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Note

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A novel tri-unsaturated highly branched isoprenoid (HBI) alkene from the marine diatom *Navicula salinicola*

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ABSTRACT

A novel tri-unsaturated C_{25} highly branched isoprenoid (HBI) alkene has been identified in a laboratory culture of the diatom *Navicula salinicola* and its structure determined using a combination of NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS). This represents the first report of a C_{25} HBI in a marine diatom from the *Navicula* genus, although a different tri-unsaturated C_{25} HBI has been reported previously in the freshwater species *N. sclesvicensis* and unspecified HBIs have been identified in the brackish *N. phyllepta*. The newly characterised HBI contains a relatively unusual conjugated diene sub-unit, a structural feature only previously reported in some HBIs biosynthesised by a further marine diatom, *Haslea ostrearia*.

Keywords: highly branched isoprenoid; alkene; diatom; *Navicula salinicola*

1. Introduction

C_{25} 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*, *Navicula*, *Rhizosolenia*, *Pleurosigma, Berkeleya* and *Pseudosolenia* (Volkman et al., 1994; Belt et al., 1996, 2000, 2001; Sinninghe Damsté et al., 1999; Grossi et al., 2004; Brown et al., 2014; Kaiser et al., 2016). A single tri-unsaturated C_{25} HBI (Structure 6; Fig. 1) has been identified in the freshwater diatom *Navicula sclesvicensis* (Belt et al., 2001), and the mainly brackish species *N. phyllepta* has also been reported as an HBI-producing diatom (Sinninghe Damsté et al., 2004), although no structures were given. In contrast, no HBIs have as yet been reported in any marine species within the *Navicula* genus. Here, we identify a novel tri-unsaturated C_{25} HBI isolated from a laboratory culture of the marine diatom *N. salinicola* and report its structure based on analysis by NMR spectroscopy and gas chromatography–mass spectrometry (GC–MS).

2. Experimental

The benthic diatom *N. salinicola* was collected from a coastal marine environment (M. Kulikovskiy, June 2016 at Nha Trang, Vietnam, 12°13'14.5"N 109°12'18.3"E) and kept as strain BTD1 in the Laboratory of Molecular Taxonomy of Aquatic Plants Institute of Plant Physiology (RAS). (Further taxonomic information can be found in the Supplementary Information). Initially, *N. salinicola* was cultured at 15 °C, 150 µmol m^{-2} s^{-1} continuous light in small flasks (150ml). Samples were harvested by filtration using MF-Millipore™ membrane filters (25 mm diameter, 0.3 µm pore size) during the exponential and stationary growth phases (Fig. 2). Large-scale cultures were then set up in several 2 l conical flasks under the same growth conditions. 80 l of such cultures were harvested by centrifugation (4000 rpm, 10 mins) during the stationary growth phase. For

the small- and large-scale culture experiments, we used enriched f/2 medium (Guillard and Ryther 1962), along with the following nutrient concentrations: 2646 μM $NaNO_3$, 318 μM Na_2SiO_3, 108 μM NaH_2PO_4. The filtered and centrifuged biomass was freeze dried and the resulting dry material extracted via sonication using hexane (3 ml (small-scale cultures); 25 ml (large-scale culture)). The total hexane extract (THE) was then concentrated by removing hexane under a stream of nitrogen and partially purified using column chromatography (SiO_2). The hydrocarbon fraction (hexane) was analysed by GC–MS using an Agilent 7890 gas chromatograph equipped with a HP_{5MS} fused-silica column (30 m; 0.25 μm film thickness; 0.25 mm internal diameter) coupled to an Agilent 5975 series Mass Selective Detector (MSD). NMR data were obtained using a JEOL ECP-400 NMR spectrometer with chemical shifts measured relative to those of $CDCl_3$ (^{1}H: 7.24 ppm; ^{13}C: 77.0 ppm). NMR data were collected on the THE obtained from the large-scale culture. The purity of the newly reported $C_{25:3}$ HBI (see Section 3) in this THE was estimated to be ca. 90% based on its relative peak area (GC–MS; Supplementary Information) and by the relative integration values of H-23 (Fig. 1) versus the alkenic protons of the co-occurring polyunsaturated linear alkenes (δ = ca. 5.3 ppm) in the ^{1}H NMR spectrum.).

3. Results and discussion

Following extraction of several small-scale cultures of *N. salinicola* from the exponential and stationary growth phases, analysis of partially purified THEs by GC–MS revealed the presence of a suite of closely eluting polyunsaturated linear alkenes (e.g. heneicosa-3,6,9,12,15,18-hexaene; *n*-$C_{21:6}$), trace amounts of a di-unsaturated HBI (**2**; Fig. 1) and a further compound exhibiting similar mass spectral properties to a range of C_{25} HBIs characterised previously. However, although the retention index (RI) of this

component (RI_{HP5ms} = 2141) did not match that of any previously reported C_{25} HBIs, hydrogenation of an aliquot of one THE resulted in the formation of the parent HBI alkane $C_{25:0}$, thus confirming the C_{25} carbon skeleton. Interestingly, this new HBI was only detected in cultures harvested during the stationary phase and was identified as tri-unsaturated on the basis of its molecular ion ($M^{+\cdot}$ 346; Fig. 3). At this point, it is not clear why this new HBI and the co-occurring diene **2** were not detected during the exponential growth phase, although we note that some variability in cellular HBI concentrations and distributions have been reported in a small number of previous studies (e.g. Wraige et al., 1997,1998,1999; Brown et al., 2020). From the large-scale culture of *N. salinicola*, we obtained ca 0.2 mg of the partially purified HBI triene (**3**; Fig. 1), which enabled full structural characterisation using ^{1}H and ^{13}C NMR spectroscopy. A conjugated diene sub-structure (C22–C25; C23–C24; Fig 1) could be readily identified through a particularly characteristic low field resonance in the ^{1}H NMR spectrum due to H-23 (Wraige et al., 1997; Allard et al., 2001), together with further low field resonances that could be attributed to alkenic methylene protons at C24 and C25 (Table 1). The third double bond could also be identified from its methylene protons at C17. Alternative positions for this third double bond at C1–C2 or C14–C15 can be discounted due to the observation of two isopropyl groups in the ^{1}H and ^{13}C NMR spectra (Table 1). Further, a double bond at C10–C18 leaves a solitary methyl group at C17 whose ^{13}C chemical shift would be at ca. 15.5 ppm, by comparison with related compounds (e.g. **1**; Belt et al., 2012). In contrast, isolated methyl groups at C18 in previously characterised HBIs resonate at ca. 19–20 ppm (e.g. 19.8 ppm for HBIs **1** and **2**; Fig. 1) (Johns et al., 1999; Belt et al., 2012), consistent with that observed for HBI **3** (i.e. 19.9 ppm; Table 1). The ^{13}C NMR spectrum of HBI **3** also contained individual resonances due to the six magnetically inequivalent alkenic

carbon nuclei (Table 1) and complete ^{13}C resonance assignments could be proposed by comparison with structurally similar HBIs characterised previously (Fig. 1; Belt et al., 1996, 2012; Wraige et al., 1997; Allard et al., 2001), some of which contain a conjugated diene sub-unit (i.e. **7**–**8**). The GC RI of HBI **3** (RI_{HP5ms} = 2141) is substantially higher than those of some other HBI trienes (e.g. RI_{HP5ms} = 2114 (**4**); 2109 (**5**); 2090 (**6**)) but is consistent with that reported for the structurally related tetraene **7** (RI_{HP-5} = 2159; Allard et al., 2001).

The identification of a C_{25} HBI in *N. salinicola* represents the first example of HBI production within a marine *Navicula* species despite the near-ubiquity of this genus within natural diatom populations. Since the *Navicula* and *Haslea* genera are quite similar, phylogenetically, with the latter well-known as an HBI-producing genus, the new finding is probably not surprising; however *Navicula* is a far more common genus, potentially making it a more important source of some HBIs in marine sediments. In terms of its structure, the conjugated diene sub-structure (C22–C25; C23-C24; Fig. 1) is somewhat unusual, although there is some previous precedent for such a feature in other HBIs isolated from a small number of cultures of the marine diatom *Haslea ostrearia* (Wraige et al., 1997; Allard et al., 2001). In fact, HBI **3** is a close structural analogue of HBI **7** identified previously in *H. ostrearia*, albeit as a minor component. Since the retention index of HBI **3** (RI_{HP5ms} = 2141) is similar to two HBI tetraenes identified in some cultures of *H. ostrearia* (*viz*. RI_{HP-5} = 2143–2146; Allard et al., 2001), one of which also contained HBI **7**, it possible that HBI **3** may also have been present in the corresponding lipid extracts, but not identified due to co-elution. We are unware of any geochemical reports of HBI **3** although its characterisation described herein may, in the future, lead to its positive identification in sedimentary archives, an outcome that may potentially add to

the use of HBIs as palaeoenvironmental indicators (c.f. HBIs **1** and **2** for Arctic and Antarctic sea ice; see Belt, 2018 for a review).

4. Conclusions

We report the structural identification of a novel C_{25} HBI biomarker in the marine diatom *N. salinicola*, the first example of HBI production within a marine *Navicula* species, thus expanding the potential number of sources of HBIs in the environment. Further studies into *N. salinicola* and related species may prove valuable in the use of HBIs as palaeoenvironmental proxies.

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References

Allard, W.G., Belt, S.T., Massé, G., Naumann, R., Robert, J.-M., Rowland, S.J., 2001. Tetra-unsaturated sesterterpenoids (Haslenes) from *Haslea ostrearia* and related species. Phytochemistry 56, 795–800.

Belt, S.T., Cooke, D.A., Robert, J.-M., Rowland, S.J., 1996. Structural characterisation of widespread polyunsaturated isoprenoid biomarkers: A C_{25} triene, tetraene and pentaene from the diatom *Haslea ostrearia* Simonsen. Tetrahedron Letters 37, 4755–4758.

Belt, S.T., Allard, W.G., Massé, G., Robert, J.-M., Rowland, S.J., 2000. Highly branched isoprenoids (HBIs): identification of the most common and abundant sedimentary isomers. Geochimica et Cosmochimica Acta 64, 3839–3851.

Belt, S.T., Massé, G., Allard, W.G., Robert, J.-M., Rowland, S.J., 2001. Identification of a C_{25} highly branched isoprenoid triene in the freshwater diatom *Navicula sclesvicensis*. Organic Geochemistry 32, 1169-1172.

Belt, S.T., Brown, T.A., Cabedo-Sanz, P., Navarro-Rodriguez, A., 2012. Structural confirmation of the sea ice biomarker IP_{25} found in Arctic marine sediments. Environmental Chemistry Letters 10, 189–192.

Brown, T.A., Belt, S.T., Cabedo-Sanz, P., 2014. Identification of a novel di-unsaturated C_{25} highly branched isoprenoid in the marine tube-dwelling diatom *Berkeleya rutilans*. Environmental Chemistry Letters, 12, 455–460.

Brown, T.A., Rad-Menéndez, C., Ray, J.L., Skaar, K.S., Thomas, N., Ruiz-Gonzalez, C., Leu, E., 2020. Influence of nutrient availability on Arctic sea ice diatom HBI lipid synthesis. Organic Geochemistry 141, 103977.

Grossi, V., Beker, B., Geenevasen, J.A.J., Schouten, S., Raphel, D., Fontaine, M.-F., Sinninghe Damsté, J.S., 2004. C_{25} highly branched isoprenoid alkenes from the marine benthic diatom *Pleurosigma strigosum*. Phytochemistry 65, 3049–3055.

Guillard, R.R. L., and Ryther, J.H., 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology 8, 229–239.

Johns, L., Wraige, E.J., Belt, S.T., Lewis, C.A., Massé, G., Robert, J.-M., Rowland, S.J., 1999. Identification of a C_{25} highly branched isoprenoid (HBI) diene in Antarctic

sediments, Antarctic sea-ice diatoms and cultured diatoms. Organic Geochemistry 30, 1471–1475.

Kaiser, J., Belt, S.T., Tomczak, T., Brown, T.A., Wasmund, N., Arz, H.W., 2016. C_{25} highly branched isoprenoid alkenes in the Baltic Sea produced by the marine planktonic diatom *Pseudosolenia calcar-avis*. Organic Geochemistry 93, 51–58.

Sinninghe Damsté, J.S., Schouten, S., Rijpstra, W.I.C., Hopmans, E.C., Peletier, H., Gieskes, W.W.C., Geenevasen, J.A.J., 1999. Structural identification of the C_{25} highly branched isoprenoid pentaene in the marine diatom *Rhizosolenia setigera*. Organic Geochemistry 30, 1581-1583.

Sinninghe Damsté, J.S., Muyzer,G., Abbas, B., Rampen, S.W., Massé, G., Allard, W.G., Belt, S.T., Robert, J-M., Rowland, S.J., Moldowan, J.M., Barbanti, S.M., Fago, F.J., Denisevich, P., Dahl, J., Trindade, L.A.F., Schouten, S., 2004. The rise of the rhizosolenid diatoms. Science 304, 584–587.

Volkman, J.K., Barrett, S.M., Dunstan, G.A., 1994. C_{25} and C_{30} highly branched isoprenoid alkenes in laboratory cultures of two marine diatoms. Organic Geochemistry 21, 407–414.

Wraige, E.J., Belt, S.T., Lewis, C.A., Cooke, D.A., Robert, J.-M., Massé, G., Rowland, S.J., 1997. Variations in structures and distributions of C_{25} highly branched isoprenoid (HBI) alkenes in cultures of the diatom, *Haslea ostrearia* (Simonsen). Organic Geochemistry 27, 497–505.

Wraige, E.J., Belt, S.T., Massé, G., Robert, J.-M., Rowland, S.J., 1998. Variations in distributions of C_{25} highly branched isoprenoid (HBI) alkenes in the diatom, *Haslea ostrearia*: influence of salinity. Organic Geochemistry 28, 855–859.

Wraige, E.J., Johns, L., Belt, S.T., Massé, G., Robert, J.-M., Rowland, S.J., 1999. Highly branched C_{25} isoprenoids in axenic cultures of *Haslea ostrearia*. *Phytochemistry* 51, 69–73.

Figure legends

Figure 1. Structures of C_{25} HBIs referred to in the text. HBIs are numbered in order of increasing unsaturation.

Figure 2. Growth curve of the small-scale culture of the marine diatom *N. salinicola*. Cell densities were estimated by measuring in vivo fluorescence between 460 nm to 670 nm using the SpectraMax® iD3 Multi-Mode Microplate Reader. Cultures were harvested during the exponential (empty arrow) and/or stationary (solid arrow) growth phases (n=1).

Figure 3. Structure and mass spectrum of HBI **3**.

Figure 1.

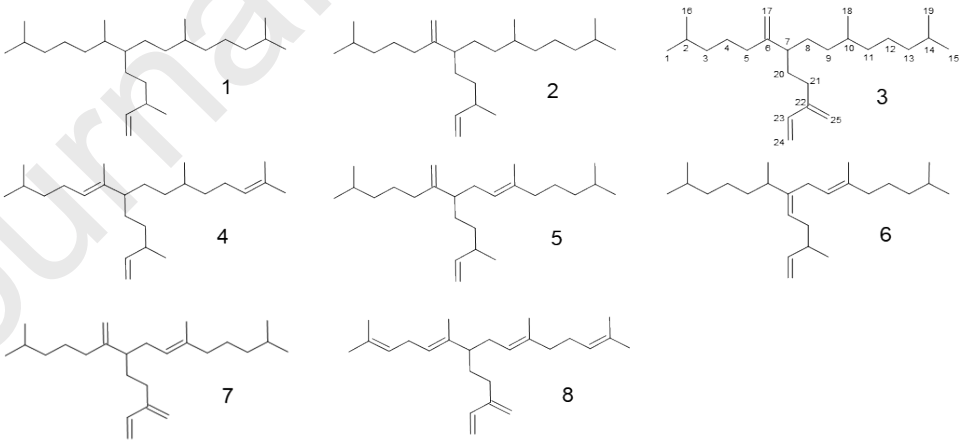

Figure 2.

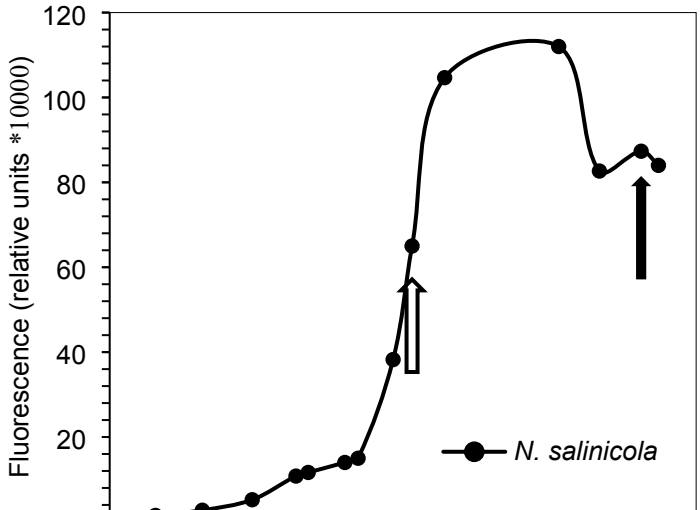

Figure 3.

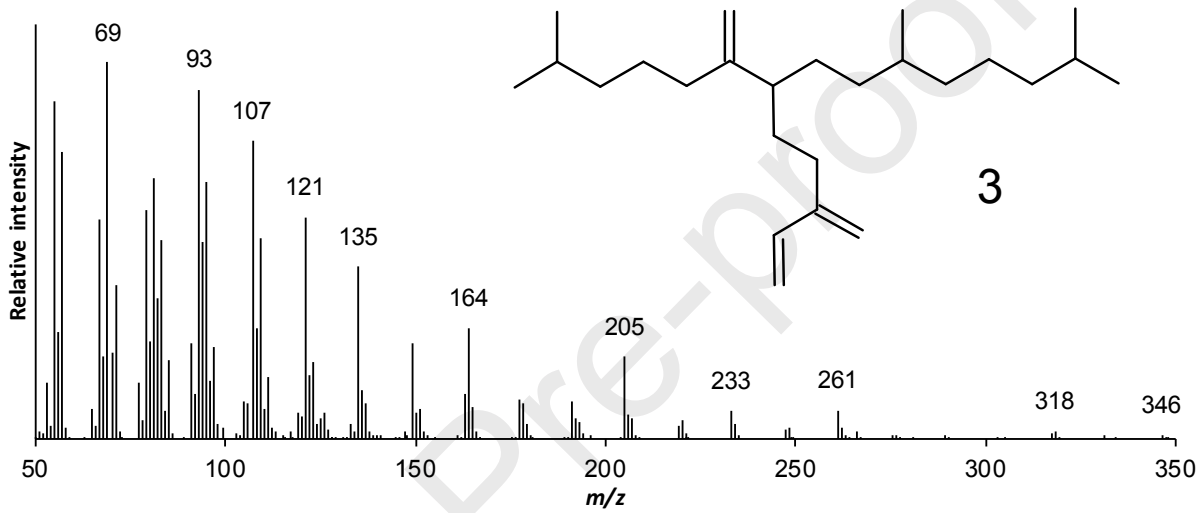

Table 1. Key NMR data for HBI **3**.

	Carbon shift (δ/ppm)	Proton Number	Proton shift (δ/ppm)
1,16*	22.8, 22.7*	1,15,16,19	0.85 (12H, m)
2	28.0	5,7,21	2.05 (5H, m)
3	39.1	17	4.77, 4.72 (2H, 2 x s, br)
4	25.6	18	0.82 (3H, t, J=6.9 Hz)
5	33.0	23	6.33 (1H, dd, J=17.6, 11.0 Hz)
6	152.3	24a	5.01 (1H, d, J=11.0 Hz)
7	47.1	24b	5.16 (1H, d, J=17.6 Hz)
8	29.8	25	4.96 (2H, m, br)
9	34.9		
10	33.0		
11	37.1		
12	24.8		
13	39.4		
14	28.0		
15,19*	22.8, 22.7*		
17	109.0		
18	19.9		
20	29.4		
21	32.0		

22	146.9
23	139.0
24	113.1
25	115.5

*Assignments may be interchanged

Highlights

1. The first report of a C_{25} HBI in a marine diatom from the *Navicula* genus
2. A new tri-unsaturated C_{25} HBI structure is characterised using NMR spectroscopy
3. The new C_{25} HBI has a relatively unusual conjugated diene sub-structure

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: