Organic Geochemistry 68 (2014) 31–38

Contents lists available at ScienceDirect

Organic Geochemistry

journal homepage: www.elsevier.com/locate/orggeochem

Diagenetic degradation products of bacteriohopanepolyols produced by *Rhodopseudomonas palustris* strain TIE-1

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ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form 20 December 2013
Accepted 8 January 2014
Available online 16 January 2014

ABSTRACT

Hopanoids are pentacyclic triterpenoids commonly found in soils and sediments and are used as biomarkers for recent or past bacterial communities. While hopanes are the ultimate degradation products of bacteriohopanepolyols (BHPs), only a few BHPs, such as 32,35-anhydrobacteriohopanetetrol (anhydroBHT), have been identified as early diagenetic degradation products. Here, we report on diagenetic degradation products of BHPs produced by *Rhodopseudomonas palustris* strain TIE-1 after exposure to elevated pressure and temperature. While bacteriohopanetetrol (BHT) was degraded to various anhydroBHT isomers, new N-containing degradation products were discovered and tentatively assigned as anhydroaminotriols.

1. Introduction

Bacteriohopanepolyols (BHPs) are pentacyclic triterpenoids, belonging to the class of hopanoids, that are ubiquitous in soils and sediments (Ourisson et al., 1979; Ourisson and Albrecht, 1992) and are exclusively synthesized by bacteria. They can be used to fingerprint recent bacterial communities (e.g. Talbot and Farrimond, 2007; Cooke et al., 2008a; van Winden et al., 2012) and, given the relatively high resistance of their carbon skeleton to thermal degradation, their degradation products are amongst the oldest extractable compounds found in the rock record (Brocks et al., 2005). Apart from biodegradation, the preservation potential of BHPs depends mainly on their resistance to abiotic degradation at elevated temperature and pressure over geological time.

It is widely accepted that most sedimentary hopanoids are degradation products of BHPs or derive from the C_{30} hopanoids diploptterol and diploptene (Ourisson et al., 1979; Rohmer et al., 1984). Sedimentary hopanoids recognized as early diagenetic products of BHPs include 32,35-anhydrobacteriohopanetetrol (anhydroBHT, **VI**; see Fig. 1 for structures) and 31-hydroxyanhydroBHT (e.g. Bednarczyk et al., 2005; Talbot et al., 2005; Schaeffer et al., 2008, 2010). AnhydroBHT was discovered in the sponge *Plakortis simplex* and was thought to be a biogenic product of either bacteria living within the sponge, or from the sponge itself converting dietary BHPs to anhydroBHT (cf. Costantino et al., 2001). Later, an abiogenic diagenetic origin for anhydroBHT was proposed, resulting from dehydration and cyclization of bacteriohopane-32,33,34,35-tetrol (BHT) **I** (Bednarczyk et al., 2005), which explains a commonly observed increasing content of anhydroBHT and a concurrent decrease of BHT in sediments with increasing depth (Watson, 2002; Bednarczyk et al., 2005; Saito and Suzuki, 2007; Cooke et al., 2008b). This suggestion was later confirmed by laboratory experiments in which anhydroBHT was abiotically formed via acid catalysis of BHPs produced by the bacterium *Zymomonas mobilis*, as well as from purified BHT and composite BHTs (Schaeffer et al., 2008, 2010). In that experiment, however, 35-aminobacteriohopanetriol **III** (aminotriol), another common BHP in the environment, was unaffected.

Here, we have used *Rhodopseudomonas palustris* strain TIE-1, a model organism for the study of hopanoid biosynthesis and function (Rashby et al., 2007; Welander et al., 2009, 2010, 2012; Doughty et al., 2011; Eickhoff et al., 2013; Kulkarni et al., 2013), to investigate the degradation of its BHPs during experiments simulating diagenetic conditions (elevated pressure and temperature, *P*/*T*).

2. Material and methods

2.1. Growth conditions

R. palustris strain TIE-1 (Jiao et al., 2005) is a purple non-sulfur bacterium belonging to the Alphaproteobacteria. It is metabolically versatile and can grow under varying conditions. Here, it was grown photoautotrophically by cultivation in anoxic mineral medium with H_2 as the electron donor as described by Eickhoff et al. (2013). The culture headspace was exchanged with H_2/CO_2 (8:2 v/v) once every 3 days and cultures were harvested after

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http://dx.doi.org/10.1016/j.orggeochem.2014.01.002

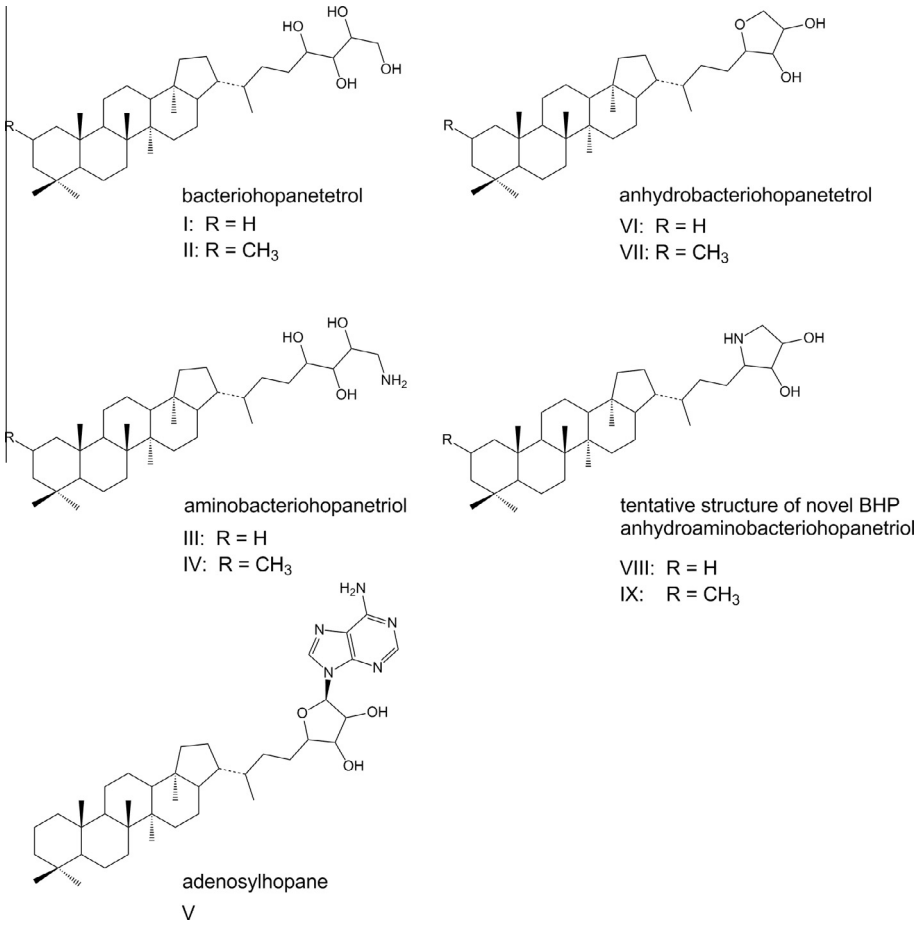

Fig. 1. Bacteriohopanepolyols produced by *R. palustris* TIE-1 and their degradation products after exposure to elevated pressure and temperature.

14 days incubation. Cells were harvested by centrifugation at 10,000*g* and freeze-dried. The material from three independent cultures, 500 ml each, was combined to obtain the required amount of biomass for lipid analysis. Because cultures were not analyzed separately, the data represent an average of the combined triplicates.

2.2. Experimental setup

Freeze-dried samples were gently homogenized in a mortar and split into three subsamples. One was extracted without further treatment (no *P*/*T*), while the other two were exposed to *P*/*T* treatment in Au capsules prior to extraction. The Au tubing was baked at 800 °C for 4 h before use to remove processing oil. For *P*/*T* exposure, the freeze-dried samples were transferred to, and compacted into, the Au tubing, which was crimped mechanically and sealed by arc welding, thereby excluding larger amounts of O_2 remaining in the capsules (cf. Posth et al., 2013). One capsule was exposed to 170 °C and 120 MPa, the other to 250 °C and 120 MPa, each for 7 days in a high pressure autoclave. Extracts from before and after *P*/*T* exposure were analyzed using high performance liquid chromatography–mass spectrometry under atmospheric pressure chemical ionization conditions (HPLC–APCI–MS) and gas chromatography–mass spectrometry (GC–MS).

2.3. Extraction and derivatization of lipids

All glassware was baked at 400 °C for 4 h before use. The freeze-dried cells of the untreated samples, as well as the opened Au capsules with the biomass exposed to *P*/*T* were extracted (3×) with dichloromethane (DCM)/MeOH (3:1 v/v) by sonicating for 15 min at room temperature. The extracts were combined and washed with DCM-extracted water. The organic phases were collected, combined and dried with Na_2SO_4. The solvent was removed under reduced pressure via rotary evaporation. Aliquots of the total lipid extract (TLE) were acetylated by reaction with $(Ac)_2O$/pyridine (1:1 v/v) for 1 h at 60 °C and subsequent incubation at room temperature overnight.

2.4. HPLC–APCI–MS

BHPs in the acetylated TLE in MeOH/propan-2-ol (60:40 v/v) were analyzed at Newcastle University as described previously (van Winden et al., 2012; Eickhoff et al., 2013). Compounds were separated using a Thermo Finnigan Surveyor HPLC system equipped with a Phenomenex Gemini C_{18} 5 µm column (150 mm, 3.0 mm i.d.) and a security guard column of the same material. The programme was: 0.5 ml/min at 30 °C with 90% A and 10% B (starting at 0 min); 59% A, 1% B and 40% C (at 25 min); isocratic (to 40 min), returning to starting conditions over 5 min and stabilizing for 15 min, with A = MeOH, B = water and C = propan-2-ol. The HPLC system was connected to a Thermo Finnigan LCQ ion trap MS instrument equipped with an APCI source operated in positive ion mode. HPLC–MS was used in data dependent mode with two scan events: SCAN 1 over the whole spectrum (*m*/*z* 500–1300) and with SCAN 2, a data-dependent MS^2 spectrum recorded of the most abundant ion found in SCAN 1.

The relative abundances of BHPs were corrected for differences in response factors. N-containing BHPs showed a response 1.5× that of non-N-containing BHPs (cf. van Winden et al., 2012 and references therein). No internal standard was added for BHP analysis. BHP quantification was achieved indirectly by correlating GC–MS and HPLC–APCI–MS data (cf. Eickhoff et al., 2013). Therefore, GC-amenable BHT or anhydroBHT isomer 4 (the most abundant anhydroBHT isomer after *P*/*T* exposure; see also Fig. 2) were used as references for the untreated sample and samples after *P*/*T* treatment, respectively. They were quantified using GC–MS with an internal standard (5α-cholestane) as described by Eickhoff et al. (2013). The compounds quantified via GC were assumed to equal the HPLC content, although a recent study reported that BHP content measured with HPLC–MS is at least 2× that measured using GC–MS (Sessions et al., 2013). The contents of the other, non GC-amenable BHPs were then determined on the basis of their relative abundance in the HPLC runs. Standards were not available for each hopanoid, so caution is advised when comparing these values with other values in the literature due to the large potential error in quantification.

2.5. GC–MS

Each acetylated TLE was analyzed using GC–MS with an Agilent 7890 A GC system coupled to an Agilent 5975 C inert MSD mass spectrometer at the University of Vienna. Compounds were separated on a DB-5HT fused silica column (15 m × 0.25 mm i.d., 0.10 μm film thickness) with He as carrier gas. The following

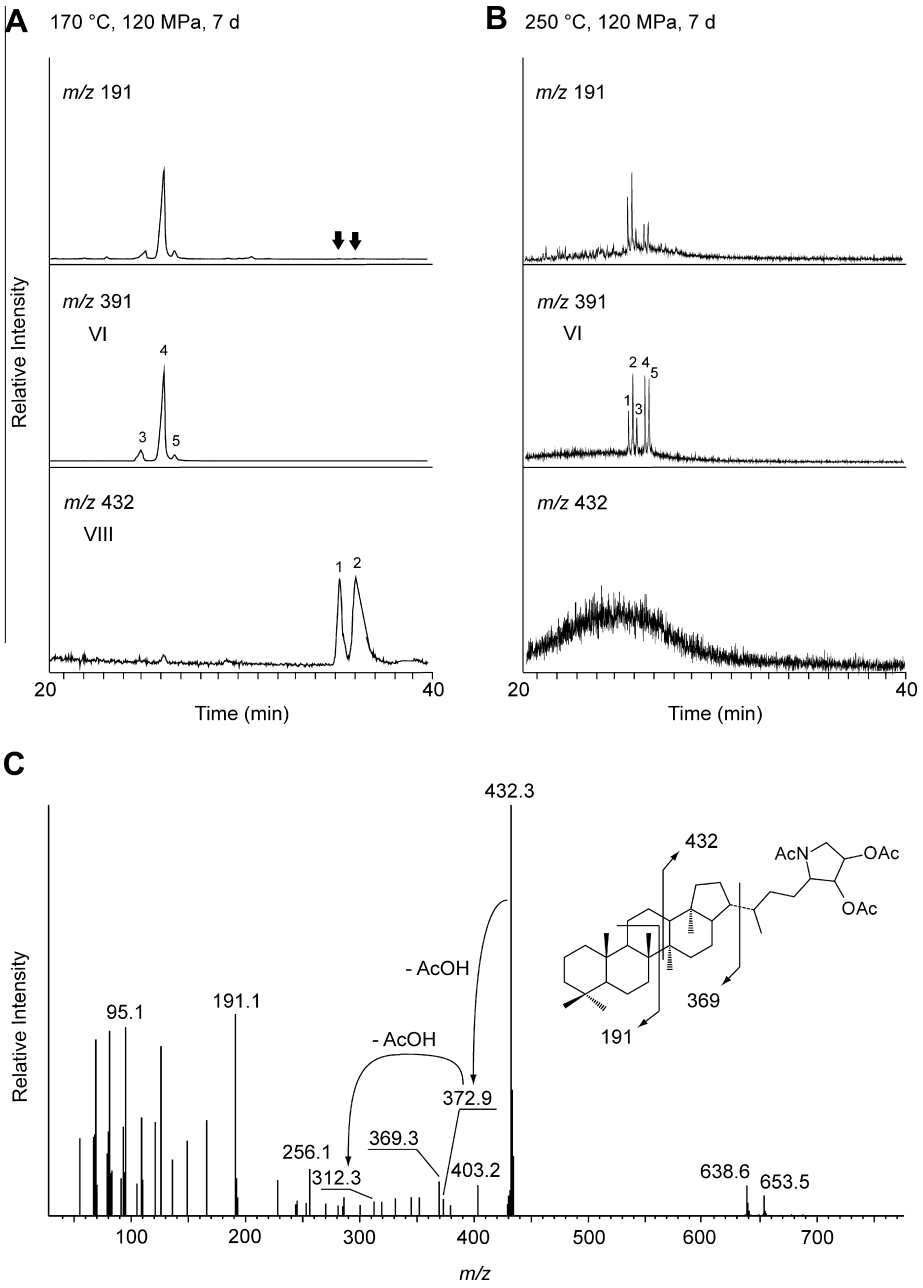

Fig. 2. GC–MS analysis of acetylated total lipid extract of *R. palustris* TIE-1 after exposure to (A) 170 °C and 120 MPa for 7 days and (B) 250 °C and 120 MPa for 7 days, showing single ion chromatograms and (C) the mass spectrum of compound **VIII** (2) under EI conditions. Numbered compounds: **VI**, anhydroBHT; **VIII**, tentatively assigned anhydroaminotriol. Arabic numbers indicate different isomers in order of elution on GC. For structures see Fig. 1. Please note that samples at 170 °C and 250 °C were run with different temperature programmes (Section 2.5).

temperature programme was used for the untreated and the *P*/*T* 170 °C sample: 80 °C (3 min) to 200 °C at 15 °C/min, then to 250 °C at 10 °C/min, then to 345 °C (held 20 min) at 15 °C/min. The *P*/*T* 250 °C sample was run with the following temperature programme: 80 °C (3 min) to 200 °C at 15 °C/min, then to 250 °C at 10 °C/min, then to 360 °C (held 10 min) at 15 °C/min. All acetylated TLEs were run in parallel in full scan (*m*/*z* 50–800) and selected ion monitoring mode (*m*/*z* 191, 391, 432) for analysis of BHPs.

3. Results and discussion

3.1. BHP inventory of TIE-1

The total hopanoid inventory of TIE-1 under the chosen conditions has been described and discussed in detail by Eickhoff et al. (2013). In brief, TIE-1 produced 19.4 mg BHPs per g of dry cellular biomass (Table 1). The most abundant BHP was adenosylhopane **V**, at 61% of all BHPs. TIE-1 also produced significant amounts of BHT **I** and aminotriol **III**; 2-Me-BHT **II** was found only in trace amount and C-2 methylated homologues of aminotriol or adenosylhopane were absent.

3.2. BHT is degraded to anhydroBHT during P/T exposure

The amount of BHPs decreased significantly after *P*/*T* exposure (Table 1). Small amounts of the compounds might have been oxidized by residual O_2 in the Au capsules (cf. Posth et al., 2013), but most of the loss can probably be accounted for by thermal degradation and transformation to macromolecular substances, which were not accessible with the analytical protocol used. Transformation of BHPs to polar hopanoids (e.g. hopanols, hopanoic acids) after *P*/*T* treatment could be excluded, because no hopanols or hopanoic acids were found in the alcohol and carboxylic acid fraction (not shown).

After exposure to 170 °C and 120 MPa, the small amount of 2-methyl-anhydroBHT **VII** (Table 1; Fig. 3) was obviously derived from 2-methyl-BHT **II**, the only C-2 methylated biosynthetic BHP in the original cell mass. The main products after *P*/*T* treatment at 170 °C were two stereoisomers of anhydroBHT **VI** measured with HPLC–APCI–MS (Fig. 3) and three stereoisomers with the GC–MS (Fig. 2; see also Section 3.4), as well as two isomers of a novel BHP with a characteristic base peak ion at *m*/*z* 654 (BHP-654 **VIII**; Fig. 3) with HPLC–APCI–MS, assumed to be $[M+H]^+$ from comparison with the APCI spectra of other BHPs (cf. Talbot et al., 2003a,b). Together, these structures accounted for 60% and 26% of extractable BHPs, respectively (Table 1). They were not detected in the bacterium prior to *P*/*T* treatment, indicating that the treatment induced their formation. For anhydroBHT **VI**, this is in agreement with studies suggesting anhydroBHT as a degradation product of both BHT **I** (Talbot et al., 2005; Saito and Suzuki, 2007; Schaeffer et al., 2008) and other composite BHTs such as BHT-cyclitol ether and BHT-glucosamine (Schaeffer et al., 2010) via dehydration and cyclization of the BHT side chain. In contrast to the experiments of Schaeffer et al. (2008, 2010), acid treatment or a clay catalyst were not used, so elevated temperature and pressure alone were sufficient for the transformation of BHT to anhydroBHT.

AnhydroBHT was probably derived mainly from BHT degradation. However, adenosylhopane **V** might be another potential source of anhydroBHT, as it disappeared completely after *P*/*T* treatment (Table 1; Fig. 3). Previous studies have suggested biogenic or diagenetic reductive removal of the adenine group from adenosylhopane and the resulting conversion to anhydroBHT (cf. Costantino et al., 2001; Talbot et al., 2005). A diagenetic origin is in agreement with a study of environmental samples in which adenosylhopane concentration decreased, with a concurrent increase in anhydroBHT with increasing sediment depth, suggesting formation of anhydroBHT from adenosylhopane (Cooke et al., 2008b). In our experiments, reduced cellular biomolecules such as cytochromes or hydroquinones that were released from the biomass upon exposure to simulate diagenetic conditions could potentially have acted as reducing agents. However, our experiments could not prove this hypothesis, and the suggestion has to be regarded with care.

After exposure to 250 °C and 120 MPa, the only remaining BHPs were three isomers of anhydroBHT **VI** measured with HPLC–APCI–MS (Table 1; Fig. 3), but five isomers were found when measured with GC–MS (Fig. 2; see Section 3.4), demonstrating its stability under these conditions. The results are supported by comparison

Table 1
Bacteriohopanepolyol (BHP) inventory (rel. abundance, % of total BHPs) of *R. palustris* strain TIE-1 before and after exposure to elevated pressure and temperature (*P*/*T*; letters in parentheses indicate different isomers in the order of elution on HPLC).

Compound[a]		No *P*/*T*	170 °C 120 MPa	250 °C 120 MPa
Biosynthetic BHPs[b]				
V	Adenosylhopane	61		
I	BHT[c] (b)	18		
II	2-Me-BHT[c]	<1		
III	Aminotriol	20		
BHPs after P/T exposure				
I	BHT[c] (a,b,c)		3	
III	Aminotriol (a,b)		3	
IV	2-Me-aminotriol (a,b)		2	
VI	AnhydroBHT[c] (c)		57	29
VI	AnhydroBHT[c] (b)		3	19
VI	AnhydroBHT[c] (a)			52
VII	2-Me-anhydroBHT[c] (a,b)		<1	
VIII	BHP-654 (b)		20	
VIII	BHP-654 (a)		6	
IX	2-Me-BHP-668 (b)		4	
IX	2-Me-BHP-668 (a)		2	
Absolute amount of all BHPs				
In mg/g dry sample weight		19.4	2.1	0.7

[a] For structures see Fig. 1.
[b] Modified from results by Eickhoff et al. (2013), Table 1.
[c] BHT, bacteriohopanetetrol.

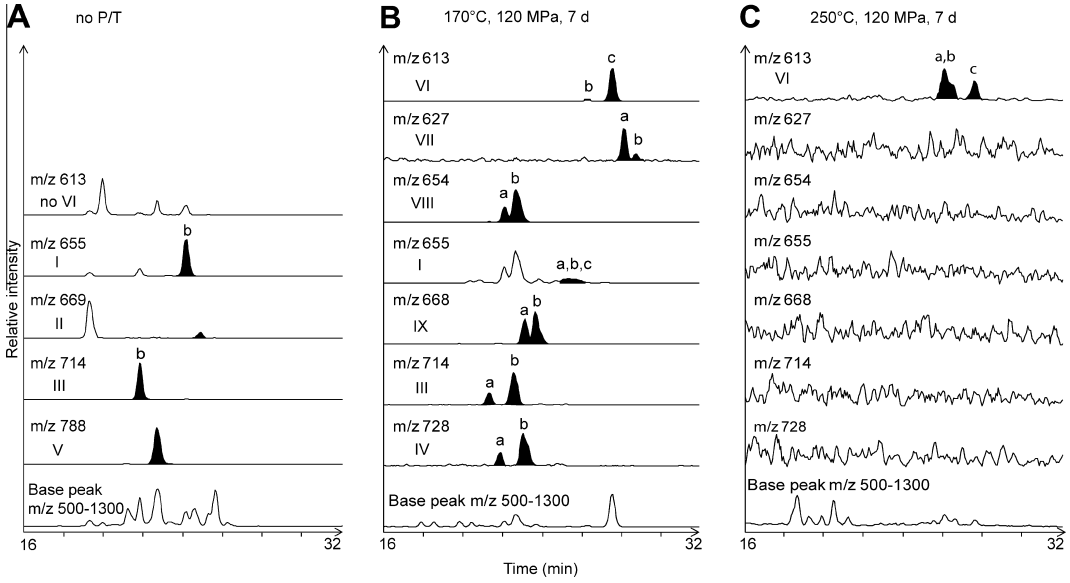

Fig. 3. HPLC–APCI–MS partial mass chromatograms showing bacteriohopanepolyols in acetylated total lipid extracts of *R. palustris* TIE-1 (A) before (modified from Eickhoff et al. (2013), Fig. 2A) and after exposure to (B) 170 °C and 120 MPa for 7 days or (C) 250 °C and 120 MPa for 7 days. Numbered compounds (peaks highlighted in black): **I**, bacteriohopanetetrol (BHT); **II**, 2-methyl-BHT; **III**, aminotriol; **IV**, 2-methyl-aminotriol, **V**, adenosylhopane; **VI**, anhydroBHT; **VII**, 2-methyl-anhydroBHT; **VIII**, BHP-654 (anhydroaminotriol), **IX**, 2-methyl-BHP-668 (2-methyl-anhydroaminotriol); letters indicate different isomers in the order of elution on HPLC. For structures see Fig. 1.

with environmental samples, in which abundant anhydroBHT was found in various recent (e.g. Watson, 2002; Bednarczyk et al., 2005; Saito and Suzuki, 2007) and ancient (Bednarczyk et al., 2005; van Dongen et al., 2006) sediments. Both laboratory experiments and environmental samples indicate the formation of anhydroBHT during diagenetic conditions, and its presence has been reported in sediments of low maturity older than 100 million years (Bednarczyk et al., 2005).

3.3. Novel degradation products from aminotriols

In cases where a ribose moiety is not present, the diagenetic formation of anhydroBHT **VI** seems to require an oxygenated functionality at C-35 (Schaeffer et al., 2008), as in BHT **I**. However, the functionality at C-35 can also be an amine group, as in the common BHP aminotriol **III**. Natural degradation products of aminotriol **III** have not been found to date. In previous experiments, no anhydroBHT was found after exposure of aminotriol to acid catalysis (Schaeffer et al., 2008). Based on the results of the present study, a different degradation pathway from that of BHT is suggested for aminotriol. TIE-1 produced aminotriol which constituted 20% of the extractable BHPs (Table 1). Aminotriol was the only biosynthetic BHP in the original culture with a terminal amine at C-35. After exposure of TIE-1 to 170 °C and 120 MPa, two novel BHPs with a base peak at *m*/*z* 654 (acetylated; BHP-654 **VIII**), and two novel BHPs with a base peak at *m*/*z* 668 (acetylated; BHP-668 **IX**) were found, suggesting non-methylated and methylated homologues. These compounds were the only N-containing degradation products (Table 1; Fig. 3), as indicated by the even *m*/*z* value of the base peak ion, indicating an odd number of N atoms in the parent molecule.

Minor amounts of an apparent C-2 methylated homologue of aminotriol **IV** (based on relative retention times) were present after exposure of the biomass to 170 °C and 120 MPa (Table 1, Fig. 3). This suggests the presence of C-2 methylated aminotriol as a biosynthetic product of TIE-1 prior to *P*/*T* exposure. However, it has not been found in this or any previous TIE-1 culture study (e.g. Rashby et al., 2007; Welander et al., 2009, 2010; Eickhoff et al., 2013; Sessions et al., 2013). Based on the presence of C-2 methylated aminotriol **IV** after *P*/*T* exposure, the compound is suggested as a biosynthetic, yet "difficult-to-extract" BHP that is released only at higher temperature. Previous studies documented at least one instance of a methylated structure, which was not extracted via normal solvent extraction protocols (Herrmann et al., 1996).

Isomers of BHP-654 **VIII** and 2-methylated BHP-668 **IX** are apparently degradation products of aminotriol **III** and 2-methyl-aminotriol **IV**, respectively. Moreover, while the relative abundances of aminotriol and BHP-654 (Table 1) supported this assumption, the potential presence of C-2 methylated aminotriol prior to *P*/*T* exposure could not be demonstrated. After exposure to 250 °C and 120 MPa, BHP-654 and 2-methylated BHP-668 disappeared (Table 1; Fig. 3).

Tentative structures for the degradation products of aminotriol **III** and C-2 methylated aminotriol **IV**, with base peak at *m*/*z* 654 and *m*/*z* 668, respectively, are proposed (Fig. 4). By analogy with the formation of anhydroBHT, anhydroaminobacteriohopanetriol **VIII** (anhydroaminotriol; Fig. 1 and 4) and the C-2 methylated homologue **IX** are believed to be formed via dehydration and cyclization of the side chain as described for anhydroBHT **VI** from BHT **I**. In the proposed structures, the heterocyclic N would be susceptible to acetylation (three acetylations in total), but significant fragmentation of BHP-654 **VIII** and 2-methyl-BHP-668 **IX** in the MS^2 spectra represents loss (CH_3COOH, 60 Da) or partial loss ($COCH_2$, 42 Da) of one or two acetylated groups (Fig. 3; Talbot et al., 2003b) and not loss of a N-containing functionality. Indeed, the observed loss of 42 Da (e.g. *m*/*z* 612 via loss of 42 from *m*/*z* 654; Fig. 4C and D) is pronounced compared with the alternative loss of 60 Da (e.g. *m*/*z* 594 via loss of 60 from *m*/*z* 654, Fig. 4C and D), which is usually the more pronounced loss in other BHPs (e.g. *m*/*z* 654 via loss of 60 from *m*/*z* 714; Fig. 4A and B; Talbot et al., 2003a,b). This agrees with the presence of an atypical group in the hopanoid, i.e. acetylated heterocyclic N, which loses ketene ($COCH_2$, 42 Da), but cannot be lost as a N-containing moiety due to the stability of the protonated N. Indeed, it has been shown for typical BHPs that loss of the acetylated amine is either very minor or not observed in MS^2 spectra (e.g. for aminotriol **III**; Fig. 4A) due to the stability of the protonated amine functionality under positive APCI conditions (Talbot et al., 2003b). We assume a similar behaviour for the two

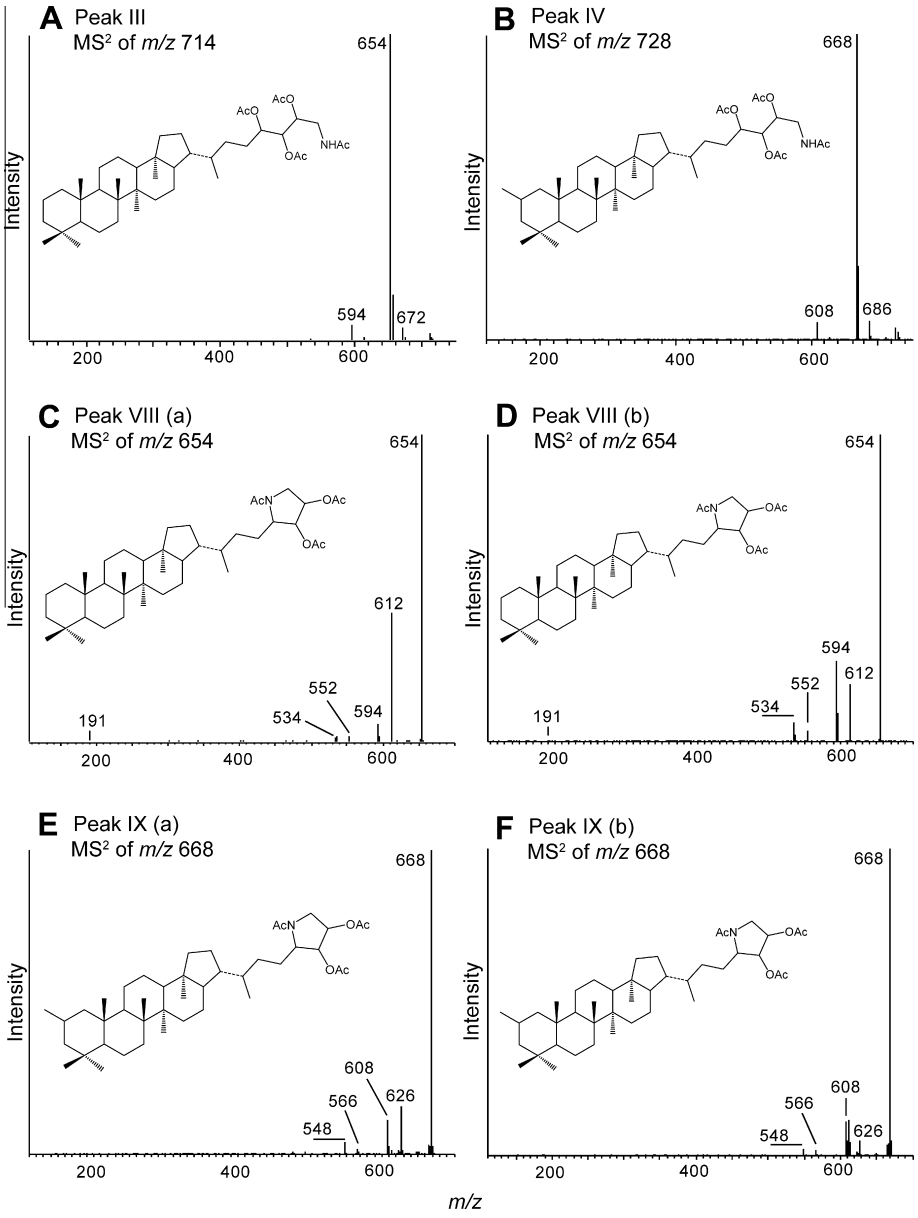

Fig. 4. APCI-MS^2 spectra of peracetylated regular ββ isomers of (A) aminotriol and (B) 2-methyl-aminotriol; two isomers of tentatively assigned anhydroaminotriol (C and D) and two isomers of methylated anhydroaminotriol (E and F). Letters in parentheses indicate isomers in order of elution on HPLC. Peak numbers refer to Fig. 2.

novel N-containing isomers of BHP-654 **VIII** and 2-methyl-BHP-668 **IX**. The APCI fragmentations of BHP-654 **VIII** and 2-methyl-BHP-668 **IX** would thus be consistent with previous studies of known BHPs and agree with the tentative structures.

The information from HPLC–APCI–MS allowed interpretation of the novel compounds as degradation products of 2-methyl-aminotriol. In APCI–MS spectra of N-containing BHPs, the ions indicative of the hopanoid ring structure are commonly not found (Talbot et al., 2003a) or are only found in traces (this study; Fig. 4C and D). To verify the tentative structure of the two BHP-654 isomers, the compounds were also analyzed using GC–MS. The *m*/*z* 191 chromatogram showed two small peaks eluting after anhydroBHT, with retention times of 35–36 min (Fig. 2A) and $M^{+\cdot}$ at *m*/*z* 653 (Fig. 2C; corresponding to $[M+H]^+$ at *m*/*z* 654 in APCI–MS). Both peaks showed nearly identical spectra, pointing to two isomers of the same compound. The EI spectrum of BHP-654 **VIII** showed fragments indicative of the hopanoid ring structure (*m*/*z* 191 and *m*/*z* 369; Fig. 2C). The base peak was at *m*/*z* 432, most likely comprising ring D and E plus side chain, resulting from cleavage of ring C, analogous to the base peak at *m*/*z* 391 for anhydroBHT (cf. Bednarczyk et al., 2005). The *m*/*z* 391 and *m*/*z* 432 ions verified the identity of the respective compound (Fig. 2A and C). Fragmentation due to loss of two acetic acid groups, as discussed above, was also apparent in the GC–MS spectrum via fragments at *m*/*z* 372 (432 − 60) and *m*/*z* 312 (432 − 2 × 60; Fig. 2C). The GC–MS data therefore confirmed the structure of BHP-654 suggested on the basis of the HPLC–APCI–MS results. Due to the very low concentration of the isomers of the novel 2-methyl-BHP-668 **IX**, no characteristic EI spectra for these compounds were obtained with GC–MS.

We can exclude artificial production for the newly described BHP-654 **VIII**, since BHP-654 was neither found in GC, nor in HPLC measurements in the original, untreated sample (no *P*/*T*). Interestingly, the novel BHP-654 has been found in up to 4 Ma old ODP sediments from the Benguela Upwelling Region (Site 1084A, 25°31′S, 13°2′E). At the time, its origin, presence and structure could not be explained (H.M. Talbot, unpublished data).

3.4. Formation of stereoisomers during P/T exposure

Upon exposure to increased temperature and pressure at 170 °C, three stereoisomers of anhydroBHT **VI** (3–5) and two isomers of anhydroaminotriol **VIII** (1–2) were observed upon GC–MS (Fig. 2A). At 250 °C, five stereoisomers of anhydroBHT **VI** (1–5) were observed (Fig. 2B), while no anhydroaminotriol **VIII** was detected. All stereoisomers of anhydroBHT from 170 °C and 250 °C have similar EI mass spectra with a base peak corresponding to ring D and E plus side chain, resulting from ring C cleavage. The ratio of the two most abundant ions at *m*/*z* 391 and *m*/*z* 191 (191/391: between 0.6 and 0.7) revealed a 17β,21β-configuration of all stereoisomers identified (cf. Peters et al., 2005). Since isomerization at C-17 and C-21 can be excluded, the formation of five anhydroBHTs **VI** can most likely be explained by isomerization of all asymmetric centers (C-32, C-33 and C-34) of the hydroxylated tetrahydrofuran ring. In this case, six isomers may have been formed in total. Since we found only five after treatment at 250 °C, we assume that one of them co-eluted with one of the other five. Since the various stereoisomers cannot be assigned to specific asymmetric centers, they were numbered according to their retention times on the GC–MS.

Upon HPLC measurements of the same fractions, only two peaks corresponding to anhydroBHT (**VI** b, c) after exposure to 170 °C, and three peaks (**VI** a, b, c) after exposure to 250 °C were found (Fig. 3), indicating poorer separation of the anyhdroBHT isomers under the HPLC–APCI–MS conditions. The assignment of the peaks is even more difficult than for the GC–MS results. Most likely GC–MS peak **VI** (4) corresponds to HPLC–APCI–MS peak **VI** (c), since these represent the most prominent peaks, respectively. Moreover, we suggest that isomer **VI** (5) measured with the GC–MS is most likely the C-32 epimer of compound **VI** (4) based on comparison of relative GC–MS retention times reported by Schaeffer et al. (2008) for C-32 anhydroBHT epimers. The precise stereochemistry of the other anhydroBHT isomers was not assigned.

The tentative degradation products of aminotriol **III** and C-2 methylated aminotriol **IV**, BHP-654 **VIII** and 2-methyl-BHP-668 **IX** were also found as two isomers after exposure to 170 °C (Table 1, Fig. 2), but none of the isomers withstood exposure to 250 °C. By analogy with the anhydroBHT isomers, the EI spectra of the BHP-654 isomers revealed that the relative intensity of the characteristic ion at *m*/*z* 432 was significantly higher than that of *m*/*z* 191 (191/391: 0.5), implying that both compounds also exhibited a 17β,21β-configuration, excluding the formation of stereoisomers at C-17 and C-21. The isomers are likely also C-32 epimers, as suggested for anhydroBHT **VI**, but the precise stereochemistry could not be clarified with certainty based on the EI spectra. Formation of C-32 epimers is also likely for 2-methyl-BHP-668. However, due to the low amounts of these compounds no information regarding the stereochemistry at C-17 and C-21 could be obtained from the EI mass spectra.

4. Conclusions

The fate of the bacteriohopanepolyol (BHP) inventory of *R. palustris* strain TIE-1 during simulated diagenesis was studied to obtain new insights into transformation and degradation products of BHPs by analyzing lipid extracts of bacterial cells before and after exposure to high pressure and temperature. This allowed correlation of biosynthetic BHPs and their "diagenetic" products. AnhydroBHT was a degradation product of BHT and possibly adenosylhopane, whereas aminotriol was degraded to a BHP-654, tentatively assigned as anhydroaminotriol. The identification of this novel BHP adds to current knowledge of BHPs and should help to better interpret findings of BHPs in the environment. The method applied here offers potential for future studies of degradation products from various other BHPs and from different BHP-producing bacteria, increasing the utility of these compounds as proxies in environmental studies.

Acknowledgments

The work was supported by grants from the "Deutsche Forschungsgemeinschaft" (DFG) to A.K. (KA 1736/4-1 and 12-1). We thank S. Büßecker for preparation of media and harvesting of biomass, H. Schulz for freeze-drying, C. Giehl and M. Nowak for providing the arc welder, I. Köhler, E. Swanner and A. Picard for help with the high pressure autoclave, K. Heindel for help with lipid extraction and F. Dunn for technical assistance. The Thermo Finnigan LCQ ion trap mass spectrometer (Newcastle University) was funded by the Science Research Infrastructure Fund (SRIF) from HEFCE. We thank C. Hallmann for helpful discussions and two anonymous reviewers for insightful comments that improved the manuscript.

Associate Editor—**P. Schaeffer**

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