Organic Geochemistry 91 (2016) 100–108

Contents lists available at ScienceDirect

Organic Geochemistry

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

Identification and mode of formation of hopanoid nitriles in archaeological soils

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ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form 19 October 2015
Accepted 22 October 2015
Available online 18 November 2015

Keywords:
Hopanoids
Nitriles
Soils
Ammonium
Hopanoid side-chain shortening
Nitrogen incorporation

ABSTRACT

In the course of investigation of lipid extracts from archaeological soils from two Merovingian tombs (7th Century, Ichtratzheim, France) in an agricultural area, a series of N-containing hopanoids was detected. They were postulated to correspond to C_{31}–C_{33} hopanoid nitriles on the basis of mass spectrometry. The C_{32} homologue was unambiguously identified by comparison with a standard obtained by synthesis. The mode of formation of the compounds was investigated using laboratory simulation experiments involving either *N*-acetyl-aminobacteriohopanetriol as a model compound representative of bacterial hopanoids with polyfunctionalized side chains, or C_{32} hopan-32-al. The experiments led us to propose that the formation of the nitriles from C_{35} biohopanoid polyols could result from a two step process involving initially an oxidation step leading to the shortening of the functionalized side chain and a second step during which N is incorporated. In the context of the soils containing archaeological wood remains from tombs and found in an agricultural area, oxidants would certainly be available, notably in the form of O_2 diffusing from the surface or H_2O_2 produced during wood degradation by wood-rotting fungi. Possible N sources could be residual N from the decomposing body within the tomb or manure and fertilizers used for field amendment. The laboratory experiments also gave a better insight into the diagenetic processes leading to the side chain shortening of the hopanoids. In particular, it could be shown that C_{31} hopanoids also derive from tetrafunctionalized hopanoids upon diagenesis and not solely from pentafunctionalized hopanoids as generally suggested in the literature.

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1. Introduction

Given their ubiquity in the sub-surface, their specific bacterial origin and their hydrocarbon skeleton prone to isomerization upon dia- and catagenesis, hopanoids have been proven to be useful biomarkers for biogeochemical studies dealing with microbial diversity in sediments (e.g. Talbot and Farrimond, 2007; Sáenz et al., 2011), climatic change (e.g. Cooke et al., 2008a, 2009), geochemical processes (e.g. Birgel and Peckmann, 2008), (palaeo) environmental assessment (e.g. Ourisson and Albrecht, 1992; Peters et al., 2005), early life evolution (e.g. Summons et al., 1999), oil–source or oil–oil correlation studies (e.g. Seifert and Moldowan, 1978; Peters et al., 2005), or maturation studies (e.g. Seifert and Moldowan, 1980; Peters et al., 2005). Regarding hopanoids in recent environments, the number of studies devoted to their occurrence and significance in soils is rather limited (e.g. Ries-Kautt and Albrecht, 1989; Bull et al., 1998; Winkler et al., 2001; Shunthirasingham and Simpson, 2006; Cooke et al., 2008b; Xu et al., 2009; Rethemeyer et al., 2010; Zhu et al., 2011) compared with those dealing with sedimentary settings. Whereas investigation of hopanoids in soils can give clues, notably to bacterial diversity (e.g. Cooke et al., 2008b; Xu et al., 2009; Rethemeyer et al., 2010; Zhu et al., 2011) or to the relative contribution from bacterial biomass to soil organic matter (e.g. Shunthirasingham and Simpson, 2006), their use as molecular markers in soils has been limited by a lack of knowledge concerning their specific diagenetic transformations in soils and their dependence on environmental conditions.

In this context, we came across a series of unknown C_{31}–C_{33} hopanoids while investigating the lipid extracts from archaeological soils containing wood remains from Merovingian tombs

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http://dx.doi.org/10.1016/j.orggeochem.2015.10.013
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(7th Century, Ichtratzheim, SE France). The compounds [**1**–**3**; numbers refer to structures in Fig. A.1 (Appendix); Fig. 1] were shown to bear a nitrile on the side chain, as demonstrated by synthesis of a reference C_{32} compound (**2**). Furthermore, laboratory experiments using model hopanoids (**4**–**5**) were carried out in order to investigate their possible mode of formation, which led us to propose different mechanistic pathways explaining N incorporation and side chain shortening in biohopanoids that might occur during early diagenesis in oxidative environments.

2. Experimental

2.1. Samples

The samples comprised three archaeological soils containing wood remains and which were collected in 2011 from two Merovingian tombs dating back to the 7th Century in an archaeological site at Ichtratzheim (NE France; Fossurier, 2013).

2.2. Analytical methods

2.2.1. Gas chromatography (GC)

GC was carried out with a Hewlett Packard 6890 gas chromatograph equipped with an on-column injector, flame ionisation detector and HP-5 fused silica column (30 m × 0.32 mm; 0.25 μm film thickness). H_2 was the carrier gas (constant 2.5 ml/min) and the oven was programmed as follows: 70–200 °C (10 °C/min), 200–300 °C (4 °C/min), isothermal at 300 °C.

2.2.2. GC–mass spectrometry (GC–MS)

GC–MS was carried out with a Thermo Scientific Trace Ultra gas chromatograph coupled to a Thermo Scientific TSQ Quantum mass spectrometer. The source was at 220 °C. The mass spectrometer was operated in the electron impact (EI) mode at 70 eV. GC separation was performed with a HP5-MS column (30 m × 0.25 mm; 0.1 μm film thickness) using He as carrier gas. The temperature program was: 70 °C (1 min), 70–200 °C (10 °C/min), 200–300 °C (4 °C/min), isothermal at 300 °C.

2.2.3. Nuclear magnetic resonance (NMR)

The NMR spectra of compounds **2** and **5** were recorded with a Bruker Avance I 500 MHz spectrometer (500 MHz for ^1H; 125 MHz for ^{13}C). The chemical shifts are reported in ppm relative to tetramethylsilane, with the solvents used as internal standard [$CHDCl_2$, δ ^1H 5.32 ppm; CD_2Cl_2, δ ^{13}C 53.8 ppm (**2**) or pyridine-D4, δ ^1H 7.22, 7.58, 8.74 ppm; pyridine-D5, δ ^{13}C 123.9, 135.9, 150.2 ppm (**5**)] and the coupling constants are expressed in Hz.

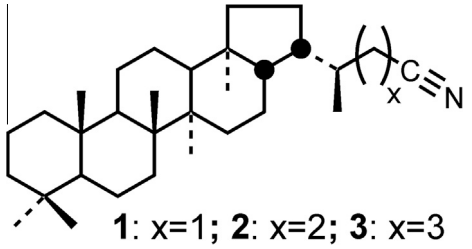

Fig. 1. Structures of C_{31}–C_{33} hopanoid nitriles (**1**–**3**) identified in archaeological soils containing wood remains from Merovingian tombs (7th Century, Ichtratzheim, France).

2.3. Extraction and fractionation of lipid extract from archaeological wood remains

The archaeological samples were extracted using sonication (25 min) with CH_2Cl_2/CH_3OH (1:1, v/v; 2 × 400 ml). The crude extracts obtained from filtration on Celite were successively acetylated (Ac_2O/pyridine 1:1 v/v, 60 °C, 2 h) and, after removal of the solvent and excess reagent under reduced pressure, esterified using diazomethane in Et_2O. The derivatized extract was fractionated using liquid chromatography on silica gel, eluting successively with CH_2Cl_2/EtOAc (8:2 v/v) and CH_2Cl_2/CH_3OH (1:1 v/v), leading respectively to a moderately polar fraction analysed using GC–MS, and a polar fraction not further investigated.

2.4. C_{32} hopanoid nitrile **2** ()Fig. 2

C_{32} hopan-32-al **4** was first prepared by way of oxidation of 35-aminobacteriohopane-32,33,34-triol **6a** (isolated from a culture of a *Streptomyces* sp.) with H_5IO_6 according to the procedure described by Rohmer et al. (1984). A 30% solution of NH_3 in water (250 μl) and a solution of I_2 (12 mg, 47.4 μmol, 1.43 eq.) in tetrahydrofuran (THF; 250 μl) were successively added under stirring to C_{32} hopan-32-al **4** (15 mg, 33.0 μmol) in THF (500 μl). The mixture was stirred at room temperature (2 h), poured into a saturated solution of sodium thiosulfate (10 ml) and the organic mixture extracted with CH_2Cl_2 (2 × 10 ml). The organic extract was evaporated to dryness under vacuum and chromatographed over a silica gel column (cyclohexane/CH_2Cl_2, 1/1, v/v), yielding the C_{32} hopanoid nitrile **2** (11 mg, 24.4 μmol, 74%).

*Compound **2***

GC–MS (EI, 70 eV, see also Fig. 4b) *m/z* (relative intensity) 451 ($M^{+\cdot}$, 4%), 436 (7), 369 (14), 230 (100), 202 (10), 191 (73), 149 (15), 95 (24). ^1H NMR (500 MHz; CD_2Cl_2): 0.73 (s, 3H), 0.80 (s, 3H), 0.83 (s, 3H), 0.88 (s, 3H), 0.960 (d, *J* = 6.0 Hz, CH_3-29), 0.965 (s, 6H), 2.24 (ddd, *J* = 5.0, 8.5, 17.5 Hz, 1H, —CH_2CN), 2.36 (ddd, *J* = 8.5, 8.5, 17.5 Hz, 1H, —CH_2CN). ^{13}C NMR (125 MHz; CD_2Cl_2): 14.9, 16.2, 16.3, 16.8, 17.0, 19.3, 19.3, 19.6, 21.5, 21.9, 23.4, 24.6, 28.1, 30.3, 32.1, 33.7, 33.9, 34.3, 37.0, 38.0, 40.9, 42.0, 42.3, 42.4, 42.7, 44.9, 46.3, 49.9, 51.0, 55.0, 56.7, 120.9.

2.5. N-acetyl-35-aminobacteriohopane-32,33,34-triol **5**

35-Aminobacteriohopane-32,33,34-triol tetraacetate **6b** (36 mg; 50.4 μmol) was treated with Amberlyst A26 resin (242 mg) in CH_3OH/Et_2O (14 ml, 1/1; v/v) for 2.5 h (Reed et al., 1981; Neunlist et al., 1985). *N*-acetyl-aminobacteriohopanetriol **5** (28 mg; 47.7 μmol; 95%) was obtained after filtration (cotton + sand) and evaporation of the solvent.

*Compound **5***

APCI-MS *m/z* (relative intensity) 588 ($[M+H]^+$, 100%), 570 ($[M+H-H_2O]^+$, 5%), ^1H NMR (500 MHz; pyridine-D5): 0.71 (s, 3H, CH_3), 0.83 (s, 6H, $2CH_3$), 0.90 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 1.07 (d, *J* = 6.0 Hz, 3H, CH_3-29), 2.10 (s, 3H, —COCH_3), 4.11 (m, 3H, —CH—OH), 4.37 (s, 1H, —CH_2—NHAc), 4.46 (s, 1H, —CH_2—NHAc), 6.37 (s, *J* = 3.0 Hz, 1H, —OH), 6.49 (d, *J* = 3.0 Hz, 1H, —OH), 6.57 (d, *J* = 3.0 Hz, 1H, —OH), 8.99 (s, 1H, —NH—). ^{13}C NMR (125 MHz; pyridine-D5): 16.4, 16.4, 17.0, 17.1, 19.3, 19.3, 20.8, 21.6, 22.1, 23.3, 23.4, 24.5, 28.3, 31.1, 32.7, 33.7, 33.8, 33.9, 34.3, 37.6, 37.9, 40.8, 42.2, 42.2, 42.3, 42.6, 44.2, 44.9, 47.1, 49.9, 51.0, 55.0, 56.7, 74.9, 75.1, 75.8, 172.1.

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