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Novel tri- and tetra-unsaturated highly branched isoprenoid (HBI) alkenes from the marine diatom *Pleurosigma intermedium*.

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ABSTRACT

Two previously uncharacterised C₂₅ highly branched isoprenoid (HBI) alkenes have been identified in a laboratory culture of the diatom *Pleurosigma intermedium* and their structures determined using a combination of NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS). These new tri- and tetra-unsaturated HBI alkenes are stereoisomers of previously characterised HBIs, with a *Z* stereochemistry for the C5–C6 double bond in each case. HBIs with an isomeric (*E/Z*) C5–C6 double bond and with 1–4 double bonds, in total, have extremely similar mass spectra and a consistent difference in retention index (Δ RI 28), with a *Z* before *E* elution order in all cases.

Keywords: highly branched isoprenoid; alkene; diatom; *Pleurosigma intermedium*

1. Introduction

C₂₅ highly branched isoprenoid (HBI) alkenes are common components of marine and lacustrine sediments worldwide and are biosynthesised by certain diatoms mainly belonging to the genera *Haslea*, *Rhizosolenia*, *Pleurosigma* and *Berkeleya* (Volkman et al., 1994; Belt et al., 1996, 2000; Sinninghe Damsté et al., 1999; Grossi et al., 2004; Brown et al., 2014). C₂₅ HBIs contain between one and six double bonds (DB) and occur in a number of regio- and stereo-isomeric forms. Individual structures have been established mainly through diatom culturing and analysis of purified extracts using NMR spectroscopy (e.g. Belt et al., 1996, 2000; Sinninghe Damsté et al., 1999; Grossi et al., 2004; Brown et al., 2014). Here, we identify two novel C₂₅ HBIs isolated from a culture of the diatom *Pleurosigma intermedium* and report their structures following analysis of partially purified extracts using NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS).

2. Experimental

P. intermedium (RA120910 P1B3) was obtained from the Roscoff Culture Collection (<http://roscoff-culture-collection.org/>) and cultured in the laboratory (15°C, 100 µmol m⁻² s⁻¹, 14/10 L/D cycle in f/2 enriched water). After 21 days, cells were filtered (GF/F), sonicated (DCM; 5 ml), re-filtered through a small plug cotton wool, dried (N₂), then partially purified using column chromatography (SiO₂). The hydrocarbon fractions (hexane; 5 ml) were analysed by GC–MS using a Hewlett-Packard 5890 Series II gas chromatograph, fitted with a 30 m fused silica HP_{5ms} column (0.25 mm i.d.,

0.25 μm film) coupled to a 5970 Series Mass Selective Detector (MSD) (Brown et al., 2014). For further separation of HBIs we used Ag-ion chromatography (Supelco discovery® Ag-Ion; 0.1 g) to obtain saturated hydrocarbons (hexane; 3 ml), HBI I (acetone/DCM; 50/50; 5 ml) and HBI III (acetone; 5 ml). NMR data were obtained using a JEOL ECP-400 NMR spectrometer with chemical shifts measured relative to those of CDCl_3 (^1H : 7.24 ppm; ^{13}C : 77.0 ppm).

3. Results and discussion

Extraction of a culture of *P. intermedium*, followed by a two-stage purification (see Experimental) yielded sufficient quantities of two HBI alkenes for analysis by GC–MS and NMR spectroscopy. These HBIs were identified as tri- and tetra-unsaturated C_{25} HBIs on the basis of the M^+ (m/z 346 and 344, respectively) in their mass spectra (Fig. 1).

The ^1H NMR spectrum of the HBI triene (I; Fig. 1) exhibits multiplets at 5.63 and 4.89 ppm (Table 1) characteristic of a vinyl moiety at C23–C24, a structural feature found in virtually all previously reported HBI alkenes (Belt et al., 1996, 2000; Sinninghe Damsté et al., 1999; Grossi et al., 2004; Brown et al., 2014). Two further alkene protons (H5 and H9) could also be identified from their distinctive chemical shifts and multiplicities (Table 1). Alternative positions for the two tri-substituted DB at C2–C3 or C13–C14 could be discounted due to the observation of two isopropyl groups at 0.85 and 0.84 ppm, totalling 12H (Table 1). The ^{13}C NMR spectrum of triene I was also consistent with the presence of a vinyl group (C23–C24), two tri-substituted DB (C5–C6, C9–C10) two isopropyl CH_3 (C1&C16, C15&C19) and three further CH_3 groups

(C17, C18, C25) (see Table 1 for details). However, since a C₂₅ triene with DB at C5–C6, C9–C10 and C23–C24 has been characterised previously (Wraige et al., 1999), but with a different retention index (RI) to that of the triene investigated here, we believed that the latter likely represented a stereoisomer of the former, which has known *E* stereochemistry for both DB (structure II; Fig. 1; Wraige et al., 1999). Confirmation of this, and identification of the stereochemistry for each DB, was achieved by further inspection of the ¹H and ¹³C NMR data. Specifically, it has been shown previously that the ¹³C chemical shifts for C11 and C18 in other C₂₅ HBI trienes are particularly characteristic of the stereochemistry at C9–C10, with C11 occurring at ca. 40 ppm and 32 ppm for the *E* and *Z* isomers, respectively, while C18 appears at 15.7 ppm (*E*) and 23.5 ppm (*Z*) (Belt et al., 2000). Since C11 for the triene in our *P. intermedium* culture appears at 40.1 ppm, with a further resonance at 16.4 ppm attributed to C18, the stereochemistry of the C9–C10 DB (*E*) could be deduced, and this was confirmed by identification of a singlet at 1.55 ppm in the ¹H spectrum due to H18 (c.f. 1.69 ppm when the C9–C10 DB is *Z*; Belt et al., 2000). In contrast, the resonance attributed to C17 (18.3 ppm) is shifted to lower field compared to that of its counterpart with known (*E*) stereochemistry (11.8 ppm; Wraige et al., 1999), while the corresponding ¹H resonance (H17) is also low field shifted (1.49 ppm) relative to the *E* isomer (1.43 ppm; Wraige et al., 1999). Finally, the resonance for the alkenic proton H5 in I (5.14 ppm) is low field shifted compared to the corresponding proton in II and to H9 in both (5.02 ppm). As such, the NMR data indicate that the stereochemistry of the C5–C6 DB is *Z* and we also provide chromatographic and mass spectrometric

data in support of this assignment. Belt and Cabedo-Sanz (2015) demonstrated that geometric isomers of C₂₅ HBI monoenes and dienes with C5–C6 DB are well resolved by GC–MS, with *Z* isomers eluting before their *E* counterparts and a difference in RI (Δ RI) of ca. 28. Significantly, therefore, the HBI triene identified here (I; RI_{HP5ms} 2075), elutes before the corresponding *E* isomer (RI_{HP5ms} 2103) with a Δ RI of 28. Finally, the mass spectrum of I is extremely similar to that of II (Fig. 1), also consistent with observations made previously for other HBI geometric isomers (Belt et al., 2000; Belt and Cabedo Sanz, 2015).

Although a much lower abundance in the purified fraction prevented us from obtaining satisfactory ¹³C NMR data, the structure of the co-occurring HBI tetraene (III) can be proposed on the basis of related ¹H NMR and GC–MS data to those found for triene I. Most notably, the ¹H NMR spectrum of tetraene III confirmed the presence of only one isopropyl moiety (0.85 ppm), two singlets due to H15 (1.65 ppm) and H19 (1.58 ppm), and two additional allylic protons (compared to triene I) at ca. 2 ppm. The *Z* stereochemistry of the C5–C6 DB was again evident with characteristic resonances for H17 (1.49 ppm) and H5 (5.15 ppm). An additional (compared to triene I) alkene proton resonance provided evidence for a tri-substituted DB at C13–C14, while an alternative location for this additional DB at C2–C3 can be discounted on the basis of an absence of any double-allylic protons (i.e. H4) that would resonate at ca. 2.8 ppm (e.g. Sinninghe Damsté et al., 1999). Further, the mass spectral similarity between III (this study) and IV (Allard et al., 2001) (Fig. 1) and a

Δ RI of 28 are consistent with other HBI isomers with *E/Z* isomerism at C5–C6 as described here and by others (Belt and Cabedo-Sanz, 2015).

Previously, HBIs containing C9-C10 DB with *Z* stereochemistry have been identified in cultures of a different strain of *P. intermedium* (Belt et al., 2000), and several other HBIs exhibiting *E/Z* isomerism have been reported in sediments and in laboratory rearrangement reactions (Belt and Cabedo-Sanz, 2015). Our new findings, however, represent the first example of biogenic HBIs possessing a C5–C6 DB with *Z* stereochemistry. Finally, the organic carbon content of *P. intermedium* (14%) and intra-cellular concentrations of HBI I (0.5 pg cell⁻¹) and III (1 pg cell⁻¹) are similar to those reported for other HBI-producing diatoms (Volkman et al., 1994; Belt et al., 1996; Grossi et al., 2004; Brown et al., 2014).

4. Conclusions

The structures of two previously uncharacterised C₂₅ HBI alkenes have been identified following laboratory culturing of the diatom *P. intermedium* and analysis of partially purified hydrocarbon extracts using NMR spectroscopy and GC–MS. This study adds to the existing suite of structurally characterised HBI alkenes and it will be interesting, in the future, to see if the same biomarkers can be identified in environmental or geochemical samples.

Acknowledgments

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Figures and Tables

Figure 1. (a) Partial GC–MS chromatogram of the hydrocarbon fraction obtained from an extract of *P. intermedium*. (b) Structures and mass spectra of HBI alkenes described in the text.

Table 1. NMR data for HBI I and III. (Note: ¹³C NMR data for HBI I only)

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Figure 1

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Carbon number	Carbon shift (δ /ppm)	Proton number	Proton shift (δ /ppm)
1	22.6	C_{25:3} (HBI I)	
2	27.7	1,15,16,19	0.84 and 0.85 (12H, 2 x d, $J = 7$ Hz)
3	38.6	25	0.94 (3H, d, $J = 7$ Hz)
4 ^a	25.4	2,3,12,13,14,20,21	1.05-1.37 (12H, m)
5	127.1	17	1.49 (3H, s)
6	136.8	18	1.55 (3H, s)
7	46.6	4,7,8,11,22	1.87-2.08 (8H, m)
8	32.2	24	4.89 (2H, m)
9	123.2	9	5.02 (1H, d, $J = 7$ Hz)
10	134.3	5	5.14 (1H, d, $J = 7$ Hz)
11	40.1	23	5.63 (1H, ddd, $J = 17, 10, 7$ Hz)
12 ^a	25.7		
13	38.6	C_{25:4} (HBI III)	
14	27.7	1,16	0.85 (6H, d, $J = 7$ Hz)
15	22.6	25	0.94 (3H, d, $J = 7$ Hz)
16	22.6	2,3,20,21	1.1-1.4 (7H, m)
17	18.3	17	1.49 (3H, s)
18	16.4	18	1.56 (3H, s)
19	22.6	19	1.58 (3H, s)
20	29.9	15	1.65 (3H, s)
21	34.6	4,7,8,11,12,22	1.86-2.06 (10H, m)
22	38.6	24	4.89 (2H, m)
23	144.9	9,13	5.05 (2H, m)
24	112.5	5	5.14 (1H, t, $J = 7$ Hz)
25	20.5	23	5.63 (1H, ddd, $J = 17, 10, 7$ Hz)

^a Resonances may be interchanged

Table 1

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Dr John Volkman
Editor, Organic Geochemistry

November 2015

Dear John

Please find attached a revised manuscript: “Novel tri- and tetra-unsaturated highly branched isoprenoid (HBI) alkenes from the marine diatom *Pleurosigma intermedium*” that addresses (accepts) all of the relatively small number of editorial changes requested by the reviewer (Joe Curiale, now acknowledged). Exceptionally, we have opted to remove the ‘See Table 1’ addition from the Figure 1 legend as these represent different data.

Regards,

Simon

Professor Simon Belt
University of Plymouth