The enigmatic structure of the crenarchaeol isomer

Jaap S. Sinninghe Damstè a,b,⇑, W. Irene C. Rijpstra a, Ellen C. Hopmans a, Mimi J. den Uijl c, Johan W.H. Weijers c, Stefan Schouten a,b

⇑ Corresponding author at: NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands

a NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry, and Utrecht University, PO Box 59, 1790 AB Den Burg, The Netherlands

b Utrecht University, Faculty of Geosciences, Department of Earth Sciences, P.O. Box 80.021, 3508 TA Utrecht, The Netherlands

c Shell Global Solutions International B.V., Grasweg 31, 1031 HW Amsterdam, The Netherlands

E-mail addresses: jaap.damste@nioz.nl, damste@nioz.nl (J.S. Sinninghe Damstè).

Abstract

Isolation of crenarchaeol and its isomer from marine surface sediments, followed by ether cleavage and GC–MS characterization using supersonic molecular beam (SMB) ionization of the biphytanes formed, revealed that the crenarchaeol isomer comprises a tricyclic biphytane that is stereochemically different from the tricyclic biphytane of crenarchaeol. This isomeric tricyclic biphytane was also released from the crenarchaeol isomer in extant Thaumarchaeotal biomass. Reinterpretation of previously obtained 13C NMR data of the crenarchaeol isomer suggested that the cyclopentane moiety adjacent to the cyclohexyl moiety of the tricyclic biphytane of the crenarchaeol isomers possesses the unusual cis stereochemistry in comparison to the trans stereochemistry of all cyclopentane moieties in crenarchaeol. This stereochemical difference likely affects the packing of lipid membranes of Thaumarchaeota and therefore provides a biophysical explanation for the role of the crenarchaeol isomer in the TEX86 palaeothermometer based on fossilized Thaumarcheotal lipids.

© 2018 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

Glycerol dibiphytanyl glycerol tetraethers (GDGTs) form the core membrane lipids of many archaea and can therefore be used as biomarker lipids. A ubiquitous group of archaea in marine environments (e.g. Karner et al., 2001) are the Thaumarchaeota, which perform nitrification, i.e. oxidation of ammonium (Könneke et al., 2005; Wuchter et al., 2006). Their membrane lipids contain a specific GDGT, called crenarchaeol (see Fig. 1 for structures), containing an uncommon cyclohexane moiety in addition to four five-membered rings (Sinninghe Damsté et al., 2002). So far, crenarchaeol has only been found in cultures of Thaumarchaeota (see for a review Schouten et al., 2013b) but not in other archaeal cultures, indicating that it can be used as specific marker for Thaumarchaeota. This is confirmed by studies of the marine water column where the abundance of specific genes of Thaumarchaeota and crenarchaeol containing polar head groups show the same depth and seasonal profiles (e.g. Pitcher et al., 2011a, 2011b).

Thaumarcheotal GDGTs find an application in paleoceanography and paleoclimatology since their fossilized GDGTs in both marine and lacustrine sediments are widely used in TEX86 palaeothermometry (Schouten et al., 2002). Thaumarchaeota regulate the chemical composition of their membrane to influence its physical properties to optimally function at a specific temperature. The TEX86 palaeothermometer is based on the fractional abundances of four GDGTs that typically occur in lower abundances than crenarchaeol and GDGT-0 (i.e. a GDGT with no cyclopentane moieties). These four GDGTs are GDGT-1, -2, -3, and an isomer of crenarchaeol, which typically occurs in low abundance relative to crenarchaeol (i.e. up to 5%), although in some Thaumarcheotal species they can be much more abundant (Pitcher et al., 2010; Sinninghe Damsté et al., 2012). The crenarchaeol isomer plays an important role in the TEX86 proxy as its abundance increases substantially in tropical regions and plays a key role in reconstructing sea surface temperature from past greenhouse periods (e.g. O’Brien et al., 2017).

The crenarchaeol isomer is characterized by a later retention time than crenarchaeol but with an identical mass spectrum as obtained by atmospheric pressure chemical ionization (APCI) mass spectrometry (Sinninghe Damsté et al., 2002). It has been proposed to be a so-called regiosomer of crenarchaeol, i.e. where the glycerol units are not in a parallel but an anti-parallel configuration or vice versa, because the carbon signals in its 13C NMR spectrum were identical to those in the 13C NMR spectrum of crenarchaeol (Sinninghe Damsté et al., 2002; Schouten et al., 2013b). Recently,
however, Liu et al. (2018) presented data based on the tentative identification of presumed partial degradation products of both crenarchaeol and its isomer indicating that both possess predominantly the parallel configuration. Liu et al. (2018) also characterized the biphytane (BP) moieties of the crenarchaeol isomer and suggested that it may possess a different ring configuration of the tricyclic BP. Here, we have repeated the experiments of Liu et al. (2018) on purified (>95%) crenarchaeol and its isomer and studied the structure of these BPs by gas chromatography-mass spectrometry (GC–MS) using supersonic molecular beam (SMB) ionization, which is a much softer ionization technique than the commonly used electron impact ionization (Fialkov et al., 2008). In addition, we re-examined our previously obtained NMR data to evaluate the hypothesis of Liu et al. (2018) that the structure of the crenarchaeol isomer is characterized by a different ring configuration compared to crenarchaeol.

2. Materials and methods

Crenarchaeol isomer was previously isolated from Arabian Sea sediments as described by Sinninghe Damsté et al. (2002). Crenar-
Crenarchaeol was isolated from Arabian Sea surface sediments following techniques described previously (Schouten et al., 2013a). Their purity was checked by dissolving them in hexane/propanol (99:1, v/v), filtered over a 0.45 μm polytetrafluorethylene filter, and analysis by high performance liquid chromatography/APCI mass spectrometry (HPLC/APCI-MS) for GDGTs using a method described elsewhere (Hopmans et al., 2016) and by NMR (see Schouten et al., 2013a).

Aliquots of the isolated crenarchaeol and the crenarchaeol isomer were subjected to ether cleavage (57% HI) as described by Lengger et al. (2014). The resulting alkyl iodides were reduced to hydrocarbons with H2/PtO2 (Kaneko et al., 2011), which were analyzed with gas chromatography (GC) after on-column injection on an Agilent 7890B GC instrument and in splitless mode with GC–MS using an Agilent 7890A GC instrument equipped with a Agilent 5975C VL MSD detector. A CP Sil SCB column was used (25 m x 0.32 mm i.d.; film thickness 0.12 μm; He carrier gas). Samples were injected at 70 °C and the GC oven was programmed at 20 °C min⁻¹ to 130 °C and increased at 4 °C min⁻¹ to 320 °C (held 10 min).

GC–SMB-MS analysis was carried out with an Agilent 7890A GC instrument, an Agilent 5975S-SMB 101–09 SMB interface (cf. Fialkov et al., 2008) and an Agilent 5975C MSD. A fused silica Zebron ZB-1HT Inferno (25 m x 0.19 mm; 0.1 μm film thickness) was used with He as a carrier gas at a constant flow rate of 2 ml min⁻¹. Samples (1 μl) were injected on column at 70 °C (held 1 min). The oven temperature was then ramped to 130 °C and the temperature was increased to 320 °C (held 1 min) at 3 °C min⁻¹. The temperature of the transfer line of the SMB interface was 320 °C and He make up gas was added at a flow of 80 ml min⁻¹. The compounds were ionized in a fly-through dual cage electron ionization (EI) source at both 70 and 20 eV. The MSD was run in full scan mode over the range m/z 50–600.

3. Results and discussion

Crenarchaeol and the crenarchaeol isomer, isolated from Arabian Sea surface sediments, were subjected to ether cleavage with HI treatment followed by reduction of the formed BP iodides by H2/PtO2 (Kaneko et al., 2011; Lengger et al., 2014). The hydrocarbons formed were analyzed by GC and GC–MS. The products of crenarchaeol were a bicyclic (I) and tricyclic (II) BP in a 1:1 ratio (Fig. 2a) and their EI mass spectra were in good agreement with those reported previously (Schouten et al., 1998). On the basis of the full structural identification of crenarchaeol by two dimensional NMR spectroscopy (Sinninghe Damsté et al., 2002), they can be unambiguously identified as the bicyclic BP I, containing two cyclopentane moieties, and the tricyclic BP II, containing one cyclohexane and two cyclopentane moieties (see Fig. 1 for structures), GC–SMB-MS analysis was applied to confirm the molecular weight of the bi- and tricyclic BPs and to provide more details on high molecular weight fragmentation products. Fig. 3a shows the 20 eV mass spectrum of the bicyclic BP I. It reveals a dominant molecular ion at m/z 558 and abundant fragments ions at m/z 390 (M−168; −C15H24), 362 (M−196; −C14H28), 194 (C14H28), and 166 (C12H24). These fragmentations are related to cleavages of relatively weak C=C bonds with associated hydrogen transfer as often is observed in “cold” EI mass spectra as obtained with the SMB ionization technique (Fialkov et al., 2008). The 20 eV SMB mass spectrum of the tricyclic BP II generated from crenarchaeol is shown in Fig. 3b. It reveals a molecular ion at m/z 556, an M−15 fragment ion at m/z 541 and abundant fragments ions at m/z 292 (M−264; −C15H28), 262 (M−294; −C14H28), and 164 (C12H24). These losses of a methyl and the formation of the two most abundant fragments ions can all be related to cleavages of C=C bonds of the only quaternary carbon atom in the tricyclic BP II (Fig. 3b). This is fully consistent with the structural identification of crenarchaeol (Sinninghe Damsté et al., 2002), in which BP II forms one of the alkyl moieties, and, in fact, confirms the position of the methyl group attached to the cyclohexane moiety. In the 70 eV EI spectrum of BP II (Schouten et al., 1998), the m/z 292 fragment ion is only very minor, probably because of the much more extensive fragmentation under these conditions (cf. Fialkov et al., 2008).

When the crenarchaeol isomer was subjected to ether cleavage and subsequent hydrogenation of the formed iodides, three BPs were formed (Fig. 2b). The first peak was the bicyclic BP I (50%), which had an identical EI and 20 eV SMB spectrum (Fig. 3b) and retention time (Kovats retention index RI = 3758) as determined for BP I derived from crenarchaeol. The two other BPs (labeled III and IV) eluted later (RI = 3871 and 3858) than the tricyclic BP II formed from crenarchaeol (RI = 3826). This confirms the results reported by Liu et al. (2018), who observed two late-eluting BPs formed from the crenarchaeol isomer enriched from a Cretaceous
black shale from the proto North Atlantic Ocean. A slight difference between our results and those of Liu et al. (2018) is that in their ether cleavage mixture of the crenarchaeol regioisomer tricyclic BP IV is slightly more abundant than BP III, whereas in our case the later eluting BP III is more dominant (i.e. 60% of the tricyclic BPs; Fig. 2b). Both BP III and IV had identical 70 eV EI mass spectra, which were virtually identical to those of BP II, in agreement with the results of Liu et al. (2018). Both these authors and we were, however, not able to conclusively confirm the molecular weight of these BPs. Therefore, GC–SMB-MS analysis was applied to determine the molecular weight of these BPs. The 20 eV SMB mass spectrum of these BPs (III and IV) are shown in Fig. 3c and d. They reveal a molecular ion at m/z 556, an M<sup>+</sup> −15 fragment ion at m/z 541 and abundant fragments ions at m/z 292 (M<sup>+</sup> −264; −C<sub>13</sub>H<sub>30</sub>), 262 (M<sup>+</sup> −294; −C<sub>21</sub>H<sub>42</sub>), and 164 (C<sub>12</sub>H<sub>22</sub>), and are virtually identical to that of BP II derived from crenarchaeol. This is consistent with our earlier report on HI cleavage of the crenarchaeol isomer (Schouten et al., 2002), although in that case no mention was made of two tricyclic BP isomers with a different retention time. Our data, however, contradicts the interpretations of Liu et al. (2018), who pointed out that there were some differences in the high molecular weight area of their 70 eV mass spectra (the insets in their Fig. 6). Based on our own 70 eV EI and, in particular, 20 eV SMB mass spectra we are inclined to conclude that the ions shown by Liu et al. (2018) (which all represent <0.1% of the base peak) represent background and cannot be interpreted to reveal structural differences between the different tricyclic BPs.

To check if the unusual BP III and IV were also formed from the crenarchaeol isomer present in extant biomass, we subjected the Bligh Dyer extract of “Ca. Nitrosotenuis uzonensis”, a nitrifying Thaumarchaeote enriched from a thermal spring (Lebedeva et al., 2013), to ether cleavage. This archaeon produced equal amounts of crenarchaeol and its isomer under certain growth conditions, together contributing 60% of the GDGTs (Palatinszky and Sinninghe Damsté, unpublished results). In addition to BP I, both BP II and III were formed in equal amounts (Fig. 2c) but, surprisingly, BP IV was not detected.

Liu et al. (2018) indicated that the identification of two additional tricyclic BPs III and IV from the crenarchaeol isomer disproves the earlier suggestion that it may represent a regio isomer of crenarchaeol (Sinninghe Damsté et al., 2002). This suggestion was based on the isolation of two GDGT-4 isomers from the archaeon Sulfolobus solfataricus that were well separated by LC

Fig. 3. 20 eV SMB mass spectra of BPs I–IV released by ether cleavage of GDGTs. The m/z 400–600 range is expanded revealing the molecular ion and loss of the methyl group attached to the quaternary carbon atom of the cyclohexane moiety. For details of BPs with roman numerals I–III see Fig. 1.
but showed identical $^{13}$C NMR spectra (Sinninghe Damsté et al., 2002). Gräther and Arigoni (1995) previously showed by specific chemical degradation that regio isomers of GDGTs exist in a number of archaea. Comparison of the $^{13}$C NMR data of crenarchaeol and its isomer revealed nearly identical spectra (Schouten et al., 2013b), in line with the suggestion that the crenarchaeol isomer is a regio isomer although it remained unclear which of the two had the parallel configuration. The findings of Liu et al. (2018), which are confirmed here, are clearly not in line with this suggestion since ether cleavage should then result in the generation of identical BPs from both crenarchaeol and its isomer. This prompted a critical re-evaluation of the $^{13}$C NMR data obtained in 2002 for the crenarchaeol isomer. At that time only 0.3 mg of the isomer was isolated using a slightly less evolved separation procedure than applied here for crenarchaeol and even though Attached Proton Test (APT) spectra were accumulated for a number of days, the signal to noise ratio was low. Nevertheless, when the data are compared with those of crenarchaeol (Table 1), it becomes evident that for most of the carbon atoms the $^{13}$C chemical shifts and multiplicities are the same. This also holds for most of the carbon atoms of the cyclohexane moiety (i.e. $A_{12}', A_{13}', A_{14}', A_{15}', A_{19}'$, $A_{20}'$), indicating that the position of this ring and its stereochemical configuration must be the same as in crenarchaeol. This is in good agreement with the SMB-MS data that reveal only fragment ions related to cleavages of C-C bonds of the quaternary carbon atom ($A_{15}'$) of the cyclohexane moiety for all three tricyclic BPs (Fig. 3). However, the $^{13}$C chemical shifts of the cyclopentane moiety adjacent to the cyclohexane moiety and carbon $A_{11}'$ of the cyclohexane moiety differ by 0.06–2 ppm from the corresponding moieties of this cyclopentane moiety in crenarchaeol (Table 1). These assignments are tentative since they were not confirmed by HMBC and HMQC experiments as in case of the structural assignments of the $^{13}$C carbon atoms of crenarchaeol (Sinninghe Damsté et al., 2002). Nevertheless, this observation strongly

Table 1
Revised assignment of $^{13}$C chemical shifts of the crenarchaeol isomer and comparison with those of crenarchaeol.

| Carbon number | $^{13}$C shifts (ppm)$^a$ | Crenarchaeol$^b$ | Crenarchaeol isomer$^c$ | $\Delta$ | $\Delta$ |
|---------------|-----------------------------|-----------------|----------------------|---------|---------|
|               | CH$_3$ CH$_2$ CH C | CH$_3$ CH$_2$ CH C |                      |         |         |
| A1,B1'        | 70.09                      | 70.06           |                      |         |         |
| A1',B1'       | 68.56                      | 68.58           |                      |         |         |
| A2,B2'        | 36.58                      | 36.54           |                      |         |         |
| A2',B2'       | 37.03                      | 37.05           |                      |         |         |
| A3,B3,B3'     | 29.71                      | 29.76           |                      |         |         |
| A3'           | 29.71                      | 29.72           |                      |         |         |
| A4,A4',B4,B4' | 37.23                      | 37.25           |                      |         |         |
| A5,B5,B5'     | 25.86                      | 25.83           |                      | 0.06    | 0.06    |
| A5'           | 25.86                      | 25.92           |                      |         |         |
| A6,A6',B6,B6' | 37.13                      | 37.15           |                      |         |         |
| A7,B7,B7'     | 39.08                      | 39.08           |                      |         |         |
| A7'           | 38.85                      | 39.18           |                      | 0.33    | 0.33    |
| A8,B8,B8'     | 33.36                      | 33.37           |                      | 0.07    | 0.07    |
| A8'           | 33.30                      | 33.37           |                      |         |         |
| A9,B9,B9'     | 31.18                      | 31.20           |                      | 0.31    | 0.31    |
| A9'           | 31.23                      | 31.54           |                      |         |         |
| A10,B10,B10'  | 44.74                      | 44.78           |                      | 1.27    | 1.27    |
| A10'          | 45.66                      | 46.93           |                      |         |         |
| A11,B11,B11'  | 38.18                      | 38.20           |                      | 1.44    | 1.44    |
| A11'          | 39.08                      | 37.64           |                      |         |         |
| A12,B12,B12'  | 35.68                      | 35.70           |                      |         |         |
| A12'          | 32.11                      | 32.14           |                      |         |         |
| A13,B13,B13'  | 24.39                      | 24.42           |                      |         |         |
| A13'          | 22.24                      | 22.25           |                      |         |         |
| A14            | 37.39                      | Nd$^d$          |                      |         |         |
| A14'          | 43.97                      | 43.95           |                      |         |         |
| B14,B14'      | 37.56                      | 37.61           |                      |         |         |
| A15            | 33.54                      | 33.59           |                      | 33.03   | 33.03   |
| A15'          | 33.07                      | Nd$^d$          |                      | 33.09   | 33.09   |
| B15,B15'      | 34.22                      | 34.24           |                      |         |         |
| A16            | 29.97                      | Nd$^d$          |                      |         |         |
| A16'          | 37.64                      | 37.64           |                      |         |         |
| B16,B16'      | 35.93                      | 35.97           |                      | 2.04    | 2.04    |
| A17,A17',B17,B17' | 19.74                 | 19.77           |                      |         |         |
| A18,B18,B18'  | 36.43                      | 38.47           |                      |         |         |
| A18'          | 17.73                      | 17.73           |                      |         |         |
| A19,B19,B19'  | 43.94                      | 43.87           |                      | 0.07    | 0.07    |
| A20,B20,B20'  | 19.93                      | 19.88           |                      |         |         |
| A20'          | 22.39                      | 22.38           |                      |         |         |
| C1,C1'        | 63.06                      | 63.08           |                      |         |         |
| C2,C2'        | 78.36                      | 78.39           |                      |         |         |
| C3,C3'        | 71.11                      | 71.15           |                      |         |         |

$^a$ Numbers refer to carbon atoms indicated in Fig. 1.
$^b$ As determined by an APT spectrum measured in CDCl$_3$ on a Bruker DRX600; chemical shifts are reported relative to tetramethylsilane (TMS); assignments of the crenarchaeol isomer are tentative since they have not been backed up with 2D NMR techniques.
$^c$ The difference in shift when >0.05 ppm (which is the error in the shift values).
$^d$ Data previously published by Sinninghe Damsté et al. (2002).
$^e$ Nd = not detected; signal to noise ratio was too low to assign this carbon atom.
suggests that there must be a difference in the structure of the cyclopentane moiety adjacent to cyclohexane moiety. Since the shifts for carbon atoms A1, A2, A3, A4, A5, A6, and A17 are hardly affected (Table 1), the position of the cyclopentane moiety within the isoprenoid carbon skeleton should remain the same. This would also be in line with the known biosynthetic capabilities of archaea since cyclopentane moieties in GDGTs are only known to occur at two positions, i.e. by ring closure of carbon atoms A10 and A18, and A6 and A17 (e.g. Schouten et al., 2013b and references cited therein). However, this latter position of a cyclopentane moiety is only observed in (hyper)thermophilic archaea, in combination with the presence of four cyclopentane moieties in the more “common” position. Hence, a different position for the cyclopentane moiety is also unlikely from the biosynthetic point of view.

A possibility that remains is that the cyclopentane moiety adjacent to the cyclohexane possesses a different stereochemistry. The stereochemistry of cyclopentane moieties in GDGTs has been shown to be trans (Sinninghe Damsté et al., 2002), so the crenarchaeol isomer could possess the cis stereochemistry for the cyclopentane moiety adjacent to the cyclohexane moiety. Indeed, reported $^{13}$C chemical shifts for cis and trans 1,3-methylcyclopentane (Christl et al., 1971) are different and the shifts of C-2 (corresponding to A18) shift ca. 2 ppm to lower field, just as is observed for A18 (Table 1). This change in stereochemistry of the cyclopentane moiety may also explain the change of the chemical shift of A11 (Table 1), the carbon atom of the cyclohexane ring to which the cyclopentane moiety is attached. Hence, based on the current data it seems likely that the crenarchaeol isomer contains a tricyclic BP where the stereochemistry of the cyclopentane moiety adjacent to the cyclohexane is not trans but cis. This may affect the physical properties of the tricyclic BP in such a way that it elutes substantially later on a GC column, while it would likely also affect the retention time on the LC.

A problem that remains, however, is that in cases where the crenarchaeol isomer present in sediments was subjected to ether cleavage, two, partially separated, tricyclic BPs are formed upon ether cleavage of the crenarchaeol isomer rather than one (Fig. 2b). Liu et al. (2018) suggested that this may be explained by the presence of two crenarchaeol isomers that are not separated by LC in addition to crenarchaeol, each with a unique, although the presence of two crenarchaeol isomers that are not separated by GC (e.g. 13,16-dimethyloctacosane, Chappe et al., 1980; C25 highly branched thiophene; Sinninghe Damsté et al., 1989). In contrast with this explanation, BP IV was not detected in the experiment with the GDGTs of “Ca. Nitrosotenuis uzonensis” (Fig. 2c), which excludes the possibility that it is formed through isomerization during the ether cleavage reaction. Perhaps, this isomerization takes place in the sediment as all other experiments have been performed on GDGTs present in sediments. More experimental work is clearly required to test this.

Summarizing, based on our analyses and reinterpretation of the NMR data of the crenarchaeol isomer, we agree with Liu et al. (2018) that the crenarchaeol isomer used in the TEX$_{86}$ palaeothermometer is not the regioisomer of crenarchaeol. Most likely, it represents an isomer in which the cyclopentane moiety adjacent to the cyclohexane moiety possesses the uncommon cis stereochemistry. Our data on “Ca. Nitrosotenuis uzonensis” demonstrate that this unusual stereochemistry is already present in living archaeal cells and is not a product of diagenesis. Indeed, some thermophilic Thaumarchaeota (Pitcher et al., 2010) and soil Thaumarchaeota grown at high temperature (Sinninghe Damsté et al., 2012) contain the crenarchaeol isomer in relatively high amounts (up to 24% of the core lipids), suggesting that production of the crenarchaeol isomer is a biological adaptation especially to high temperature. Marine Thaumarchaeota also produce higher fractional abundances of the crenarchaeol isomer at higher temperatures (Kim et al., 2010), explaining why it is used in the TEX$_{86}$ palaeothermometer (Schouten et al., 2002). Apparently, the change in the stereochemical structure of the specific cyclopentane moiety results in different physical properties of the crenarchaeol isomer affecting the overall membrane fluidity in such a way that it makes the membrane more suitable for use at higher temperatures. This interpretation actually provides a better explanation for the observed relationship of the crenarchaeol isomer with temperature than if it would represent the regioisomer since variations in the fractional abundance of regioisomers are much less likely affecting the packing of the membrane than the stereochemistry of bulky groups in the alkyl chains of the GDGTs. Similar to the proposition of the cyclohexane ring being an adaptation of GDGT membranes to mesophilic temperatures (Sinninghe Damsté et al., 2002), the introduction of a cis-configuration could allow for better packing of the Thaumarchaeotal membrane at higher temperatures.

Acknowledgements

We thank Elise Jonkheim and Lennart van Maldegem for their help in isolating crenarchaeol and its isomer, Wim Genuit for assistance in obtaining the SMB mass spectra, Marton Palatinszky and Michael Wagner for a kind gift of biomass of “Ca. Nitrosotenuis uzonensis”, and two anonymous referees for helpful suggestions. JSSD, ECH, and SS receive funding from the Netherlands Earth System Science Center (NESSC) though a gravitation grant (024.002.001) from the Dutch ministry for Education, Culture and Science.

Associate Editor—Andrew Revill

References

Chappe, B., Michaelis, W., Albrecht, P., 1980. Molecular fossils of Archaeabacteria as selective degradation products of kerogen. Physics and Chemistry of the Earth 12, 265–274.

Christl, M., Reich, H.J., Roberts, J.D., 1971. Nuclear magnetic resonance spectroscopy. Carbon-13 chemical shifts of methycyclopentanes, cyclopentanols, and cyclopentyl acetates. Journal of the American Chemical Society 93, 3463–3468.
Fialkov, A.B., Gordin, A., Amirav, A., 2008. Hydrocarbons and fuels analyses with supersonic gas chromatography mass spectrometry – the novel concept of isomer abundance analysis. Journal of Chromatography A 1195, 127–135.

Gräther, O., Arigoni, D., 1995. Detection of regiosomeric macrocyclic tetrateraethers in the lipids of *Bacteroides* thermoautotrophicus and other archaeal organisms. Journal of the Chemical Society, Chemical Communications, 405–406.

Hopmans, E.C., Schouten, S., Sinninghe Damsté, J.S., 2016. The effect of improved chromatography on CDT-based palaeoproxies. Organic Geochemistry 93, 1–6.

Karner, M.B., DeLong, E.F., Karl, D.M., 2001. Archaeal dominance in the mesopelagic zone of the Pacific Ocean. Nature 409, 507–510.

Kaneyko, M., Kirjaimi, F., Naraoka, H., 2011. Stable hydrogen isotope measurement of marine hydrocarbons. Organic Geochemistry 42, 166–172.

Kim, J.H., Van der Meer, J., Schouten, S., Helmke, P., Willmott, V., Sangiorgi, F., Koç, N., Hopmans, E.C., Sinninghe Damsté, J.S., 2010. New indices and calibrations derived from the distribution of crenarchaeal isoprenoid tetrateraether lipids: implications for past sea surface temperature reconstructions. Geochimica et Cosmochimica Acta 74, 4639–4654.

Könneke, M., Bernhard, A.E., Józef, R., Walker, C.B., Waterbury, J.B., Stahl, D.A., 2005. Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437, 543–546.

Lebedeva, E.V., Hatzenpichler, R., Pelletier, E., Schuster, N., Haunzayer, S., Bulaev, A., Naderzda, V., Grigor'eva, V., Calushko, A., Schmid, M., Palatinszky, M., Le Paslier, D., Holger Daims, H., Wagner, M., 2013. Enrichment and genome sequence of the group I.1a ammonia-oxidizing archaeon “Ca. Nitrosotenuis uzonensis” representing a clade globally distributed in thermal habitats. Plos one 8, e80835.

Lengger, S.K., Lipswers, Y.A., De Haas, H., Sinninghe Damsté, J.S., Schouten, S., 2014. Lack of 13C-label incorporation suggests low turnover rates of thaumarchaeotal intact polar tetrateraether lipids in sediments from the Iceland shelf. Biogioscences 11, 201–216.

Liu, X.L., Lipp, J.S., Birgel, D., Summons, R.E., Hinrichs, K.U., 2018. Predominance of parallel glycerol arrangement in archaeal tetrateraethers from marine sediments: structural features revealed from degradation products. Organic Geochemistry 115, 12–23.

O’Brien, C.L., Robinson, S.A., Pancost, R.D., Sinninghe Damsté, J.S., Schouten, S., Lunt, D.J., Alsenz, H., Bornemann, A., Bottini, C., Brassell, S.C., Farnsworth, A., Forster, A., Huber, B.T., Inglis, G.N., Jenkyns, H.C., Linnert, C., Littler, K., Markwick, P., MCArena, A., Mutterlose, J., Naafs, B.D.A., Püttermann, W., Sluijs, A., van Helmond, Niels A.G.M., Vellekoop, J., Wagner, T., Wrobel, N.E., 2017. Cretaceous seasurface temperature evolution: constraints from TEX86 and planktonic foraminiferal oxygen isotopes. Earth Science Reviews 172, 224–247.

Pitcher, A., Rychlik, N., Hopmans, E.C., Speek, E., Rijpstra, W.L.C., Ossebaar, J., Schouten, S., Wagner, M., Sinninghe Damsté, J.S., 2010. Crenarchaeal dominates the membrane lipids of *Candidatus Nitrososphaera garrensis*, a thermophilic Group I.1b Archaeon. ISME Journal 4, 542–552.

Pitcher, A., Villanueva, L., Hopmans, E.C., Schouten, S., Reichart, G.-J., Sinninghe Damsté, J.S., 2011a. Niche segregation of Ammonia-oxidizing Archaea and Anammox bacteria in the Arabian Sea oxygen minimum zone. ISME Journal 5, 1896–1904.

Pitcher, A., Wuchter, C., Siedenberg, K., Schouten, S., Sinninghe Damsté, J.S., 2011b. Crenarchaeal tracks winter blooms of planktonic, ammonia-oxidizing Thaumarchaeota in the coastal North Sea. Limnology Oceanography 56, 2308–2318.

Schouten, S., Hoefs, M.J., Koopmans, M.P., Bosch, H.J., Sinninghe Damsté, J.S., 1998. Structural characterization, occurrence and fate of archaeal ether-bound acyclic and cyclic biphytanes and corresponding diols in sediments. Organic Geochemistry 29, 1305–1319.

Schouten, S., Hopmans, E.C., Schefuß, E., Sinninghe Damsté, J.S., 2002. Distributional variations in marine crenarchaeotal membrane lipids: a new tool for reconstructing ancient sea surface temperatures? Earth and Planetary Science Letters 204, 265–274.

Schouten, S., Hopmans, E.C., Rosell-Melé, A., Pearson, A., Adam, P., et al., 2013a. An interlaboratory study of TEX86 and BIT analysis of sediments, extracts, and standard mixtures. Geochemistry, Geophysics, Geosystems 14, 5263–5283.

Schouten, S., Hopmans, E.C., Sinninghe Damsté, J.S., 2013b. The organic geochemistry of glycerol dialkyl glycerol tetraether lipids: a review. Organic Geochemistry 54, 19–61.

Sinninghe Damsté, J.S., van Koert, E.R., Kock-van Dalen, A.C., de Leeuw, J.W., Schenck, P.A., 1989. Characterisation of highly branched isoprenoid thiophenes occurring in sediments and immature crude oils. Organic Geochemistry 14, 555–578.

Sinninghe Damsté, J.S., Schouten, S., Hopmans, E.C., van Duin, A.C., Geenevasen, J.A., 2002. Crenarchaeal: the characteristic core glycerol dibiphytanylglycerol tetraether membrane lipid of cosmopolitan pelagic crenarchaeota. Journal of Lipid Research 43, 1641–1651.

Sinninghe Damsté, J.S., Rijpstra, W.L.C., Hopmans, E.C., Jung, M.Y., Kim, J.G., Rheo, S.K., Stiegmeier, M., Schleper, C., 2012. Intact polar core glycerol dibiphytanylglycerol tetraether lipids of group I.1a and I.1b Thaumarchaeota in soil. Applied and Environmental Microbiology 78, 6866–6874.

Wuchter, C., Abbas, B., Coolen, M.J., Herfort, L., van Bleijswijk, J., Timmers, P., Strous, M., Teira, E., Herndl, G.J., Middelburg, J.J., Schouten, S., Sinninghe Damsté, J.S., 2006. Archaeal nitrification in the ocean. Proceedings of the National Academy of Sciences 103, 12317–12322.