## ARTICLES

*Chinese Science Bulletin* 2006 Vol. 51 No. 14 1736—1740

DOI: 10.1007/s11434-006-2036-x

# The biomarkers of 2,6,10,15, 19-pentamethylicosenes and their carbon isotopic composition in the sediments from the Gulf of Mexico

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Received June 1, 2005; accepted August 2, 2005

**Abstract** A group of 2,6,10,15,19-pentamethylicosenes (PMIΔ) containing 1—5 unsaturated double bonds has been identified in the sea floor sediments from the Gulf of Mexico at the Green Canyon 238 site. These PMIΔ compounds are distributed between $nC_{22}$ and $nC_{24}$ on the mass chromatogram of aliphatic fraction. Their $\delta^{13}C$ values are very much depleted in $^{13}C$ and in the range of –86.7‰ to –115.5‰, whereas the $\delta^{13}C$ values of companion *n*-alkanes range from –28.4‰ to –34.6‰. These unsaturated PMIΔ compounds are typical biomarkers derived from the anaerobic oxidation of methane mediated by methane-oxidizing archaeal bacteria and indicative of the gas seeps or even the occurrence of gas hydrates in the deep sea sediments.

**Keywords: sediments from the Gulf of Mexico, methanogenic archaea, PMIΔ biomarkers, sea floor gas seepage, gas hydrates.**

The methanogenic archaea and sulfate reduction bacteria are flourishing in the sediments associated with gas venting and gas hydrate settings on the sea floor, where the anaerobic oxidation of methane (AOM) mediated by these bacteria is the dominant pathway for the methane consumption in the sediments and therefore plays an important role in the carbon cycle and in the growth and metabolism of microbial communities[1—6]. Some biomarkers originating from the AOM are regarded as exclusive biogeochemical products of these bacteria growth and metabolism and have been widely considered as an important indication of the occurrence of the marine gas seepage and gas hydrates[7—19]. Therefore, the study on these archaeal biomarkers has been a major interest of organic geochemistry on marine gas venting and gas hydrates.

The previous investigation on the molecular biomarkers in the sediments and cold seep carbonates associated with gas vent ecosystems and gas hydrate settings along continental margins in the world's oceans indicates that these sediments contain two typical types of "tail-to-tail" linked acyclic $C_{20}$ and $C_{25}$ isoprenoids, i.e. 2,6,11,15-tetrmethylhexadecane (or crocetane), 2,6,10,15,19-pentamethylicosane (PMI) and a group of corresponding unsaturated PMI compounds, namely 2, 6, 10, 15, 19-pentamethylicosenes (PMIΔ) containing 1—5 double carbon-carbon bonds. These biomarkers are likely to be derived from methanogenic archaea mediated AOM during the bacteria growth and metabolism and show a significantly $^{13}C$ depleted composition[13,15,16,20—23]. Among these biomarkers, PMI is structurally stable and has been found in both ancient sediments and crude oils and indicative of the AOM occurring in the ancient marine sediments[24—26].

This paper reports a group of containing 1—5 double bond 2,6,10,15,19-pentamethylicosenes and their identification mass spectra and carbon isotopic composition found in a marine sediment sample and accompanied carbonate sample from the Gulf of Mexico. The identification of this kind of biomarkers is important to the exploration of gas hydrates in South China Sea and to the study of microbial methane oxidation and their natural products in the gas seeps and gas hydrate settings of the marine sediments.

## 1 Samples and experiments

The sediment samples were collected in 2002 by Johnson-Sea-Link from the Green Canyon (GC) 238 site at a depth of 703.19 m in the Gulf of Mexico where gas venting and gas hydrates are in abundance. The water temperature at the sea floor was 6.12℃ with a salinity of 34.89‰. S-1 sample was black mud sediment, while S-4 sample is a piece of carbonate associated with S-1 sediments. After freezing dried and grounded, the powder samples were subjected to Soxhlet extraction using a mixture of dichloromethane and methanol. The extracts were first treated with normal

hexane to remove asphaltene, then the rest of extracts were divided into aliphatic, aromatic and polar fractions through alumina/silica column chromatography separation using normal hexane, a mixture of n-hexane and dichloromethane (6:4) and a mixture of dichloromethane and methanol (5:5) as elutes, respectively. The aliphatic fraction was initially analyzed by Gas Chromatography and then was divided into *n*-alkanes and branched and cyclic (b and c) alkanes via urea adduction.

A Thermo Finnigan Trace Ultra Gas Chromatography coupled with an FID detector was used for GC analysis. A JW-DB-5 fused silica capillary column (30 m × 0.25 mm i.d.× 0.25 μm film thickness) was used. The injector and detector temperatures were 290℃ and 300℃, respectively. The samples were injected in a splitless mode and nitrogen was the carrier gas. The oven temperature was initially held at 60℃ for 5 min, and then programmed in a heating rate of 3℃/min to 290℃ and maintained for 20 min.

The gas chromatography and mass spectrometry analysis (GC-MS) were performed with HP 6890 series II Gas Chromatography interfaced with an HP 5972 mass selective detector using electron impact ion source (70eV). The GC was equipped with a DB-5 fused silica capillary column (30 m × 0.25 mm i.d.×0.25 μm film thickness). The injector temperature of GC was 290℃ and the oven temperature was initially held at 60℃ for 5 min, and then programmed in a heating rate of 3℃ /min to 290℃ and maintained for 30 min. The mass scan range was *m/z* 50 to 550. Helium was the carrier gas at a flow rate of 1.2 mL/min.

The carbon isotopic analyses on aliphatic and aromatic compounds were performed on a British made-Isoprime Gas Chromatography-isotopic ratio mass spectrometry. A DB-5 fused silica capillary column (30 m × 0.25 mm i.d.×0.25 μm film thickness) was used. The injector was set at sliptless mode and 290℃ while helium was used as carrier gas. The oven temperature was initially set at 80℃ and programmed to 140℃ in a heating rate of 10℃/min, and then programmed to 290℃ in a rate of 6℃/min and held for 15 min. For the isotopic analysis of branched and cyclic alkanes, an AT-5 capillary column (50 m × 0.32 mm ×0.3 μm) was used. The oven temperature was initially set at 80℃ and programmed to 245℃ at a heating rate of 1℃/min and then to 290℃ at a rate of 10℃/min. The standard carbon isotope gas was calibrated using NS22 crude oil standard provided by IAEA and the isotopic error of parallel analysis is less than 0.5‰.

## 2 Results and discussion

### *2.1 Identification of unsaturated 2,6,10,15,19-pentamethylicosenes*

The total mass chromatograms of aliphatic hydrocarbons in S-1 mud sediments and S-4 carbonate from the Gulf of Mexico show that there is a group of unusual compounds distributed between $nC_{22}$ and $nC_{24}$ and centered on $nC_{23}$ (Fig. 1).

Fig. 2 shows the mass spectra of these compounds that were characterized by a commonly existing *m/z* 69 base peak and a second base peak of *m/z* 123, and their ionic molecular mass peaks are 350, 348, 346 and 344, respectively. According to the literature, these compounds are identified as a group of unsaturated 2,6,10,15,19-pentamethylicosenes (PMIΔ) containing 1—4 double bonds. Furthermore, five double-bond isomers were also identified from the aromatic fraction as it co-eluted with aromatic fraction due to higher polarity. These PMIΔ compounds consist of 13 isomers and their distribution is: one isomer for one double bond compound (PMI:1), 4 isomers for two double-bond compounds (PMI:2), 3 isomers for three double-bond compounds (PMI:3), 4 isomers for four double bond compounds (PMI:4) and one isomer for five double-bond compounds (PMI:5). Among these isomers, PMI: 4 are the most abundant isomers. According to the literature, the identification of a full range containing 1—5 double bond PMIΔ isomers has only been reported from the Mediterranean marine sediments associated with gas venting but the total number of isomers was less than 11, while in the other marine sediments including the Gulf of Mexico these PMIΔ mainly consisted of 1—4 or 3—5 double bonds isomers[15—17]. The previous studies show that PMIΔ are often accompanied by PMI while the absence of PMI has never been reported for the modern sediments. On the other hand, the relative abundance of PMI against that of PMIΔ and among these PMIΔ compounds vary greatly even for the sediments from the same area but different sampling sites. It should also be pointed out that PMI found in the ancient sediments and crude oils was never accompanied with PMIΔ compounds[24—26].

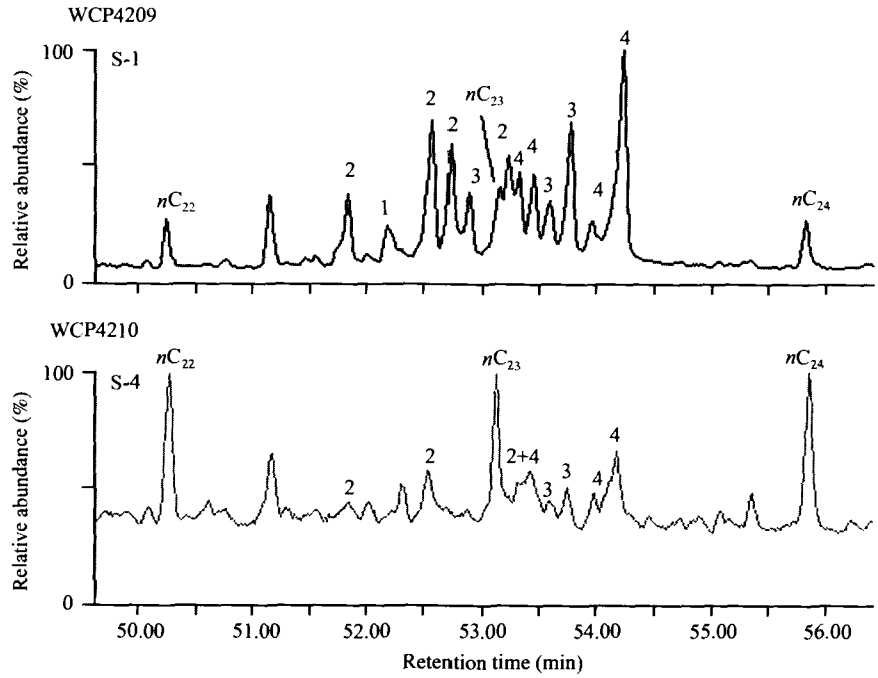


Fig. 1. Part of total ion chromatogram showing the composition and distribution of PMIΔ compounds in S-1 and S-4 samples. The number on the top of peaks indicates the number of double-bond in the PMIΔ.

### 2.2 *Carbon isotopic composition of PMIΔ biomarkers*

The carbon isotopic compositions of PMIΔ in branched and cyclic fraction, *n*-alkanes and PMI: 5 in aromatic fraction from S-1 sample were analyzed using GC-IRMS. Fig. 3 displays the isotopic mass spectra for PMIΔ compounds, while Table 1 lists the isotopic composition for the related PMIΔ compounds and as well as isotopic data for PMIΔ quoted from comparison literatures. Table 1 indicates that these PMIΔ are very much depleted in $^{13}C$. The $\delta^{13}C$ values of PMI:2, PMI:3 and PMI:4 are in the range of −107.2‰ to −115.5‰, PMI:5 is −86.7‰, while there is no measured data for PMI:1 due to its very low abundance. These carbon isotopic compositions are comparable to those of PMIΔ from Mediterranean and the Black Sea, while they are more negative compared to a $\delta^{13}C$ value of −99‰ for PMI:3 from the sediments associated with gas hydrates at GC286 site in the Gulf of Mexico[18]. These indicate that there are variation to some extent in the distribution and composition of these typical biomarkers and their isotopic composition even if these samples were collected in the same region of the ocean. These variations may reflect the differences in the sedimentary environments, the types of microbial speciation and even the mechanism of isotopic fractionation[18,23,27]. However, the geochemical implications of these variations need to be investigated in the future. On the other hand, the carbon isotopic compositions of normal alkanes in S-1 are correlated with their carbon number distribution as the $\delta^{13}C$ values of $nC_{12}$– $nC_{24}$ varies between −28.4 to −30.1‰, while the $\delta^{13}C$ value of $nC_{25}$ – $nC_{33}$ is between −29.8 to −34.6‰. The difference in $\delta^{13}C$ values between these

Table 1 The $\delta^{13}C$ values of PMIΔ biomarkers and relevant comparison

| | *n*-alkanes | PMI:1 | PMI:2 | PMI:3 | PMI:4 | PMI:5 | Reference |
|---|---|---|---|---|---|---|---|
| | | −104.7 | −105.5 | −101.0 | | | [15] |
| | | −70.7 | −73.1 | | | | [15] |
| $\delta^{13}C$ (‰) | | −67.1– −107 | | | | | [18] |
| | −26– −31 | | | −99 | | | [17] |
| | −28.4– −34.6 | | −107.2 | −115.0 | −115.5 | −86.7 | this study |

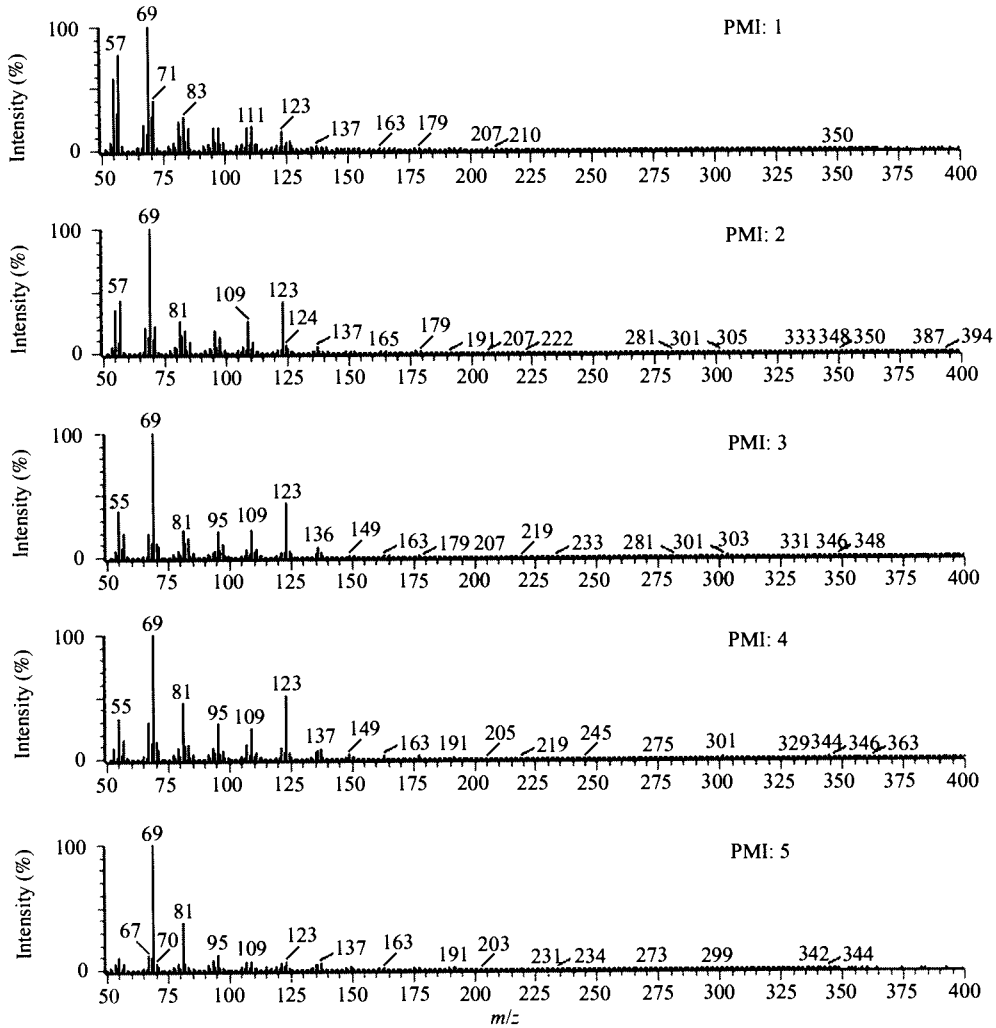


Fig. 2. Mass spectra for PMIΔ compounds identified in S-1 sample.

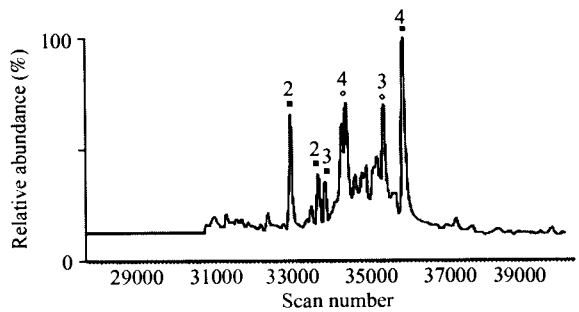


Fig. 3. Isotopic mass chromatogram of PMIΔ biomarkers in S-1sample. The number on the top of peak indicates the number of double-bond in PMIΔ, "■" marks a real $\delta^{13}C$ value for this compounds, "○" marks no reliable $\delta^{13}C$ value for this compound.

lower carbon number alkanes and these heavy alkanes reflects the difference in their source input. The huge difference in $\delta^{13}C$ values between the *n*-alkanes and PMIΔ demonstrates unambiguously that these PMIΔ biomarkers are special in their origins.

## 3 Conclusions

The identification of AOM-related archaeal biomarkers containing 1—5 double-bond PMIΔ isoprenoids from the sediments and companied carbonate in the Gulf of Mexico is not only the first study of this kind in China but also a first report of identifying a full range of PMIΔ compounds in the Gulf of Mexico. These compounds are very much depleted in $^{13}C$ and exhibit $\delta^{13}C$ values in the range of −86.7% to −115.5% and therefore were recognized as the products of AOM. Furthermore, the carbon isotopic analysis indicates that S-4 carbonate is a cold seep carbonate with a $\delta^{13}C$ value of −51‰[28,29], while cold seep

carbonate is considered to have resulted from the AOM mediated by a consortium of methanogenic archaea and sulfate reduction bacteria in the marine sediments[21,30]. Therefore, these data suggest that the sampling site of S-1 is likely associated with a methane venting or gas hydrate setting in the Gulf of Mexico.

**Acknowledgements** The authors are grateful to Dr. Chen Duofu for providing the samples from the Gulf of Mexico. Thanks are also given to Mr. Xiang Tongshou and Dr. Liu Jinzhong for their technical assistance in the GC-MS and GC-IRMS analyses and to these anonymous reviewers and editors for their useful comments and corrections. This study was supported by Chinese Academy of Science (Grant Nos. KZCX3-SW-224 & KGCX2-SW-309).

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