Presence of an unusual $17\alpha, 21\beta(H)$-bacteriohopanetetrol in Holocene sediments from Ace Lake (Antarctica)

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Abstract

Whilst investigating the intact biohopenoid (bacteriohopanopolyol, BHP) distribution in Holocene sediments from Ace Lake (Antarctica), we have identified the presence of $\alpha\beta$-bacteriohopanetetrol in sediments aged up to 9400 years BP. To our knowledge, this is the first time that an intact polyfunctionalised BHP with the “geological” $17\alpha,21\beta(H)$ configuration has been identified in a sediment. Previously, this structure has only been observed in species of the nitrogen fixing bacterium Frankia. Its presence here in the sedimentary environment has implications for the interpretation of hopanoid $\beta\beta/\alpha\beta$ ratios in the geological record.

Keywords: Ace Lake, bacteriohopanopolyols, $\alpha\beta$-bacteriohopanetetrol, Holocene
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Introduction

Biohopanoids are widely distributed components in the biosphere (Ourisson and Albrecht, 1992) and their diagenetic products are amongst the oldest molecular biomarkers, dating back to late Archean times [2.77 billion years (Ga)] (e.g. Brocks et al., 1999; 2003). Biohopanoids occur in several higher plants, ferns, mosses, fungi, protists, and particularly in bacteria (Ourisson et al., 1987). However, bacteria are the only known source of C-35 hopanepolyols (bacteriohopanepolyols [BHPs] e.g. Rohmer et al., 1984; Rohmer, 1993), which are thought to act as cell membrane rigidifiers analogues to some of the sterols in eukaryotes (Kannenberg and Poralla, 1999). BHPs have recently been reported from a wide range of environments (e.g. Talbot and Farrimond, 2007; Talbot et al., 2003a; 2007a and references therein).

Typically, BHPs are biosynthesized with the biological $17\beta,21\beta(H)$ stereochemistry in the hopanoid skeleton. During diagenesis, however, BHPs undergo a series of defunctionalisation and isomerisation reactions, including the formation of the structures with a more stable “geological” $17\alpha,21\beta(H)$ stereochemistry (e.g. Peters et al., 2005). This isomerisation has been reported to occur rapidly in some peats, probably due to highly acidic conditions (e.g. Pancost et al., 2003 and references therein).

Evidence for $17\alpha,21\beta(H)$ BHPs was previously reported by Thiel et al., (2003) who observed abundant $17\alpha,21\beta(H)$-bishomohopanol in living microbial mats from the Black Sea after periodate treatment but were not able to identify an intact $17\alpha,21\beta(H)$ precursor by GC-MS during this study.

Here we describe the identification of a highly unusual BHP which was observed during a larger combined study of intact BHP derivatives and 16S ribosomal RNA gene (16S rDNA) analysis identifying biological precursors of BHPs carried out
on the Holocene sedimentary record of Ace Lake (Vestfold Hills, Antarctica; Coolen et al., 2007 and unpublished data).

2. Experimental

Descriptions of the site, lithology, sediment ages and sampling procedure have been published previously (Coolen et al., 2004a,b).

The extraction methodology was adapted from the Kates modification (Kates, 1975) of the original Bligh and Dyer extraction (Bligh and Dyer, 1959) and has been described in full elsewhere (e.g. Talbot et al., 2007a). After addition of the internal standard (5α-androstanol) to the total lipid extract (TLE), an aliquot was acetylated using acetic anhydride and pyridine (2 mL each; heat at 50°C for 1 h), then left to stand overnight. The acetic anhydride and pyridine were removed using a rotary evaporator. The acetylated extract was then dissolved in a solution of dichloromethane for analysis by gas chromatography-mass spectrometry (GC-MS).

Hopanols and GC amenable BHPs (acetylated aliquot) were analysed by GC-MS using a Hewlett-Packard 5890 II GC system (split/splitless injector; 350°C) linked to a Hewlett-Packard 5972 mass-selective detector (electron energy 70 eV; filament current 220 μA; source temperature 270°C; multiplier voltage 2000 V; interface temperature 350°C). A 15 m DB5-HT fused silica column (0.25 mm i.d.; 0.1 μm film thickness) was used with helium as the carrier gas. The oven temperature was programmed from 50-200°C at 15°C/min (held for 1 min), from 200 to 250°C at 10°C/min (held for 1 min) and from 250 to 350°C at 5°C/min (held for 8 min).

Hopanoids were identified from full scan (m/z 50-700) analysis of selected samples, by comparison with authentic standards and published spectra and by relative retention
times. They were quantified using selected ion monitoring (SIM) from peak areas in the m/z 191 mass chromatograms and the m/z 243 peak area response of the 5α-androstanol internal standard using a relative response factor of 1.

3. Results and Discussion

3.1 Identification of 17α,21β(H)-bacteriohopanetetrol

Analysis of aliquots of acetylated total extract by GC-MS revealed the presence of a highly unusual component in the m/z 191 chromatogram (Fig. 1a) in all of the sediments analysed down to a depth of 127 cm corresponding to samples from sedimentary Units I and II (Table 1; e.g. Coolen et al., 2004a; 2007). This compound has a very similar mass spectrum as the commonly observed 17β,21β(H)–bacteriohopanetetrol (BHT; e.g. Talbot et al., 2003a and references therein), but elutes earlier. There are clear differences, however, in the relative abundance of two of the most diagnostic ions in the EI mass spectra, the D+E+side chain and A+B ring fragments. In the mass spectrum of the common (biological) 17β,21β(H) isomer, the ring D+E+side chain fragment (m/z 493) is always more intense than the A+B ring fragment (m/z 191; Fig. 1b). Here, in the earlier eluting compound, we observe a reversal of this, with the m/z 191 ion being more intense, a diagnostic feature of 17α,21β(H) hopanoids (Fig. 1c).

Assignment of this compound as 17α,21β(H)-BHT was further confirmed by GC-MS analysis of the hopanol products produced via periodate treatment of the TLE (e.g. Rohmer et al., 1984; Talbot et al., 2003a), followed by reduction with NaBH₄, which yielded only hopanol products with either the 17β,21β(H) or 17α,21β(H) stereochemistry.
The 17α,21β(H)-BHT was also observed by LC-MS\textsuperscript{n} analysis of the acetylated extract as a minor component eluting just before ββ-BHT in all Unit I samples and several from Unit II (Table 1), however, its APCI MS\textsuperscript{2} spectrum was very similar to that of ββ-BHT (Talbot et al., 2003b,c), unlike the diagnostic EI mass spectrum (Fig. 1).

3.2 Possible sources of 17α,21β(H)-bacteriohopanetetrol in the sedimentary record of Ace Lake

Hopanoid structures with the 17α,21β(H) configuration are usually considered to be indicative of diagenetic transformation of hopanoids to more stable “geohopanoids”. To our knowledge, this is the first observation of an intact polyfunctionalised 17α,21β(H) biohopanoid in the sedimentary environment although Thiel et al., (2003) reported 17α,21β(H)-bishomohopanol in a living microbial mat from the Black Sea after periodate treatment, implying the existence of a 17α,21β(H)-BHP precursor.

The fact that this compound was not observed in any of the oldest (Unit III) sediments suggests it is related to a specific biological precursor rather than a product of a diagenetic reaction (cf. rapid isomerisation of ββ-isomers in peats; e.g. Pancost et al. 2003).

Currently, only one group of bacteria, \textit{Frankia} sp., have been found to directly produce hopanoids in the more stable αβ-configuration (Rosa-Putra et al., 2001). However, none of the bacterial phylotypes identified during a recent survey of sedimentary 16S ribosomal RNA encoding genes (16S rDNA) in this lake (Coolen et al., 2007 and unpublished data) were affiliated with \textit{Frankia} spp. (order of Actinomycetales). Only one phylotype (identified as AL_Bac14; Coolen et al.,
unpublished data) clustered within this order, but was only distantly related to *Frankia* spp. and only found at depth intervals 125-127 cm and 129-131 cm where the intact polyfunctionalised $17\alpha,21\beta(H)$-BHT was absent. Phylotypes related to *Frankia* were also not reported from the recent bacterial 16S rDNA clone library obtained from sulfidic surface sediments of Ace Lake (Bowman et al., 2000). Therefore, any biological precursor of the polyfunctionalised $17\alpha,21\beta(H)$-BHT is not likely to be *Frankia* and remains unidentified.

4. Conclusions

A novel bacteriohopanepolyol, here assigned as $17\alpha,21\beta(H)$-bacteriohopanetetrol, has been identified in Holocene sediments of Ace Lake (Antarctica). To our knowledge, this is the first time that an intact polyfunctionalised BHP with the $17\alpha,21\beta(H)$ stereochemistry has been identified in environmental samples. The occurrence of this apparent biohopanoid with the more stable “geological” configuration could have implications for the interpretation of $\alpha\beta/\beta\beta$ ratios in geological samples.

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LCQ ion trap mass spectrometer. Mr Paul Donohoe is thanked for assistance with the mass spectrometer and Dr Paul Farrimond for helpful discussions.

References


Table 1. Concentrations of ββ- and αβ-BHT (µg g⁻¹ TOC) measured by GCMS

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Sediment Unit</th>
<th>TOC (%)</th>
<th>ββ-BHT (µg)</th>
<th>αβ-BHT (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-7</td>
<td>I</td>
<td>9.3</td>
<td>57</td>
<td>14</td>
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<tr>
<td>29-31</td>
<td>I</td>
<td>4.8</td>
<td>75</td>
<td>16</td>
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<tr>
<td>53-55</td>
<td>II</td>
<td>8.3</td>
<td>112</td>
<td>27</td>
</tr>
<tr>
<td>65-67</td>
<td>II</td>
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<td>35</td>
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<td>13.9</td>
<td>30</td>
<td>0.6</td>
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<td>18.9</td>
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<td>III</td>
<td>3.4</td>
<td>21</td>
<td>nd</td>
</tr>
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</table>

a Unit I – permanently stratified lake, anoxic bottom waters (age ~3000 years BP to present); Unit II – marine influenced phase (9400 – 3000 years BP); Unit III – freshwater lake (>9400 years BP).

b P = present
c nd = not detected

Figure 1. (a) GC-MS partial mass chromatograms showing relative retention times of ββ-BHT and proposed αβ-BHT. (b) EI mass spectrum of tetraacetylated ββ-BHT. (c) EI mass spectrum of tetraacetylated αβ-BHT. (Ac = COCH₃)