Presence of an unusual 17α, 21β(*H*)-bacteriohopanetetrol in Holocene sediments from Ace Lake (Antarctica)

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Abstract

Whilst investigating the intact biohopanoid (bacteriohopanepolyol, BHP) distribution in Holocene sediments from Ace Lake (Antarctica), we have identified the presence of αβ-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" 17α,21β(*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 ββ/αβ ratios in the geological record.

Keywords: Ace Lake, bacteriohopanepolyols, αβ-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 17β,21β(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" 17α,21β(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 17α,21β(H) BHPs was previously reported by Thiel et al., (2003) who observed abundant 17α,21β(H)-bishomohopanol in living microbial mats from the Black Sea after periodate treatment but were not able to identify an intact 17α,21β(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

50 on the Holocene sedimentary record of Ace Lake (Vestfold Hills, Antarctica; Coolen et
51 al., 2007 and unpublished data).
52
53 **2. Experimental**
54 Descriptions of the site, lithology, sediment ages and sampling procedure have
55 been published previously (Coolen et al., 2004a,b).
56 The extraction methodology was adapted from the Kates modification (Kates,
57 1975) of the original Bligh and Dyer extraction (Bligh and Dyer, 1959) and has been
58 described in full elsewhere (e.g. Talbot et al., 2007a). After addition of the internal
59 standard (5α-androstanol) to the total lipid extract (TLE), an aliquot was acetylated
60 using acetic anhydride and pyridine (2 mL each; heat at 50ºC for 1 h), then left to stand
61 overnight. The acetic anhydride and pyridine were removed using a rotary evaporator.
62 The acetylated extract was then dissolved in a solution of dichloromethane for analysis
63 by gas chromatography-mass spectrometry (GC-MS).
64 Hopanols and GC amenable BHPs (acetylated aliquot) were analysed by GC-
65 MS using a Hewlett-Packard 5890 II GC system (split/splitless injector; 350°C) linked
66 to a Hewlett-Packard 5972 mass-selective detector (electron energy 70 eV; filament
67 current 220 μA; source temperature 270°C; multiplier voltage 2000 V; interface
68 temperature 350°C). A 15 m DB5-HT fused silica column (0.25 mm i.d.; 0.1 μm film
69 thickness) was used with helium as the carrier gas. The oven temperature was
70 programmed from 50-200°C at 15°C/min (held for 1 min), from 200 to 250°C at
71 10°C/min (held for 1 min) and from 250 to 350°C at 5°C/min (held for 8 min).
72 Hopanoids were identified from full scan (m/z 50-700) analysis of selected samples, by
73 comparison with authentic standards and published spectra and by relative retention

74 times. They were quantified using selected ion monitoring (SIM) from peak areas in the
75 m/z 191 mass chromatograms and the m/z 243 peak area response of the 5α-androstanol
76 internal standard using a relative response factor of 1.
77
78 **3. Results and Discussion**
79 *3.1 Identification of 17α,21β(H)-bacteriohopanetetrol*
80 Analysis of aliquots of acetylated total extract by GC-MS revealed the presence
81 of a highly unusual component in the m/z 191 chromatogram (Fig. 1a) in all of the
82 sediments analysed down to a depth of 127 cm corresponding to samples from
83 sedimentary Units I and II (Table 1; e.g. Coolen et al., 2004a; 2007). This compound
84 has a very similar mass spectrum as the commonly observed 17β,21β(*H*)–
85 bacteriohopanetetrol (BHT; e.g. Talbot et al., 2003a and references therein), but elutes
86 earlier. There are clear differences, however, in the relative abundance of two of the
87 most diagnostic ions in the EI mass spectra, the D+E+side chain and A+B ring
88 fragments. In the mass spectrum of the common (biological) 17β,21β(*H*) isomer, the
89 ring D+E+side chain fragment (*m/z* 493) is always more intense than the A+B ring
90 fragment (*m/z* 191; Fig. 1b). Here, in the earlier eluting compound, we observe a
91 reversal of this, with the *m/z* 191 ion being more intense, a diagnostic feature of
92 17α,21β(*H*) hopanoids (Fig. 1c).
93 Assignment of this compound as 17α,21β(*H*)-BHT was further confirmed by
94 GC-MS analysis of the hopanol products produced via periodate treatment of the TLE
95 (e.g. Rohmer et al., 1984; Talbot et al., 2003a), followed by reduction with $NaBH_4$,
96 which yielded only hopanol products with either the 17β,21β(*H*) or 17α,21β(*H*)
97 stereochemistry.

98 The 17α,21β(*H*)-BHT was also observed by LC-MS^n analysis of the acetylated
99 extract as a minor component eluting just before ββ-BHT in all Unit I samples and
100 several from Unit II (Table 1), however, its APCI MS^2 spectrum was very similar to that
101 of ββ-BHT (Talbot et al., 2003b,c), unlike the diagnostic EI mass spectrum (Fig. 1).
102
103 *3.2 Possible sources of 17α,21β(H)-bacteriohopanetetrol in the sedimentary record of*
104 *Ace Lake*
105 Hopanoid structures with the 17α,21β(*H*) configuration are usually considered
106 to be indicative of diagenetic transformation of hopanoids to more stable
107 "geohopanoids". To our knowledge, this is the first observation of an intact
108 polyfunctionalised 17α,21β(*H*) biohopanoid in the sedimentary environment although
109 Thiel et al., (2003) reported 17α,21β(H)-bishomohopanol in a living microbial mat
110 from the Black Sea after periodate treatment, implying the existence of a 17α,21β(H)-
111 BHP precursor.
112 The fact that this compound was not observed in any of the oldest (Unit III)
113 sediments suggests it is related to a specific biological precursor rather than a product of
114 a diagenetic reaction (cf. rapid isomerisation of ββ isomers in peats; e.g. Pancost et al.
115 2003).
116 Currently, only one group of bacteria, *Frankia* sp., have been found to directly
117 produce hopanoids in the more stable αβ-configuration (Rosa-Putra et al., 2001).
118 However, none of the bacterial phylotypes identified during a recent survey of
119 sedimentary 16S ribosomal RNA encoding genes (16S rDNA) in this lake (Coolen et
120 al., 2007 and unpublished data) were affiliated with *Frankia* spp. (order of
121 Actinomycetales). Only one phylotype (identified as AL_Bac14; Coolen et al.,

122 unpublished data) clustered within this order, but was only distantly related to *Frankia*
123 spp. and only found at depth intervals 125-127 cm and 129-131 cm where the intact
124 polyfunctionalised 17α,21β(*H*)-BHT was absent. Phylotypes related to *Frankia* were
125 also not reported from the recent bacterial 16S rDNA clone library obtained from
126 sulfidic surface sediments of Ace Lake (Bowman et al., 2000). Therefore, any biological
127 precursor of the polyfunctionalised 17α,21β(*H*)-BHT is not likely to be *Frankia* and
128 remains unidentified.
129
130 **4. Conclusions**
131 A novel bacteriohopanepolyol, here assigned as 17α,21β(*H*)-bacteriohopanetetrol, has
132 been identified in Holocene sediments of Ace Lake (Antarctica). To our knowledge, this
133 is the first time that an intact polyfunctionalised BHP with the 17α,21β(*H*)
134 stereochemistry has been identified in environmental samples. The occurrence of this
135 apparent biohopanoid with the more stable "geological" configuration could have
136 implications for the interpretation of αβ/ββ ratios in geological samples.
137
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Table 1. Concentrations of ββ- and αβ-BHT (μg g^{-1} TOC) measured by GCMS

Depth (cm)	Sediment Unit[a]	TOC (%)	ββ-BHT (μg)	αβ-BHT (μg)
5-7	I	9.3	57	14
29-31	I	4.8	75	16
53-55	II	8.3	112	27
65-67	II	9.3	35	1.0
77-79	II	13.9	30	0.6
89-91	II	18.9	20	0.3
105-107	II	3.6	8.9	1.0
113-115	II	8.5	4.1	1.3
117-119	II	6.4	5.9	1.3
125-127	II	5.9	62	1.9
137-139	III	10.4	54	nd
139-141	III	5.3	30	nd
143-145	III	6.2	36	nd
147-149	III	3.4	21	nd

[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

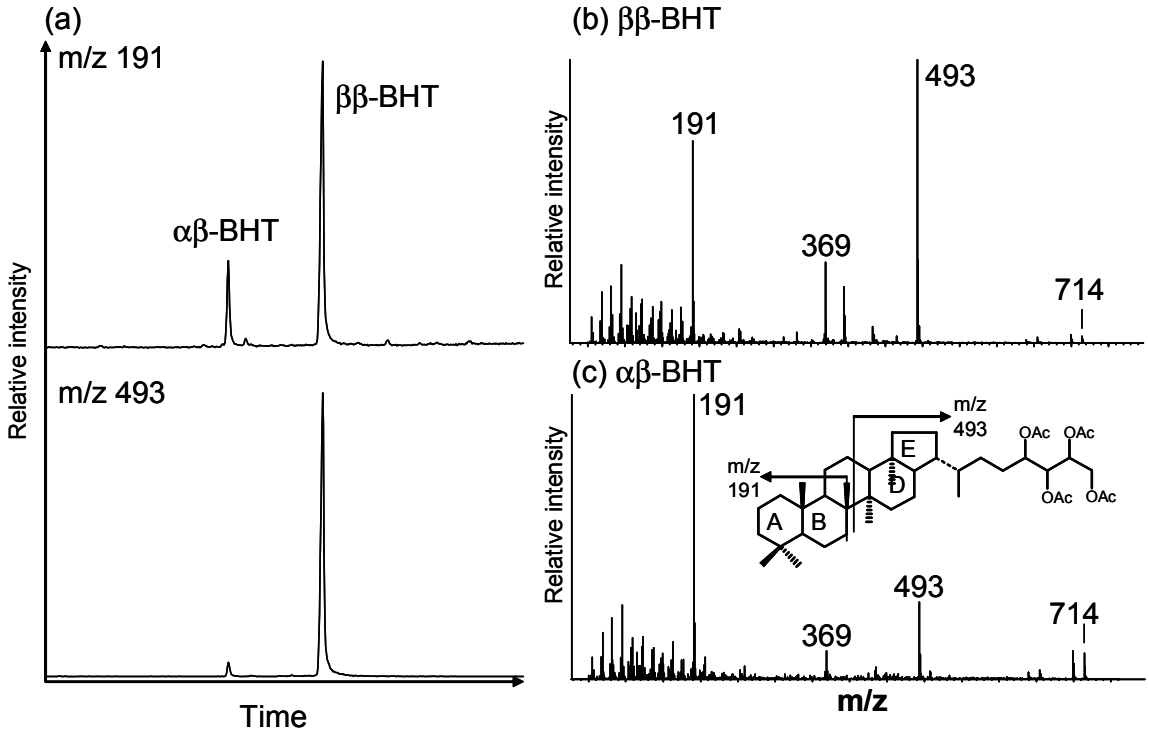

Figure 1. (a) GC-MS partial mass chromatograms showing relative retention times of ββ-BHT and proposed αβ-BHT. (b) EI mass spectrum of tetraacetylated ββ-BHT. (c) EI mass spectrum of tetraacetylated αβ-BHT. (Ac = $COCH_3$)