the center of the limestone layer.

Teshionakagawa Abeshinaigawa limestone (TA limestone)

Teshionakagawa Abeshinaigawa is located in northern Hokkaido (Fig. 1). The TA limestone has a lens shaped structure, with a diameter 10–15 m, and a thickness of 6–8 m. One lens was found in turbidite layers referred to as the Omagari Formation, which comprises the alternating layers of coarse sandstone, sandstone and mudstone. Slump structure was well developed in the turbidites with some pebbles scattered in the coarse sandstone. Ammonites and Inoceramus fossils, ranging from Coniacian to Santonian in age, were found in the turbidite layers. The upper part of this lens is micritic limestone that includes worm tubes. The lower part consists of muddy and silty calcareous breccia. The samples used in this study were collected from the upper part of the limestone lens. Hikida *et al*. (2003) found *Miltha* sp., *Thyasira* sp. and *Calyptogena* sp. within the lens and compared them to a cold-seep chemosynthetic community found in the present day marine environment. This limestone lens is an exceptionally well-preserved carbonate and includes a fossil chemosynthetic community. Samples were collected horizontally from the west (bottom of limestone lens) to east (top) at 1 m intervals. All samples were used for carbonate carbon and oxygen isotope analysis, and the clearest micritic tube worm limestone collected from under the top of the limestone lens was utilized for biomarker analysis.

ANALYTICAL PROCEDURE

Sample preparation

The massive limestone blocks were cut into 5 cm cubes with a diamond cutter and cleaned with dichloromethane/methanol (1:1, v:v). Each cube was crushed into small pieces with a hammer, and the pieces whose faces had not been exposed to the diamond cutter were collected and powdered using a vibration mill. These powdered samples were utilized for the following analysis.

Carbonate carbon and oxygen isotopes

The CO_2 for carbon and oxygen isotope analysis was obtained by reacting the sample powder with 100% orthophosphoric acid in a vacuum at 25°C. The CO_2 gas was analyzed using a Finnigan MAT 252 spectrometer.

The $\delta^{13}C$ and $\delta^{18}O$ results are reported relative to the PDB standard.

Biomarker analysis

Powdered samples (25 g) were put in a 250 ml centrifuge tube with distilled water washed by hexane and slowly dissolved using distilled hydrochloric acid (20 wt%). In order to avoid excess acidification, the addition was stopped before the carbonate had dissolved completely. The remaining residue was separated from the solution by centrifugation. After washing twice with distilled water, the samples were dried by freeze drier. The residues were extracted by ultrasonication in dichloromethane/methanol (93:7, v:v) until the solvent became colorless. The organic portion of the solvent phase was concentrated with a rotary evaporator.

The isolated lipids were divided into four fractions using silica gel column chromatography. A hydrocarbon fraction (N-1) was obtained with two column volumes of *n*-hexane, an aromatic hydrocarbon fraction (N-2) with two column volumes of *n*-hexane/dichloromethane (2:1, v:v), an aliphatic ketone and ester fraction (N-3) with seven column volumes of *n*-hexane/dichloromethane (1:1, v:v) and polar fraction (N-4) with excess dichloromethane/methanol (1:1, v:v).

The N-1 and N-3 fractions were examined by using gas chromatography-mass spectrometry (GC/MS) and combined gas chromatography-combustion-isotope ratio mass spectrometry (GC/C/IRMS). Compound identification was based on the comparison with published data. The GC/MS system was a ThermoQuest Voyager equipped with a 30 m fused silica capillary column (HP-5 ms, 0.25 mm i.d., 0.25 μm film thickness) and an on-column injector. The temperature program was: 40°C isothermal for 1 min, 40–300°C at 4°C min^{-1} and 300°C isothermal for 25 min. The GC/C/MS system consisted of a GC (HP 6890 gas chromatography equipped with a 30 m fused silica capillary column, DB-5, 0.32 mm i.d., 0.25 μm film thickness, and a splitless injector) coupled with a combustion interface (ThermoFinnigan GC combustion III) and Finnigan MAT Delta Plus isotope ratio mass spectrometer. The temperature program of GC was: isothermal at 60°C for 1 min, 40–300°C at 3°C min^{-1} and 300°C isothermal for 25 min. Stable carbon isotope compositions are given as $\delta^{13}C$ values vs. V-PDB and have an error less than 1.0‰ based on the measurements of the co-injected standard.

RESULTS

Stable isotope composition of carbonate

The $\delta^{13}C$ values of 10 TK limestone samples range from –43.4‰ to –39.9‰, and the $\delta^{18}O$ values range from –5.8‰ to –2.5‰. The $\delta^{13}C$ values of the 10 TA limestone samples range from –45.4‰ to –43.7‰ and the $\delta^{18}O$ values from –2.3‰ to +1.7‰. The carbonate carbon of both limestones is therefore significantly depleted in ^{13}C.

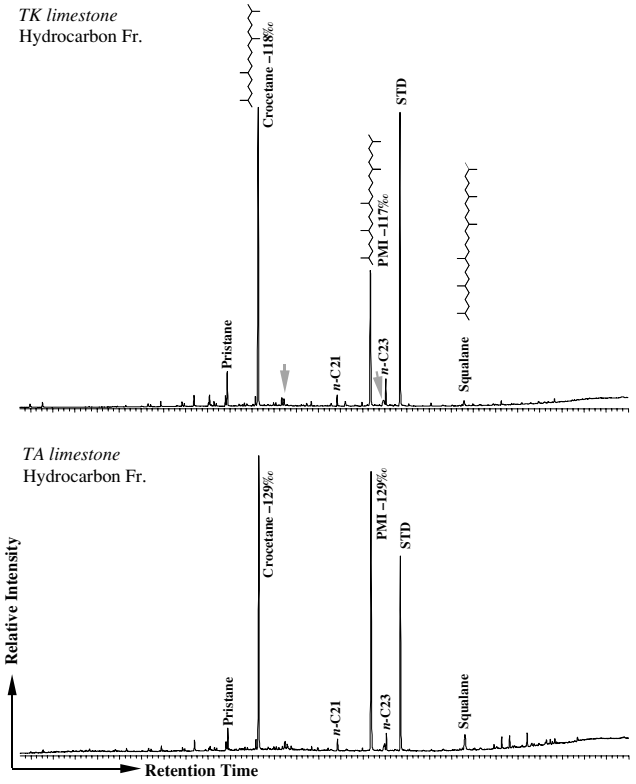

Fig. 2. Total ion chromatograms of hydrocarbon fraction from TK and TA limestones with structures and $\delta^{13}C$ values (vs. PDB). Grey spikes indicate unsaturated derivatives of crocetane and PMI.

Aliphatic hydrocarbons

TK limestone The tail-to-tail irregular C_{20} isoprenoid crocetane and its C_{25} homologue PMI were the main compounds in the aliphatic hydrocarbon fraction (N-1) of the TK limestone (Fig. 2, top). The abundance of crocetane was greater than that of PMI. Relatively small amounts of unsaturated derivatives of crocetane and PMI were detected only in TK limestone, as indicated by grey spikes. Squalane, another tail-to-tail linked isoprenoid hydrocarbon, was also detected in low abundance. Comparatively small amount of pristane was also found. In addition, relatively small quantities of *n*-alkanes were detected, these have a unimodal distribution ranging from C_{13} to C_{19}, with a maximum at C_{16}, although *n*-C_{23} and *n*-C_{21} are present in greater abundance. Stable carbon isotope analysis revealed extreme depletion of crocetane and PMI in ^{13}C, with $\delta^{13}C$ values g –118‰ and –117‰, respectively.

TA limestone Crocetane and PMI were the characteristic compounds in the aliphatic hydrocarbon fraction of the

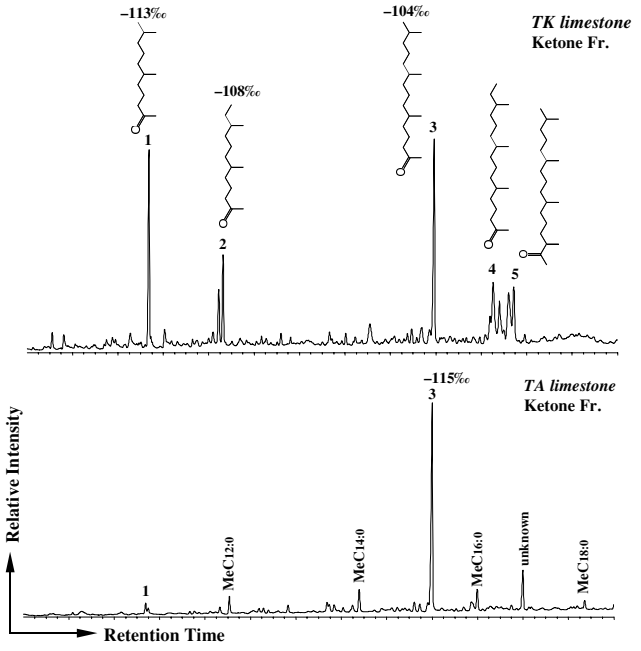

Fig. 3. Total ion chromatograms of ketone fraction from TK and TA limestones with structures and $\delta^{13}C$ values (vs. PDB). Fatty acid methyl esters are indicated as MeCx:y, where the numbers of carbon chain lengths and unsaturated bonds are denoted before and after colon, respectively.

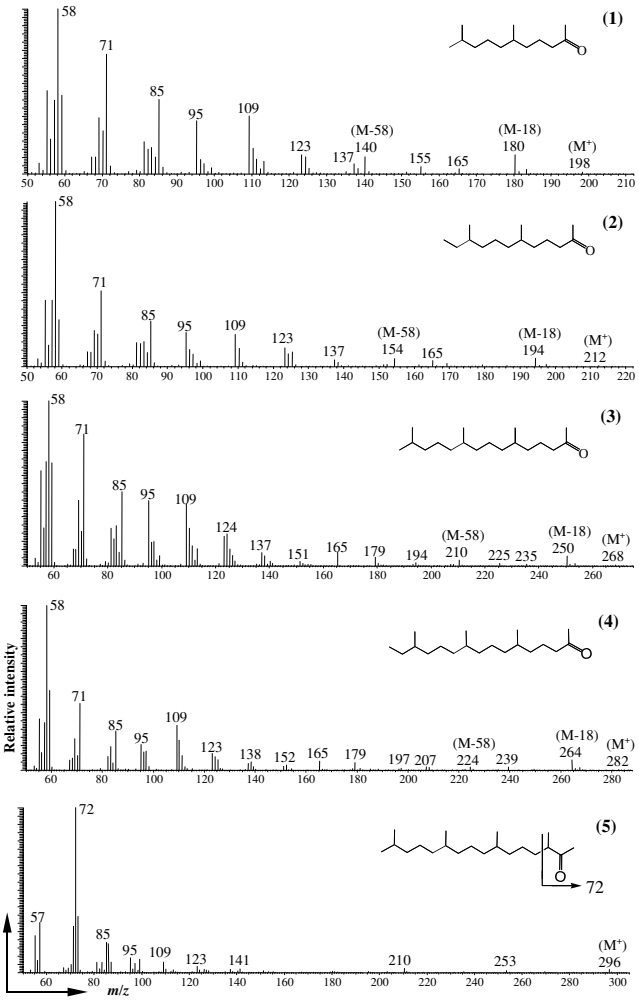

Fig. 4. Electron ionization mass spectra of 6,10-dimethyl-undecan-2-one (1), 6,10-dimethyl-dodecan-2-one (2), 6,10,14-trimethyl-pentadecan-2-one (3), 6,10,14-trimethyl-octadecan-2-one (4) and 3,7,11,15-tetramethylhexadecan-2-one (5).

TA limestone (Fig. 2, bottom), with crocetane in higher abundance. Unsaturated derivatives of PMI and crocetane were absent. Squalane and pristane were detected in relatively low abundance as in the TK limestone. The abundance of *n*-alkanes was much smaller than that of crocetane and PMI and they were difficult to detect in the Total Ion Chromatogram (TIC) trace (Fig. 2). The stable carbon isotope compositions of the lipids were similar to those of the TK limestone components, with crocetane and PMI extremely ^{13}C depleted, with $\delta^{13}C$ values as low as –129‰.

Ketones

TK limestone A distinct feature of the ketone fraction of the TK limestone lipids is the prominent occurrence of isoprenoid ketones. The dominant components were 6,10-dimethyl-undecan-2-one (C_{13} isoprenoid ketone, compound 1 in Fig. 3, top) and 6,10,14-trimethyl-pentadecan-2-one (C_{18} isoprenoid ketone, compound 3), with minor amounts of 6,10-dimethyl-dodecan-2-one (C_{14} isoprenoid ketone, compound 2), 6,10,14-trimethyl-octadecan-2-one (C_{19} isoprenoid ketone, compound 4) and 3,7,11,15-tetramethylhexadecan-2-one (compound 5). The mass spectra are shown in Fig. 4. The mass spectra of compounds 1 and 3 were identical to those found in the NIST library. The CAS number of each compound is 001604-34-8 and 000502-69-2, respectively. The structures of compounds 2 and 4 were deduced from interpretation of the mass spectrum. The mass spectra of isoprenoid alkan-2-ones are characterized by a base peak at *m/z* 58 (McLafferty rearrangement), and by peaks $(M-58)^+$ and $(M-18)^+$, the latter corresponding to loss of H_2O. Ions at *m/z* 95, 109, 123, 137, 151, 165, and 179 were explained as $M–H_2O–C_nH_{2n+1}$ species (Raymer *et al*., 1985). The mass spectrum of compound 5 is identical to that reported by Grossi *et al*. (1998). The spectra of isoprenoid alkan-2-ones with a 3-methyl substituent, such as compound 5, are characterized by a base peak ion at *m/z* 72 (Grossi *et al*., 1998). The individual isoprenoid ketones were extremely depleted in ^{13}C like crocetane and PMI, with $\delta^{13}C$ values of compounds 1, 2 and 3 of –113‰, –108‰ and –104‰, respectively.

TA limestone The C_{18} isoprenoid ketone (compound 3) was accompanied by relatively small amounts of C_{13} isoprenoid ketone (compound 1). Compounds 2, 4 and 5, present in the TK limestone were not found. The only compound with sufficient quantity for individual carbon isotopic analysis was compound 3 with a $\delta^{13}C$ value of –115‰.

Discussion

The TK and TA limestones have been well studied by paleontologists such as Hikida *et al*. (2003) and Kanie *et al*. (1996). They concluded that the bivalves found in this kind of limestone were members of chemosynthetic communities related to methane seepage. Both limestones have been identified in paleontological studies as cold-seep carbonates. In addition, carbonate isotope analyses in this study have shown that TK limestone ($\delta^{13}C$ = –43.4 to –39.9‰) and TA limestone (–45.4 to –43.7‰) are extremely ^{13}C depleted. Campbell *et al*. (1993) used the presence of both fossil chemosynthetic bivalves and depleted $\delta^{13}C$ carbonate carbon isotope values as evidence of cold-seep carbonates. According to their definition, TK and TA limestones can be identified as typical cold-seep carbonates.

Lipids in these limestones are characterized by a high crocetane content and PMI. PMI is considered to be a methanogen biomarker because it is biosynthesized by *Methanosarcina barkeri* (Holzer *et al*., 1979). Unsaturated PMIs, which are thought to be useful biomarkers for methanogenic archaea, were also identified in cultures of *Methanosarcina mazei* and *Methanolobus bombayensis* (Schouten *et al*., 1997). Both saturated and unsaturated PMIs were identified in cold-seep carbonates and ascribed to ANME (Elvert *et al*., 1999; Thiel *et al*., 2001). In fact, PMI and its unsaturated derivatives have been found in various modern and ancient sediments and are often used as biomarkers for ANME (Elvert *et al*., 2000). Furthermore, in this study, PMI and crocetane were shown to be extremely depleted in ^{13}C, with values as low as –118 to –117‰ in the TK limestone and –129‰ in the TA limestone. The carbon isotope values of crocetane and PMI in each limestone were the same or quite close, indicating the same source for PMI and crocetane. Usually, biogenic methane is depleted in ^{13}C, with $\delta^{13}C$ values in the range of –50 to –100‰ (Whiticar, 1999). The strongly depleted values for the archaeal biomarkers from the two limestones are therefore assigned to the occurrence of AOM during limestone precipitation.

The source of isoprenoid ketones in the sediments and sedimentary rocks has not been described as being particularly characteristic and has not been proposed as a specific biomarker for archaea or bacteria. On the other hand, isoprenoid ketones are not indicative compounds in marine and terrestrial environments. Those with carbon numbers 13, 18, and 23 were obtained from the chemical degradation of Green River Formation oil shale kerogen (Burlingame and Simoneit, 1969). Ikan *et al*. (1973) reported the C_{18}-isoprenoid ketone in recent marine sediment from Tanner Basin, Southern California continental shelf. Simoneit (1979) found the C_{13} and C_{18} isoprenoid ketones in DSDP core samples from Legs 5 to 15 (Black Sea, Lake Kivu and Mangrove lake) and concluded that they were microbial degradation products from phytol. Rontani and Giral (1990) argued that the C_{18} ketone could be produced by the photosensitized oxidation of some isoprenoid hydrocarbon such as pristane. Azevedo *et al*. (2001) identified C_{13}, C_{14}, C_{18} and C_{19} isoprenoid ketones in Tasmanian tasmanite bitumen and proposed that the C_{13} and C_{18} ketones were derived from phytol, and that the C_{14} and C_{19} ketones were derived from the oxidation of double bonds in the side chain of tricyclooctaprenol or tricyclooctaprenene. Rontani and Volkman (2003) reviewed the degradation pathway of phytol, and mentioned that isoprenoid ketones can be produced in several ways: (1) biotic and abiotic degradation and autoxidation of phytol, (2) photosensitized oxidation, or (3) hydrolysis of chlorophyll-*a* photoproducts and alkaline hydrolysis of tocopherols. On the other hand, Leif and Simoneit (1995) proposed a reaction scheme for the formation of *n*-alkenones during the pyrolysis of aliphatic-rich organic matter. They argued that *n*-alkenones were produced from the aliphatic kerogen network by radical cracking and that hydration of *n*-alkenes could form *n*-alkenols, with dehydrogenation of the latter forming the *n*-alkenones in an oxygen free environment.

The sources of isoprenoid ketones identified in TK and TA limestones are complicated in terms of interpretation. They are characterized by low $\delta^{13}C$ values. Extremely ^{13}C depleted compositions of bacterial or archaeal biomarkers strongly indicate that the carbon was ultimately derived from methane, but isoprenoid ketones have not been accepted in previous investigations as achaeal markers. It is therefore proposed that the ^{13}C depletion in C_{13}, C_{14} and C_{18} isoprenoid ketones is strong evidence for the components originating from ANME. However, the oxidation pathway from archaeal lipids to isoprenoid ketones is not clear.

In the case of crocetane, crocetane and crocetenes have not been observed in cultured archaea and the precise source remains unknown, but their structural similarity to PMI and occurrence in the cold-seep settings strongly suggests that these are archaeal biomarkers (Pancost *et al*., 2001). There is only circumstantial evidence showing that crocetane is a biomarker indicating ANME. As well as crocetane, the exact source and pathway to isoprenoid ketones are uncertain, although isoprenoid ketones are possible biomarker indicating ANME because of their extremely low $\delta^{13}C$ values.

CONCLUSIONS

Two Cretaceous limestone samples associated with fossil chemosynthetic biological communities, found in central Hokkaido in northern Japan, were investigated focusing on the molecular organic geochemical aspects. They are characterized by extremely ^{13}C depleted values for crocetane and PMI, which are related to anaerobic methane oxidation occurring during limestone precipitation. The C_{13}, C_{14}, C_{18}, C_{19}, and C_{20} isoprenoid ketones were also present. The C_{13}, C_{14} and C_{18} ketones had extremely low $\delta^{13}C$ values. The origin of the isoprenoid ketones is proposed to be ANME lipids, because of the $\delta^{13}C$ values, although the diagenetic pathway leading to them remains uncertain.

Acknowledgments—The author would like to thank Prof. H. Naraoka and Dr. S. Sakata for their valuable suggestions, which improved the manuscript remarkably. The author thanks Dr. J. Robert and Mr. Y. Hamada for their sampling help.

REFERENCES

Aloisi, G., Bouloubassi, J. C., Heijs, S. K., Pancost, R. D., Pierre, C., Sinninghe Damsté, J. S., Gottschal, J. C., Forney, L. J. and Rouchy, J.-M. (2002) CH_4-consuming microorganisms and the formation of carbonate crust at cold seeps. *Earth Planet. Sci. Lett.* **203**, 195–203.

Azevedo, D. A., Zinu, C. J. A., Neto, F. R. A. and Simoneit, B. R. T. (2001) Possible origin of acyclic (linear and isoprenoid) and tricyclic terpane methyl ketones in a Tasmanian tasmanite bitumen. *Org. Geochem.* **32**, 443–448.

Burlingame, A. L. and Simoneit, B. R. (1969) High resolution mass spectrometry of Green River formation kerogen oxidations. *Nature* **222**, 741.

Campbell, K. A., Carlson, C. and Bottjer, D. J. (1993) Fossil cold seep limestones and associated chemosymbiotic macroinvertebrate faunas, Jurassic-Cretaceous Great Valley group, California. *Advances in the Sedimentary Geology of the Great Valley Group, Sacramento Valley, California* (Graham, S. and Lowe, D., eds.), 37–50, The Pacific Section Society of Economic Paleontologists and Mineralogists.

Elvert, M., Suess, E. and Whiticar, M. J. (1999) Anaerobic methane oxidation associated with marine gas hydrates: Superlight C-isotopes from saturated and unsaturated C_{20} and C_{25} irregular isoprenoids. *Naturwissenschaften* **86**, 295–300.

Elvert, M., Suess, E., Greinert, J. and Whiticar, M. J. (2000) Archaea mediating anaerobic methane oxidation in deep-sea sediments at cold seep of the eastern Aleutian subduction zone. *Org. Geochem.* **31**, 1175–1187.

Grossi, V., Hirschler, A., Raphel, D., Rontani, J.-F., DeLeeuw, J. W. and Bertrand, J.-C. (1998) Biotransformation pathways of phytol in recent anoxic sediment. *Org. Geochem.* **29**, 845–861.

Hikida, Y., Suzuki, S., Togo, Y. and Ijiri, A. (2003) An exceptionally well-preserved fossil seep community from the Cretaceous Yezo Group in Nakagawa area, Hokkaido, northern Japan. *Paleontological Res.* **7**, 329–342.

Hinrichs, K. U., Hayes, J. M., Sylva, S. P., Brewer, P. and DeLong, E. F. (1999) Methane-consuming arhaebacteria in marine sediments. *Nature* **398**, 802–805.

Holzer, G., Oro, J. and Tornabene, T. G. (1979) Gas chromatography-mass spectrometric analysis of neutral lipids from methanogenic and thermacidophilic bacteria. *J. Chromatogr.* **18**, 795–809.

Ikan, R., baedecker, M. J. and Kaplan, I. R. (1973) C_{18}-isoprenoid ketone in resent marine sediment. *Nature* **224**, 1554–155.

Jensen, P., Aagard, I., Burke, R. A., Dando, P. R., Jørgensen, N. O., Kuijperts, A., Laier, T., O'Hara, S. C. M. and Schmaljohann, R. (1992) Bubbling reefs in the Kattegat: submarine landscapes of carbonate-cemented rocks support a diverse ecosystem at methane seep. *Mar. Ecol. Prog. Ser.* **83**, 103–112.

Kanie, Y., Yoshikawa, Y., Sakai, T. and Kuramochi, T. (1996) Cretaceous chemosynthetic fauna from Hokkaido. *Scientific Reports of Yokosuka City Museum* **44**, 69–74.

Kulum, L. D., Suess, E., Moore, J. C., Carson, B., Lewis, B. T., Ritger, S. D., Kadko, D. C., Thornberg, T. M., Embeley, R. W., Rugh, W. D., Massoth, G. J., Langseth, M. R., Cochrane, G. R. and Scamman, R. (1986) Oregon subduction zone: venting, fauns, and carbonates. *Science* **231**, 561–566.

Leif, R. N. and Simoneit, B. R. T. (1995) Ketones in hydrothermal petroleum and sediment extracts from Guaymas Basin, Gulf of California. *Org. Geochem.* **23**, 889–904.

Meyerdierks, A., Kube, M., Lombardot, T., Knittel, K., Bauer, M., Glockner, F. O., Reinhardt, R. and Amann, R. (2005) Insight into the genomes of archaea mediating anaerobic oxidation of methane. *Environ. Microbiol.* **7**, 1937–1951.

Neurauter, T. W. and Roberts, H. H. (1994) Three generations of mud volcanoes on Louisiana continental slope. *Geo-Mar. Lett.* **14**, 120–125.

Orphan, V. J., House, C. H., Hinrichs, K. U., McKeegan, K. D. and DeLong, E. F. (2002) Multiple archaeal groups mediate methane oxidation in anoxic cold seep sediments. *Proc. Natl. Acad. Sci. USA* **99**, 14002–14007.

Pancost, R. D., Sinninghe Damsté, J. S., De Lint, S., Van der Maarel, M. J. E. C., Gottschal, J. C. and the MEDINAUT Shipboard Scoentific Party, 2000 (2001) Biomarker evidence for widespread anaerobic oxidation in Mediterranean sediments by a consortium of methanogenic archaea and bacteria. *Appl. Environ. Microbiol.* **66**, 1126–1132.

Peckmann, J., Thiel, V., Michaelis, W., Clari, P., Gaillard, C., Martire, I. and Reitner, J. (1999.) Cold seep deposit of Beauvoisin (Oxfordinan; Southeastern France) and Marmoto (Miocene; Northern Italy): Microbially induced authigenic carbonates. *Int. J. Earth Sci.* **88**, 60–75.

Raymer, J., Wiesler, D. and Novotny, M. (1985) Structure-retention studies of model ketones by capillary gas chromatography. *J. Chromatogr.* **325**, 13–22.

Rontani, J.-F. and Giral, P. J.-P. (1990) Significance of alkane photosensitized oxidation during the photochemical degradation of crude oil hydrocarbon fraction. *J. of Environmental Photochemistry and Photobiology* **42**, 61–68.

Rontani, J.-F. and Volkman, J. K. (2003) Phytol degradation

products as biological tracers in aquatic environments. *Org. Geochem.* **34**. 1–35.

Rontani, J.-F., Baillet, G. and Aubert, C. (1991) Production of acyclic isoprenoid compounds during the photodegradation of chlorophyll in seawater. *J. Photochem. Photobiol.* **A59**, 369–377.

Schouten, S., Van der Maarel, M. J. E. C., Hhuber, R. and Sinninghe Damsté, J. S. (1997) 2,6,10,15,19-Pentamethylicosanes in Methanolobus bombayensis, a marine methanogenic archaeon, and in *Methanosarcina mazei*. *Org. Geochem.* **26**, 409–414.

Simoneit, B. R. T. (1979) Organic geochemistry of the shales from the northwestern Proto-Atlantic, Deep Sea Drilling Project Leg 43. *Initial Reports of the Deep Sea Drilling Project, vol. 43* (Tucholke, B., Vogt, P. *et al.*, eds.), 643–649, US Government Printing Office, Washington, D.C.

Tanabe, K., Hirano, H., Matsumoto, T. and Miyata, Y. (1977) Stratigraphy of the Upper Cretaceous deposits in the Obira area, northern Hokkaido. *Mem. Fac. Sci. Kyushu Univ., (D)* **12**, 181–202 (in Japanese with English abstract).

Thiel, V., Peckmann, J., Seifert, R., Wehrung, P., Reitner, J. and Michaelis, W. (1999) Highly isotopically depleted isoprenoids: Molecular markers for ancient methane venting. *Geochim. Cosmochim. Acta* **63**, 3959–3966.

Thiel, V., Peckmann, J., Richnow, H. H., Luth, U., Reitner, J. and Michelis, W. (2001) Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and microbial mat. *Mar. Chem.* **73**, 97–112.

Tuo, J. and Li, Q. (2005) Occurrence of long-chain acyclic ketones in immature coals. *Appl. Geochem.* **20**, 553–568.

Whiticar, M. J. (1999) Carbon and hydrogen isotope systematics of bacterial formation and oxidation of methane. *Chem. Geol.* **161**, 291–314.