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Short chain ladderanes: Oxic biodegradation products of anammox lipids

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Abstract

Anammox, the microbial anaerobic oxidation of ammonium by nitrite to produce dinitrogen gas, has been recognized as a key process in both the marine and freshwater nitrogen cycles, and found to be a major sink for fixed inorganic nitrogen in the oceans. Ladderane lipids are unique anammox bacterial membrane lipids that have been used as biomarkers for anammox bacteria in recent and past environmental settings. However, the fate of ladderane lipids during diagenesis is as of yet unknown. In this study, we performed oxic degradation experiments (at 20–100 °C) with anammox bacterial biomass to simulate early diagenetic processes occurring in the water column and at the sediment–water interface. Abundances of C_{18} and C_{20} ladderane lipids decreased with increasing temperatures, testifying to their labile nature. The most abundant products formed were ladderane lipids with a shorter alkyl side chain (C_{14} and C_{16} ladderane fatty acids), which was unambiguously established using two-dimensional NMR techniques on an isolated C_{14}-[3]-ladderane fatty acid. The most pronounced production of these short-chain lipids was at 40 °C, suggesting that degradation of ladderane lipids was microbially mediated, likely through a β-oxidation pathway. An HPLC–MS/MS method was developed for the detection of these ladderane alteration products in environmental samples and positively tested on various sediments. This showed that the ladderanes formed during degradation experiments also naturally occur in the marine environment. Thus, short-chain ladderane lipids may complement the original longer-chain ladderane lipids as suitable biomarkers for the detection of anammox processes in past depositional environments.

1. INTRODUCTION

Lipids provide much information as biomarkers for specific biosynthetic pathways in source organisms (Peters et al., 2005; Volkman, 2005). However, during settling in the water column and at the sediment–water interface, lipids can undergo structural alterations by biotic and abiotic processes. Depending on structure, protection by a mineral or organic matrix, and oxygen levels, different alterations of individual biomarker lipids takes place, e.g. preferential degradation of shorter-chain *n*-alkanes (Johnson and Calder, 1973), incorporation of sulfur into functionalized lipids (Sinninghe Damsté et al., 1989), and autoxidation of alkenones (Rontani et al., 2006). Unsaturated and cyclic components are generally considered more susceptible to degradation than saturated components (Sun and Wakeham, 1994; Hoefs et al., 1998, 2002; Sinninghe Damsté et al., 2002a). Identifying the diagenetic products of biomarker lipids is an important tool in molecular biogeochemistry as it enables the reconstruction of past biomarker presence (Peters et al., 2005).

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Ladderane lipids (Sinninghe Damsté et al., 2002b) are membrane lipids specific for anammox bacteria, belonging to the *Planctomycetes*, which perform anaerobic ammonium oxidation to dinitrogen gas with nitrite as the electron acceptor (Van de Graaf et al., 1995; Strous et al., 1999). Ladderane lipids consist of either three or five linearly concatenated cyclobutane rings (Fig. 1). These lipids form a dense and highly impermeable membrane surrounding the intracellular compartment where the anammox reaction takes place: the anammoxosome (Lindsay et al., 2001; Van Niftrik et al., 2004). Such a dense membrane is thought to be required to maintain concentration gradients during the exceptionally slow metabolism of anammox bacteria, and would also protect the remainder of the cell from the highly toxic intermediate hydrazine (Sinninghe Damsté et al., 2002b; van Niftrik et al., 2010). Since its discovery in a wastewater treatment system (Mulder et al., 1995), many studies have shown that anammox bacteria are ubiquitous in the marine environment and that the anammox process constitutes a substantial sink for fixed inorganic nitrogen in the oceans (Dalsgaard and Thamdrup, 2002; Kuypers et al., 2005; Thamdrup et al., 2006; Hamersley et al., 2007), which previously was solely attributed to heterotrophic denitrification. Ladderane lipids have been detected in oxygen minimum zones (OMZs) (Hamersley et al., 2007; Jaeschke et al., 2007) and found to be exported through the water column (Jaeschke et al., 2007). However, the effect of (oxic) degradation on ladderane lipids occurring during transport through the water column and after deposition on the seafloor is presently unknown.

In this study, we investigated the effect of oxic degradation by incubating anammox cell material with Wadden Sea sediment and water under oxic conditions. We monitored the degradation of ladderane lipids and analyzed the products formed during these experiments in order to evaluate potential early diagenetic processes of ladderanes which might occur in the natural environment.

2. MATERIALS AND METHODS

2.1. Experimental setup

Two sources of anammox cell material were used in this experiment: (1) An anammox enrichment culture grown over 5 months in sequencing batch reactors (SBRs) as de-

Table 1
Protonated molecules, selected product ions, and collision energies for the SRM detection of short-chain ladderane fatty acids.

Ladderane lipid alteration product	$[M+H]^+$ *m/z*	Product *m/z*	Optimum collision energy (V)
I	233.2	117.0	37
		131.0	32
		158.9	24
		172.9	23
II	235.2	119.2	34
		133.2	32
		161.2	24
		175.2	22
III	263.2	93.5	25[a]
		121.3	25[a]
		135.2	25[a]
		171.1	25[a]

[a] Collision energy was set at 25 V for all transitions monitored for component III as no standard was available for optimization.

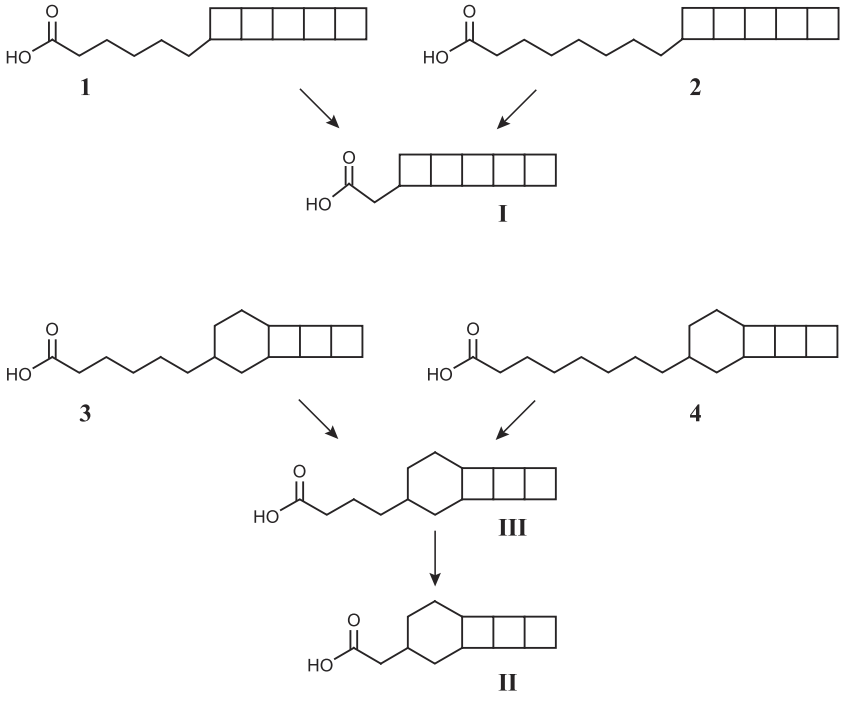


Fig. 1. Structures of ladderane lipids derived from anammox bacteria and their transformation products derived from microbial oxic degradation. (1) C_{18}-[5]-ladderane FA, (2) C_{20}-[5]-ladderane FA, (3) C_{18}-[3]-ladderane FA, (4) C_{20}-[3]-ladderane FA, (I) C_{14}-[5]-ladderane FA, (II) C_{14}-[3]-ladderane FA, (III) C_{16}-[3]-ladderane FA. Key: FA = fatty acid.

scribed by Strous et al. (1998), which contained two species of anammox bacteria, *Candidatus* Kuenenia stuttgartiensis and *Candidatus* Brocadia fulgida; and (2) biomass from the Dokhaven wastewater treatment plant, shown to contain 70% bacterial dominance of *Candidatus* Kuenenia stuttgartiensis (Schmid et al., 2000). All cell material was freeze-dried and stored at −20 °C until further use.

For each degradation experiment approximately 20–30 mg of the SBRs anammox cell material was thoroughly mixed with 1.5–2 g of freeze-dried sandy sediment derived from the Wadden Sea, a tidal flat area situated close to NIOZ, and placed in open tubes. Ca. 1–2 ml of bi-distilled water was added to obtain a thick slurry, so that the cell material could be well dispersed throughout the experiment. These samples were then incubated for 72 h at temperatures of 20, 40, 50, 60, 70, 80 and 100 °C, respectively, and gently stirred to maintain aeration during the experiments. Afterwards, the supernatant was removed and the residue was freeze-dried again. A control experiment was performed at 40 °C for 72 h using SBRs anammox cell material mixed with sterilized sediment (autoclaved for 20 min at 120 °C).

In order to isolate degradation products, a large batch degradation experiment was also performed. In an open flask, 1.5 g of anammox cell material from the Paques wastewater treatment plant was thoroughly mixed with 100 g of freeze-dried sediment. The mixture was gently stir-

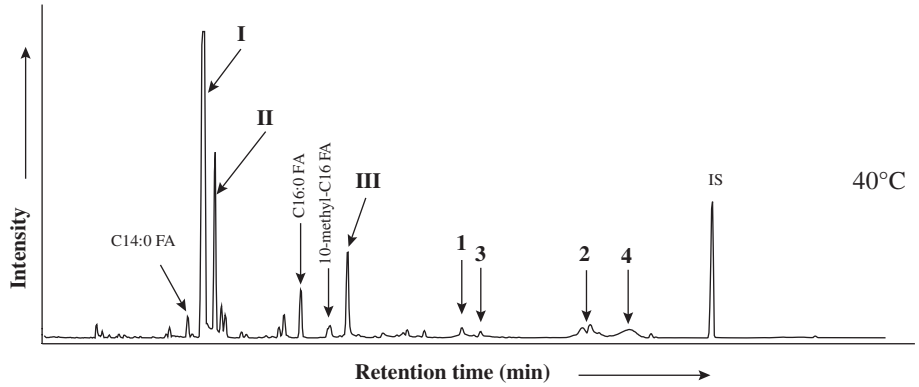


Fig. 2. Gas chromatogram of the fatty acid fraction of anammox biomass incubated for three days at 40 °C. Numbers correspond to structures shown in Fig. 1. IS denotes internal standard (*ante iso* C_{22} alkane).

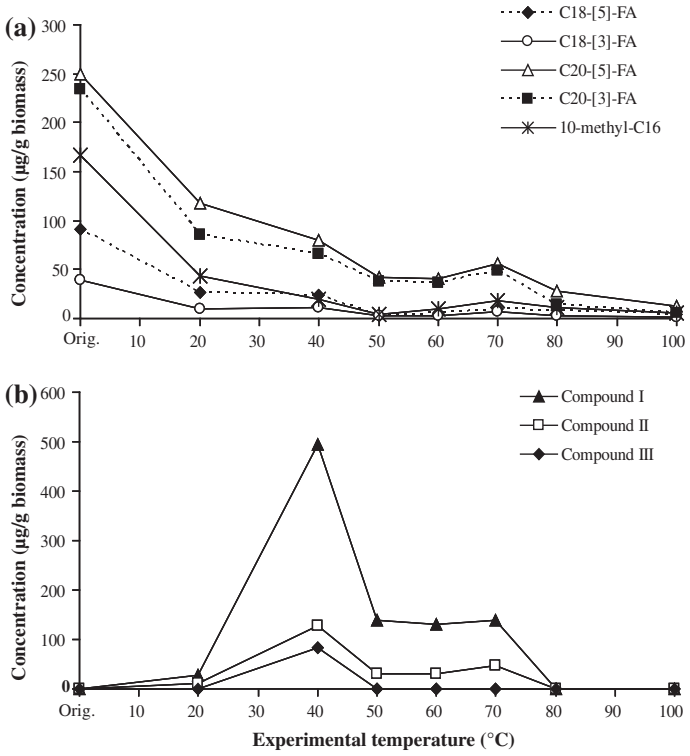


Fig. 3. Concentration of (a) known ladderane lipids and 10-methylhexadecanoic acid, and (b) short-chain ladderane transformation products as a function of temperature in the fatty acid fractions after base hydrolysis and analyzed by GC.

red at 40 °C with 100 mL of bi-distilled water, which was refilled throughout the course of the week-long experiment to replace water lost due to evaporation. After 7 days, the experiment was stopped, the supernatant was removed, and the residue was freeze-dried.

2.2. Extraction and fractionation

Samples from the degradation experiments were ultrasonically extracted five times using a dichloromethane (DCM)/methanol mixture (2:1 v/v). Combined extracts were dried using rotary evaporation yielding the total lipid extract (TLE).

Aliquots of the TLEs from the temperature experiments were saponified with aqueous 1 N KOH in methanol for 2 h at 100 °C. Fatty acids were obtained by acidifying the solution to pH 3 and extracting with DCM. The fatty acid fraction was methylated by adding diazomethane to convert fatty acids into their corresponding fatty acid methyl esters (FAMEs). To remove very polar components, aliquots of the FAMEs were eluted with ethyl acetate over a small column filled with silica. Extracts derived from sediments were further eluted with DCM over a small silver nitrate-impregnated (5%) silica column to remove polyunsaturated fatty acids, yielding a saturated fatty acid fraction. For quantification, a known amount of internal standard (*ante iso* C_{22} alkane) in ethyl acetate was added prior to gas chromatography (GC) and GC/mass spectrometry (MS) analyses.

For high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) analysis, an aliquot of the methylated TLE was dissolved in acetone and filtered through a 0.45 μm, 4 mm diameter PTFE filter.

2.3. Isolation of short-chain ladderane fatty acids

Freeze-dried residue of the large batch degradation experiment was ultrasonically extracted five times using a DCM/methanol mixture (2:1 v/v). Combined extracts were dried using rotary evaporation, yielding the TLE (1.1 g). The total extract was methylated using BF_3 in methanol. Lipids were separated over an activated Al_2O_3 large column (75 × 250 mm) using four solvent mixtures: hexane:DCM (9:1), hexane:DCM (1:1), DCM, and DCM:methanol (1:1) (v/v). The short-chain ladderane fatty acids eluted in the hexane:DCM (1:1) fraction. Two short-chain components (**I** and **II**; see Fig. 1 for structures) were further isolated by repetitive semi-preparative HPLC (high performance liquid chromatography) following conditions described by Hopmans et al. (2006). Briefly, aliquots of the hexane:DCM (1:1) fraction were injected onto two Zorbax Eclipse XDB-C_8 columns (4.6 × 150 mm, 5 μm, Agilent, coupled in series and maintained at 30 °C). Ladderane lipids were eluted using 0.4 ml/min methanol and fractions of 15 s were collected. Purity of the resulting fractions was assessed by GC and GC/MS. The purity of the isolated methyl ester C_{14}-[3]-ladderane fatty acid (**II**) was found to be 97%. The structure of this component was established using 2D-nuclear magnetic resonance (NMR) spectroscopy. The second short-chain fatty acid, C_{14}-[5]-ladderane fatty acid (**I**), was sufficiently enriched during the preparative HPLC to be used in the development of a

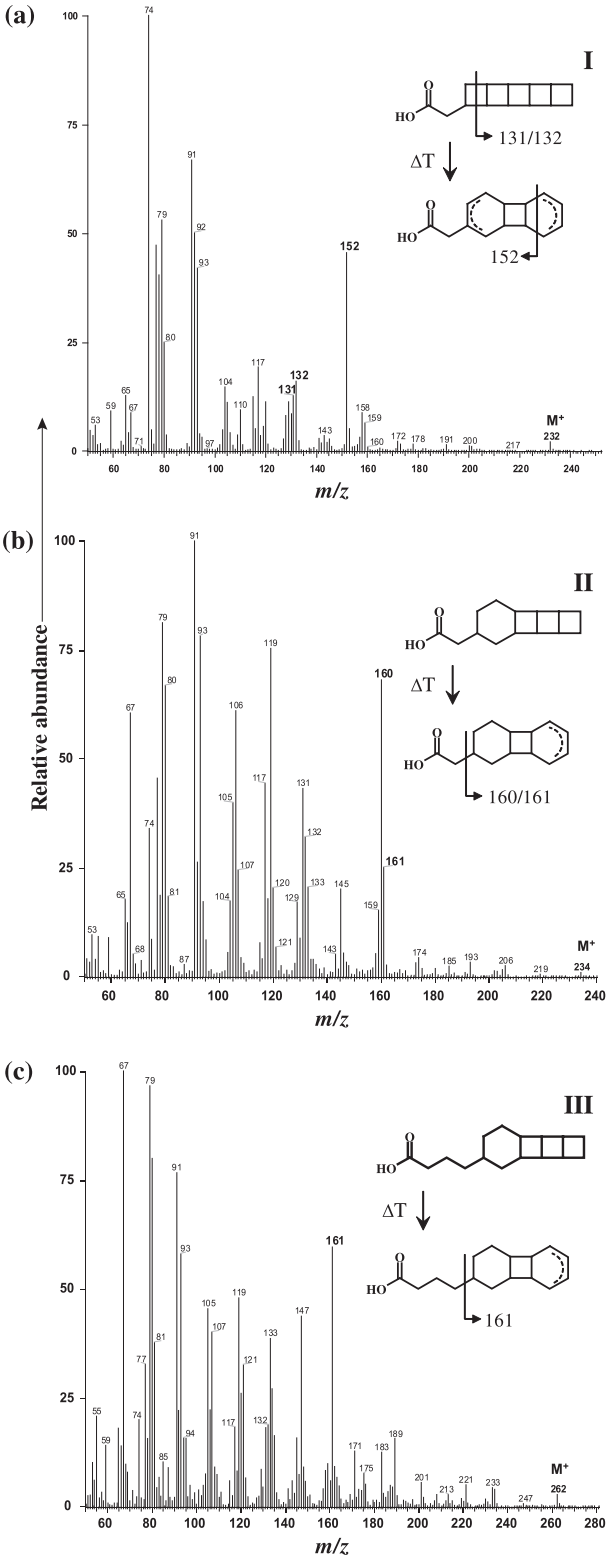


Fig. 4. GC/MS spectra of the short-chain ladderane fatty acids **I**, **II**, and **III** analyzed as their methyl ester derivatives. (a) C_{14}-[5]-ladderane fatty acid, (b) C_{14}-[3]-ladderane fatty acid, and (c) C_{16}-[3]-ladderane fatty acid. It should be noted that the mass spectra reflect the thermal degradation products formed during GC analysis as discussed by Sinninghe Damsté et al. (2005).

HPLC/APCI–MS/MS method (HPLC coupled to positive ion atmospheric pressure chemical ionization tandem mass spectrometry), but the total amount of **I** was insufficient for 2D NMR studies.

2.4. GC and GC/MS analysis

GC analysis was performed using a Hewlett-Packard 6890 instrument equipped with an on-column injector and a flame ionization detector (FID). A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.12 μm) was used with helium as carrier gas. The samples were injected at 70 °C. The GC oven temperature was subsequently raised to 130 °C at a rate of 20 °C/min, and then at 4 °C/min to 320 °C, which was held for 15 min. GC/MS analysis was carried out using a Finnigan Trace GC Ultra, Thermo Electron Corporation, interfaced with a Finnigan Trace DSQ mass spectrometer, with a mass range of *m/z* 40–800. GC conditions for GC/MS were the same as those described above for GC. The components formed during thermal destruction of the ladderane lipids during GC analysis were identified according to retention times and mass spectra described by Sinninghe Damsté et al. (2005).

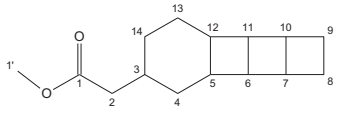
2.5. HPLC/APCI–MS/MS analysis

The HPLC/APCI–MS/MS method developed by Hopmans et al. (2006) and modified by Rattray et al. (2008) was expanded to include Selective Reaction Monitoring (SRM) transitions diagnostic for the newly detected short-chain components with concatenated cyclobutane moieties (Table 1). First, an aliquot from the 40 °C degradation experiment was analyzed using chromatographic and source conditions identical to those described by Rattray et al. (2008). Detection was achieved by MS/MS in data dependent scan mode where a positive ion scan (*m/z* 200–400) was followed by a product ion scan (collision energy 25 V, 0.8 mTorr argon, collision gas) of the base peak of the generated mass spectrum. Based on the results of this experiment, diagnostic fragments for each of the short-chain ladderane lipids (**I**, **II**, and **III**) were identified. Optimal conditions for each monitored SRM transition were determined by an automatic tuning procedure while directly infusing the isolated C_{14}-[3]-ladderane fatty acid (**II**) and the fraction enriched in C_{14}-[5]-ladderane fatty acid (**I**) (Table 1). Collision gas pressure was maintained at 1.5 mTorr for these experiments. No standard was available for component **III** and thus the collision energies for its reactions were set to 25 V (Table 1).

2.6. ^{1}H and ^{13}C NMR spectroscopy

The C_{14}-[3]-ladderane fatty acid methyl ester isolated using semi-preparative HPLC was dissolved and dried in (2×) DCM, (2×) $CHCl_3$, (2×) tetrachloroethane, and (2×) $CDCl_3$ then transferred to an NMR tube using 0.75 mL $CDCl_3$. 1D and 2D ^{1}H and ^{13}C NMR analyses were per-

Table 2
Proton and carbon NMR data for component II (C_{14}-[3]-ladderane fatty acid methyl ester), formed during the oxic degradation of ladderane biomass.



C-number	Stereo-chemistry	Proton shift (ppm)	Carbon shift (ppm)[a]				2D NMR	
			Primary	Secondary	Tertiary	Quaternary	COSY correlations	TOSCY correlations
1		–				173.62	–	–
2		2.24 (d, 2H)		42.32			3	3, 4α, 4β, 5, 13, 14α, 14β
3		1.76 (m, 1H)			29.96		2, 4β	2
4	α	1.80 (m, 1H)		33.93			4β, 5	4β, 14α
	β	1.11 (m, 1H)					3, 4α, 5	5, 6, 13, 14α
5		2.27 (m, 1H)			37.43		4α, 4β, 6, 12	4α, 4β, 13
6		2.31 (m, 1H)			49.18		5	4α, 4β, 12
7		2.75 (m, 1H)			41.38		8α, 10	8α, 8β, 9α, 9β, 10, 11, 12
8	α	2.50 (m, 1H)		26.15			7, 8β, 9α, 9β	7, 8β, 9β, 10
	β	1.84 (m, 1H)					8α, 9α, 9β	7, 8α, 9α, 10
9	α	2.42 (m, 1H)		25.39			8α, 8β, 9β, 10	7, 8β, 9β, 10
	β	1.95 (m, 1H)					8α, 8β, 9α, 10	7, 8α, 9α, 10
10		2.63 (m, 1H)			42.18		7, 9α, 9β, 11, 12	7, 8α, 8β, 9α, 9β, 11, 12
11		2.45 (m, 1H)			47.07		10, 12	7, 10, 12
12		2.31 (m, 1H)			37.03		5, 10, 11, 13	4α, 4β, 5, 6, 10, 11, 13, 14α, 14β
13		1.54 (m, 2H)		25.23			12, 14α, 14β	4α, 4β, 5, 12, 14β
14	α	1.60 (m, 1H)		27.83			13, 14β	2, 4α, 4β, 12, 13, 14β
	β	1.18 (m, 1H)					13, 14α	2, 4α, 4β, 12, 13, 14α
1′		3.68 (s, 3H)	51.38				–	–

[a] Connection was established by an HSQC experiment.

formed on a Bruker DMX-600 spectrometer equipped with a TCI CryoProbe at 298 K as previously reported by Sinninghe Damsté et al. (2005).

3. RESULTS AND DISCUSSION

3.1. Degradation experiments

The effect of oxygen and temperature on the preservation of ladderane lipids was investigated using aliquots of biomass from an anammox enrichment culture, which contained *Candidatus* Kuenenia stuttgartiensis and *Candidatus* Brocadia fulgida, mixed with surface sediment derived from the Wadden Sea. These experiments were incubated in open tubes at temperatures ranging from 20 to 100 °C for three days. The original C_{18} and C_{20} ladderane fatty acids (**1–4**; see Fig. 1 for structures), as analyzed with GC, substantially decreased in concentration with increasing experimental temperature (Figs. 2 and 3a). Compared to the original unheated anammox biomass, ladderane lipids incubated at 20 °C were 2–4 times lower in concentration, and then slowly decreased further with increasing temperature. At 100 °C, these lipids were still detectable but had decreased substantially in concentrations, leaving <10% of the original lipids.

Several new components were produced during the incubation experiments and were detected with GC–MS (Fig. 2). Components **I** and **II** were present at incubation temperatures between 20 and 70 °C, while **III** was only detected in the 40 °C experiment (Fig. 3b). At temperatures of 80 °C and above, these new components were not detected (Fig. 3b). The highest concentrations of the three components were found at a temperature of 40 °C (Fig. 3b). GC–MS analysis revealed that the mass spectra for **I–III** were similar to those of known C_{18}–C_{20} ladderane fatty acids (Sinninghe Damsté et al., 2005), i.e. loss of the alkyl chain resulting in fragments *m/z* 131, 132, and 160, 161, but with different molecular ions, i.e., *m/z* 232, *m/z* 234, and *m/z* 262 for **I**, **II**, and **III**, respectively (Fig. 4a–c). The molecular ion of **I** is 84 Da less than that of the C_{20}-[5]-ladderane fatty acid (**2**) or 56 Da less than that of the C_{18}-[5]-ladderane fatty acid (**1**), which, together with similar fragmentation patterns, suggests that **I** is a C_{14}-[5]-ladderane fatty acid. The molecular ion of **II** is 84 Da less than that

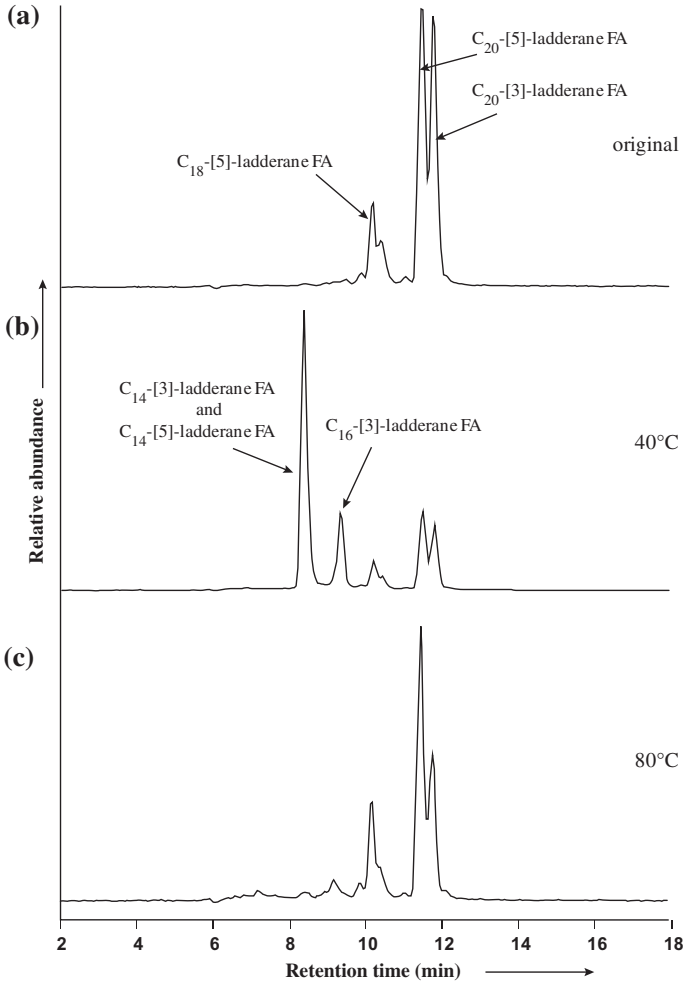


Fig. 5. HPLC base peak chromatograms of the fatty acid fraction (analyzed as their methyl ester derivatives) showing the distribution of ladderane fatty acids in anammox degradation experiments: original (a), heated for three days at 40 °C (b), and heated for 3 days at 80 °C (c).

of the C_{20}-[3]-ladderane fatty acid (**4**) or 56 Da less than that of the C_{18}-[3]-ladderane fatty acid (**3**), suggesting component **II** is a C_{14}-[3]-ladderane fatty acid. Finally, the molecular ion of **III** is 56 Da less than that of the C_{20}-[3]-ladderane fatty acid (**4**) or 28 Da less than that of the C_{18}-[3]-ladderane fatty acid (**3**), suggesting that **III** is a C_{16}-[3]-ladderane fatty acid. The ladderane moiety, which has been found to be heat labile (Sinninghe Damsté et al., 2005) and form thermally more stable products at hydrous pyrolysis temperatures of 120 °C and above (Jaeschke et al., 2008), was still intact at 100 °C.

To unambiguously establish the structure of the short-chain ladderane analysis, a large scale experiment was performed, which enabled the isolation of 1.6 mg of the most abundant component (**II**) for structural identification by high-resolution NMR spectroscopy (Table 2). ^{1}H NMR revealed no olefinic protons as well as shifts (ppm) in aliphatic protons that were akin to ladderane cyclobutane rings. ^{13}C NMR revealed 15 carbon atoms (1 carbonyl carbon, 1 primary carbon in the terminal position of an ester, 6 secondary carbons, and 7 tertiary carbons) which is consistent with a C_{14}-[3]-ladderane FAME. The identification of the ladderane moiety was established by the application of 2D NMR techniques (HSQC, COSY, TOSCY). These studies also confirmed that the conformational properties of component **II** were identical to those of the first ladderanes identified (Sinninghe Damsté et al., 2002b; Mascitti and Corey, 2004). These structural identifications imply that the new fatty acids are formed by the oxidation of the alkyl chain on the original ladderane fatty acids.

The question arises as to whether or not these short-chain fatty acids are formed abiotically or through microbial activity. The fact that ladderane lipids with shorter alkyl chains occur only at temperatures between 20 and 70 °C, and have maximum concentrations at 40 °C, strongly suggested microbial degradation. To confirm this, a control experiment was performed with anammox biomass and sterilized sediment under the same conditions (40 °C, 72 h). No C_{14} or C_{16} ladderane fatty acids were detected, demonstrating that their formation was microbially mediated.

The oxidation of alkyl side chains of cyclic components has been shown previously during microbial degradation experiments. For example, several microorganisms are able to degrade *n*-alkylcyclohexanes by an alkyl side chain oxidation pathway, the so-called β-oxidation pathway (Beam and Perry, 1974; Dutta and Harayama, 2001; Koma et al., 2003). This pathway includes four reactions that occur in repeated cycles. In each cycle, as the alkyl chain is oxidized, it is progressively shortened by two carbon atoms. Depending on whether there is an odd or even number of carbon atoms in the alkyl side chain, carboxylic or acetic acid derivatives are formed, respectively. Typically, acetic acid derivatives are metabolically recalcitrant; they cannot be further oxidized by β-oxidation and usually accumulate (Beam and Perry, 1974). Therefore, we suggest a similar degradation pathway is likely for the ladderane lipids, as their chemical structure comprises of an even number of carbons in the alkyl chain and a cyclic group, which is comparable to that of *n*-alkylcyclohexanes with even-numbered

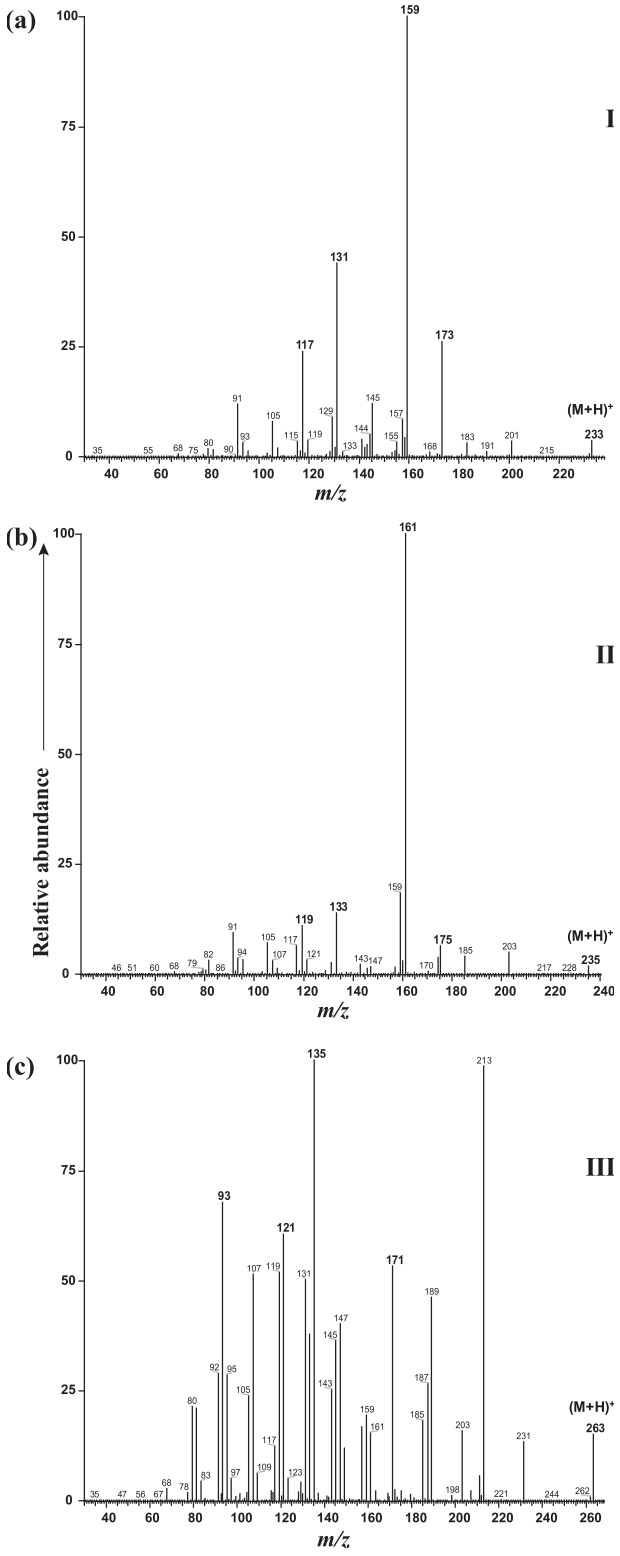


Fig. 6. APCI–MS/MS spectra of the protonated molecular ions of three different ladderane lipid transformation products: (a) component I (*m/z* 233), (b) component II (*m/z* 235), and (c) component III (*m/z* 263). Structures of the original lipids and the alteration products are depicted in Fig. 1.

side chains. The C_{14} and C_{16} ladderane fatty acids (**I**, **II**, and **III**) detected in the degradation experiments are thus derived from C_{18} and C_{20} ladderane fatty acids (**1**–**4**) via the β-oxidation route (Fig. 1) (cf. Dutta and Harayama, 2001). Component **II** could be derived from **III** by further oxidation of the alkyl side chain by two carbon atoms during ongoing degradation. It should be noted that the C_{16}-[3]-ladderane fatty acid with ($[M+H]^+$) of *m/z* 263 was reported previously by Rattray et al. (2010) in an anammox enrichment culture, suggesting that this shorter-chain fatty acid may be synthesized as an adaptation to lower cultivation temperatures. Since this lipid was not present in the starting biomass, our results, however, indicate that, in this case, the shorter-chain ladderane fatty acids were products of microbial oxidation.

3.2. Environmental occurrence of short-chain ladderane fatty acids

To investigate whether the products formed in our degradation experiments are also formed in natural environments, we analyzed a number of sediments for the presence of short-chain ladderane fatty acids. We thus aimed to expand the existing HPLC/APCI–MS/MS SRM method developed for the original ladderane lipids in the natural environment by Hopmans et al. (2006), and modi-

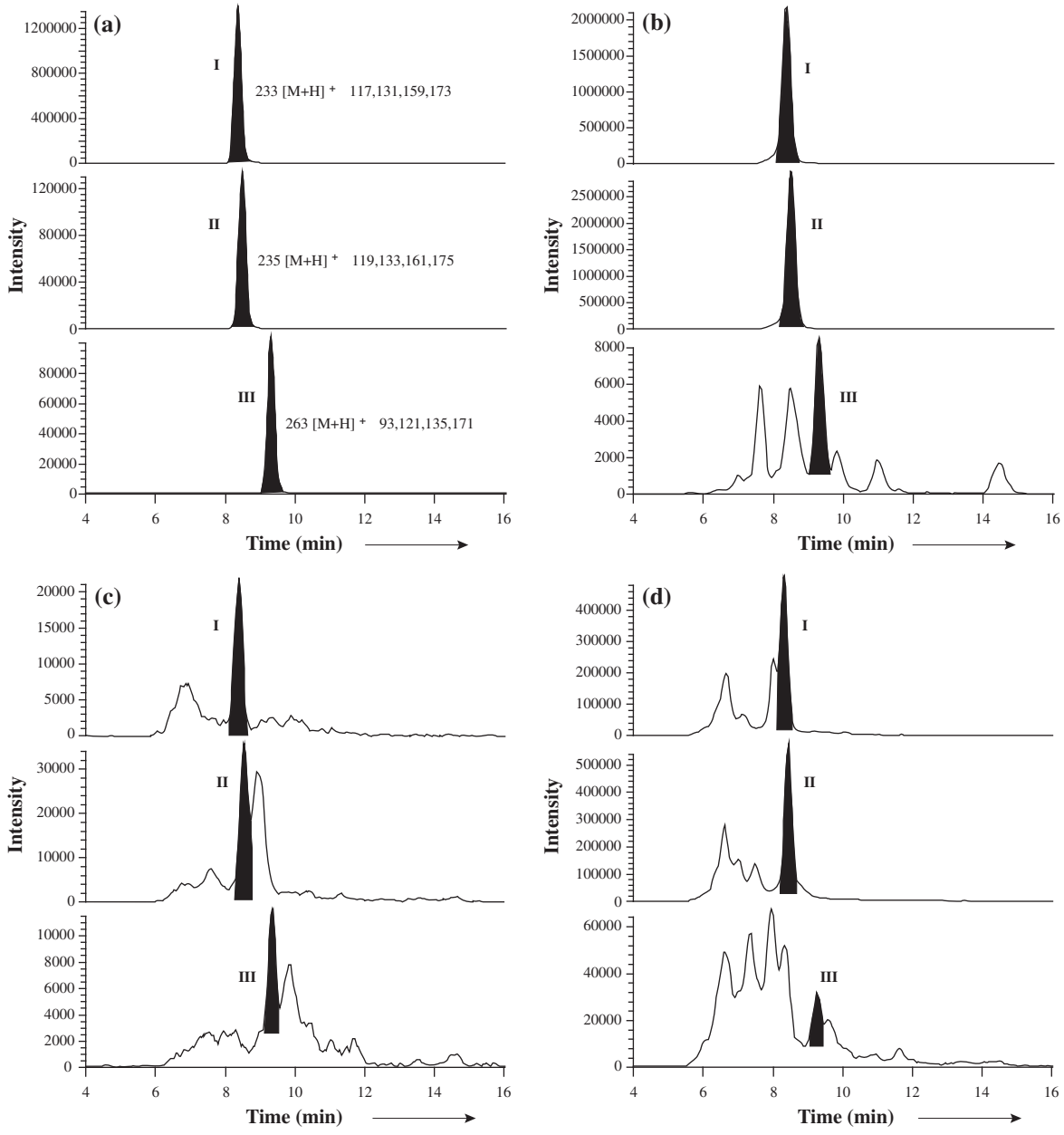


Fig. 7. SRM traces of short-chain ladderane fatty acids (analyzed as their methyl ester derivatives) in (a) anammox biomass heated at 40 °C for three days, and in sediments from (b) the Arabian Sea, (c) the Irish Sea, and (d) offshore northwest Africa.

fied by Rattray et al. (2008), to include the short-chain ladderane fatty acids (Table 1).

We first analyzed an aliquot from the 40 °C experiment, in which the biodegraded ladderane products were initially detected, by MS/MS in data dependent scan mode (Fig. 5b). The results showed two additional peaks, besides those of the original ladderane fatty acids which were found in the biomass before the degradation experiment (Fig. 5a) and in the 80 °C experiment (Fig. 5c). At 40 °C, the peak with a retention time of ca. 8 min. actually consisted of two co-eluting components with protonated molecules ($[M+H]^+$) of *m/z* 233 and 235, respectively. The peak with a retention time of ca. 9 min represented a component with a protonated molecule of *m/z* 263. The APCI–MS/MS spectra of the protonated molecules of these biodegraded ladderanes showed similar product ions to those reported to be representative of the ladderane moieties in the original ladderane fatty acids (Hopmans et al., 2006) (Fig. 6a–c, showing mass spectra for products **I**–**III**, respectively). Therefore, these peaks likely represented the C_{14} and C_{16} shorter-chain ladderane fatty acids. Confirmation came from the HPLC/MS/MS analyses of the isolated C_{14}-[3]-ladderane fatty acid and the enriched fraction of C_{14}-[5]-ladderane fatty acid, which showed the same retention times and MS/MS mass spectra as those identified in the 40 °C experiment. The selection of product ions for SRM was based on their diagnostic value for the concatenated cyclobutane moieties of the ladderane molecules as well as on their high relative abundances in the mass spectra. Parent ions, selected product ions, and respective collision energies for maximal abundance of each monitored short-chain fatty acid are listed in Table 1.

To test this modified method, it was applied on an aliquot of the methylated TLE from the 40 °C experiment (Fig. 7a). C_{14}-[5]-ladderane fatty acid (**I**) was found to be most abundant, followed by C_{14}-[3]-ladderane fatty acid (**II**), which was one order of magnitude lower in abundance. C_{16}-[3]-ladderane fatty acid (**III**) was approximately 1.5 times lower in abundance than component **II**. The method was then used to analyze sediments derived from various locations where C_{18} and C_{20} anammox lipids have previously been detected, i.e., off northwest Africa (08°54.0′N, 14°56.1W; 1–2 cmbsf), the Arabian Sea (22°32.9′N, 64°02.8′E; 193 cmbsf), and the Irish Sea (53°53.0′N, 5°35.6′W; 1–2 cmbsf) (Jaeschke et al., 2007, 2009, 2010). As shown in the partial SRM traces in Fig. 7b-d, the ladderane lipid degradation products with shorter-chain lengths could indeed be detected with the modified HPLC/APCI–MS/MS method in all three sediments analyzed. In these sediments, the C_{14}-[3]-ladderane fatty acid (**II**) was the most abundant, followed by C_{14}-[5]-ladderane fatty acid (**I**), at 1.5–2 times lower abundance. C_{16}-[3]-ladderane fatty acid (**III**) was about two orders of magnitude lower in abundance.

The occurrence of these short-chain ladderane alteration products in marine sediments suggests that ladderane lipids are degraded in the natural environment in a way similar to our simulated degradation experiments, i.e., via a microbially mediated β-oxidation pathway. Thus, the short-chain ladderane fatty acids **I**–**III** may be useful biomarkers for anammox bacteria, especially in sediments underlying oxygen minimum zones, where ladderane lipids produced in the OMZ have been re-exposed to oxygen during settling through the water column and at the sediment–water interface. Future investigations of short-chain ladderanes in natural settings should reveal the quantitative importance of these compounds as biomarkers for oxic ladderane degradation.

4. CONCLUSIONS

The results of our laboratory studies strongly suggest that under oxic conditions ladderane lipids can be microbially degraded via the β-oxidation pathway of the alkyl side chain, resulting in shorter-chain ladderane lipids. The detection of these biodegraded ladderane lipids in marine sediments using a modified HPLC/APCI–MS/MS method indicates that these components are also produced in the natural environment. These short-chain ladderane lipids may be a useful biomarker for past OMZ anammox processes in immature sediments.

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