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Identification of C_{25} highly branched isoprenoid (HBI) alkenes in diatoms of the genus *Rhizosolenia* in polar and sub-polar marine phytoplankton.

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ABSTRACT

We report the identification of a range of C_{25} highly branched isoprenoid (HBI) alkenes and certain sterols in filtered phytoplankton samples obtained from western Svalbard (Arctic) and near South Georgia (South Atlantic, sub-Antarctic) in 2016 and 2014, respectively. The C_{25} HBIs contained 3–5 double bonds and had structures identified previously from analysis of laboratory diatom cultures. The same HBIs were also identified in individual diatom taxa isolated from the mixed assemblages and with reasonably similar distributions. Thus, C_{25} HBIs were identified in *Rhizosolenia setigera* isolated from western Svalbard near-surface waters, while the same HBIs were also found in *R. polydactyla f. polydactyla* and *R. hebetata f. semispina* picked from seawater collected from a site in the South Atlantic. The main sterol composition was slightly different between the two locations, with cholesta-5,24-dien-3β-ol (desmosterol) identified as one of the major components in the sample from West Svalbard, consistent with the diatom assemblage being dominated by *R. setigera*. In contrast, the major sterol in the South Atlantic sample was cholesta-5,22-dien-3β-ol (22-dehydrocholesterol), likely reflecting the relatively high proportion of the genus *Pseudo-nitzschia*. For both locations, the suite of HBIs included a tri-unsaturated isomer (HBI III; 6*Z*-2,6,10,14-tetramethyl-9-(3'-methylpent-4-enylidene)-pentadec-6-ene), proposed in previous studies as a potential proxy measure of pelagic sea ice-edge conditions, and thus, a counterpart to the mono- and di-unsaturated HBIs IP_{25} and $IPSO_{25}$, which have been used as seasonal sea ice proxies in the Arctic and Antarctic, respectively.

HBI III has been reported previously in sediments from West Svalbard and we report here its occurrence in a small number of surface sediments from the South Atlantic. For both regions, HBI III was present as one of the major HBIs in sediments, which contrasts the HBI distributions in the filtered phytoplankton samples, where HBIs with four and five double bonds were the major components. Differences in HBI distributions between phytoplankton and sediment samples may potentially be due to the presence of other (unanalysed) diatoms in the filtered water samples, seasonal/annual variability in the production of HBIs by a range of diatoms, differential degradation of HBIs between sources and sediments, or a combination of these. Interestingly, we did not detect any C_{30} HBIs in the water samples, picked cells or sediments from either location, despite earlier reports of these lipids in laboratory cultures of *R. setigera*. This study represents the first source identification of certain C_{25} HBI lipids under *in situ* pelagic conditions.

Keywords: highly branched isoprenoid; alkene; diatom; biomarker; *Rhizosolenia*

1. Introduction

C_{25} and C_{30} highly branched isoprenoid (HBI) alkenes are common components of marine and lacustrine sediments (Rowland and Robson, 1990; Belt et al., 2000; Sinninghe Damsté et al., 2004; Belt and Müller, 2013) and are generally believed to be biosynthesised by a limited number of diatom genera. To date, C_{25} HBIs (e.g. Fig. 1) have been reported in laboratory cultures of individual species of *Haslea* (Volkman et al., 1994; Belt et al., 1996; Wraige et al., 1997; Allard et al., 2001; Poulin et al., 2004), *Navicula* (Belt et al., 2001c), *Rhizosolenia* (Volkman et al., 1994; Sinninghe Damsté et al., 1999; Belt et al., 2001a, 2002; Rowland et al., 2001), *Pleurosigma* (Belt et al., 2000; 2001b; Grossi et al., 2004) and *Berkeleya* (Brown et al., 2014a). Further, under in situ environmental conditions, a small number of C_{25} HBIs have also been identified in *Pseudosolenia calcar-avis* isolated from Baltic Sea surface waters (Kaiser et al., 2016). On the other hand, apart from a limited number of reports in sediments (e.g., Prahl et al., 1980; Barrick and Hedges, 1981) and particulate organic matter (e.g., Wakeham et al., 2002; Xu and Jaffé, 2007), C_{30} HBIs have only been identified in laboratory cultures of *R. setigera* (Volkman et al., 1994; Belt et al., 2001a, 2002; Rowland et al., 2001). For both C_{25} and C_{30} HBIs, structural determinations have been achieved largely through laboratory culturing and analysis of purified apolar lipid extracts using NMR spectroscopy (e.g., Belt et al., 1996, 2000, 2001a,b,c; Sinninghe Damsté et al., 1999; Grossi et al., 2004; Brown et al., 2014a).

In recent years, the source or environmental specificity of certain C_{25} HBI alkenes has led to their use as organic geochemical proxies for seasonal Arctic and Antarctic sea ice reconstruction (e.g., Belt et al., 2007, 2016; Massé et al., 2011; Belt and Müller, 2013). Thus, a mono-unsaturated C_{25} HBI termed IP_{25} (structure I; Fig. 1) has been used as a palaeo proxy for Arctic sea ice (e.g., Belt et al., 2007; Fahl and Stein., 2012; Belt and Müller, 2013; Knies et al., 2014; Müller and Stein, 2014; Stein et al., 2016), while a closely related di-unsaturated analogue ($IPSO_{25}$; structure II; Fig. 1) represents a likely counterpart for the Antarctic (e.g., Barbara et al., 2010, 2013; Denis et al., 2010; Massé et al., 2011; Collins et al., 2013; Etourneau et al., 2013; Belt et al., 2016). Furthermore, sources of IP_{25} and $IPSO_{25}$ have been identified following isolation of individual species from mixed sea ice algal communities and analysis of their lipid content using gas chromatography–mass spectrometry (GC–MS) (Brown et al., 2014b; Belt et al., 2016). In contrast, although a tri-unsaturated C_{25} HBI (HBI III; Fig. 1) has been suggested to be a possible proxy indicator of the retreating ice edge during spring (Collins et al., 2013; Belt et al., 2015; Smik et al., 2016a,b; Ribeiro et al., 2017), thus far, no source identification of this biomarker from such locations has been made.

In the current study, we report the occurrence of various C_{25} HBIs and certain sterols in filtered water samples collected during (ice-free) summers from West Svalbard (Arctic) and near to South Georgia (South Atlantic, sub-Antarctic) and, in particular, we identify individual species of *Rhizosolenia* that biosynthesise HBI III.

We also believe this to be the first report of HBI source identification from in situ polar and sub-polar open water (pelagic) settings.

2. Experimental

2.1. Sample collection

Water samples were collected from western Svalbard (sample V12; 78°58.52'N; 9°21.1'E) and slightly north of South Georgia in the South Atlantic (sample E103; 53°15.56'S; 38°25.01'W) as part of the annual Kongsfjorden "Climate and Ecosystem" (Norwegian Polar Institute) and JR304 (British Antarctic Survey) cruise campaigns in 2016 and 2014, respectively (Fig. 2). All sampling was carried out in ice-free open water conditions (August and December for V12 and E103, respectively). The V12 sample was collected from a single vertical tow (0–30 m) using a plankton net (HYDRO-BIOS©, Kiel, Germany) fitted with a 20 µm mesh. Approximately 50 ml of sampled seawater were filtered onto a 47 mm Whatman GF/F filter and kept frozen (–20 °C) prior to analysis. The E103 sample was obtained using a paired motion-compensated Bongo net (61 cm mouth diameter, 2.3 m length) equipped with solid cod-ends and 100 µm and 200 µm mesh sizes. Based on the area of the net's mouth and the vertical sampling interval (0–200 m), we estimate the sampled volume of seawater to be ca. 58 m^3. Of the 100 µm sample retrieved, ca. 2 l were filtered onto a 47 mm GF/F filter and kept frozen (–80 °C) prior to analysis. Further unfiltered aliquots of V12 and E103 (ca. 25–50 ml) were also collected and kept frozen for subsequent species identification and cell picking.

Surface sediment material from seven locations in the South Atlantic (Fig. 2) was taken from the upper 0–1 cm of archived box cores held at the British Antarctic Survey, UK.

2.2. Species identification

Centric diatoms of the genus *Rhizosolenia* have long cylindrical cells with many girdle bands and, usually, with a single, elongated, rimoportula or labiate process (spine) on each cell valve (Round et al., 1990; Scott and Thomas, 2005). Species identification using light microscopy is based, usually, on the shape of the valve and its process with associated otaria morphology (Priddle et al., 1990; Armand and Zielinski 2001). *Rhizosolenia setigera* is narrow in diameter (4–20 µm) with a long needle-like process lacking otaria. *R. hebetata f. semispina* is also narrow (4–25 µm), with a long tapering process, but has a small pointed otaria. In contrast, cells of *R. polydactyla f. polydactyla* are wider (15–105 µm) with a process that is also wider at the base, tapering to the tip, with a large otaria that tapers distally to the process.

2.3. Extraction and purification

Filtered water samples were extracted, partially purified and analysed using established methods (e.g., Belt et al., 2012, 2013). In brief, GF/F filters were saponified in methanolic KOH (ca. 4 ml H_2O/MeOH, 1:9; 5% KOH; 60 min, 70 °C) following addition of 9-octylheptadec-8-ene (10 ng) as internal standard to permit

quantification of HBIs. Hexane (3 × 2 ml) was added to the saponified solution, which was vortexed (1 min) and centrifuged (1 min; 2,000 rpm). The supernatant, containing apolar lipids, was transferred to a clean vial and dried (N_2 stream) to remove hexane and traces of H_2O/MeOH. The apolar fractions were re-suspended in hexane (0.5 ml) and fractionated using column chromatography (0.5 g SiO_2) to obtain HBIs (5 ml hexane) and sterols (5 ml hexane/methyl acetate (4:1, v/v)). The procedure for analysis of the picked individual diatoms was the same as for the filtered water samples, except that cells were extracted with hexane only (1 ml, ultrasonication; 5 min). Freeze-dried surface sediments (ca. 2–3 g) from the South Atlantic were extracted using dichloromethane/methanol (3 × 3 mL; 2:1, v/v) according to established methods (Belt et al., 2012), with the resulting lipid extracts treated as per the extracted water samples. Analysis of sediments from western Svalbard is described in Smik and Belt (2017).

2.4. Analytical methods

All lipid extracts were analysed using GC–MS in total ion current (TIC) or single ion monitoring (SIM) mode using an Agilent 7890a 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 5975c Series Mass Selective Detector (MSD) (Belt et al., 2012). Individual HBIs were identified based on their characteristic retention indices (RI) and mass spectra (Wraige et al., 1999; Belt et al., 2000; Brown and Belt, 2016). For HBI quantification (picked cells), individual integrated peak areas for HBIs III and

IV obtained from GC–MS SIM analyses were normalised to those of the internal standard, instrumental response factors obtained from calibrations using purified standards (Belt et al., 2000, 2012) and the number of cells extracted. Since we did not have sufficient quantity and purity of all HBIs to conduct the corresponding calibrations, we took integrated peak areas of the molecular ion for each isomer and the calibrations using HBI III and IV to provide estimates of the concentrations of all other HBI components. Sterol fractions were derivatised using N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA; 50 µl; 70 °C; 60 min) prior to analysis by GC–MS. Individual sterols were identified by comparison of the mass spectra of their TMS ethers with published data (e.g., Volkman, 1986).

3. Results

3.1. C_{25} HBIs and sterols in phytoplankton from western Svalbard and the South Atlantic

The partially purified extracts of the filtered water samples from western Svalbard (sample V12) and South Georgia in the South Atlantic (sample E103) contained a number of C_{25} HBIs that could be identified by comparison with previously reported GC–MS data. Thus, sample V12 (western Svalbard) contained HBIs III–VIII with VII present as the major component (Fig. 3a). HBIs III–VIII were also present in the filtered water sample from the South Atlantic (E103), with V and VII as the most abundant isomers in approximately equal amounts (Fig. 3b). In addition, relatively small amounts of HBI IX could also be identified in E103,

although its geometric isomer, X, was not detected (Fig. 3b). In contrast, C_{25} HBIs I (IP_{25}) and II ($IPSO_{25}$) and C_{30} HBIs could not be identified in water samples from either location. The main sterols in sample V12 were 24-methylcholesta-5,22-dien-3β-ol (epi-brassicasterol), 24-methylcholesta-5,24(28)-dien-3β-ol (24-methylenecholesterol), cholesta-5,22-dien-3β-ol (22-dehydrocholesterol), cholesta-5,24-dien-3β-ol (24-dehydrocholesterol or desmosterol), cholest-5-en-3β-ol (cholesterol) and 24-methylcholest-5-en-3β-ol (24-methylcholesterol), with desmosterol and cholesterol as the major constituents. In contrast, 22-dehydrocholesterol and cholesterol dominated the sterol composition of sample E103, with 22-dehydrocholesterol as the main component, while epi-brassicasterol, 24-methylenecholesterol and desmosterol were only present in relatively minor quantities.

3.2. C_{25} HBIs in picked cells

The taxonomic composition of sample V12 (western Svalbard) was dominated by *Rhizosolenia setigera* (> 90% of total diatom abundance) and the same HBIs identified in the mixed microphytoplankton assemblage were also identified in the picked cells of this species, and in similar distribution, especially for the three most abundant components III, V and VII (Fig. 3a, 4a). The most abundant diatom taxa in the South Atlantic Bongo net sample (E103) were *Pseudo-nitzschia lineola* (ca. 50%) and *Trichotoxon reinboldii* (ca. 22%), with *R. polydactyla f. polydactyla* (ca. 11%) and *R. hebetata f. semispina* (3%) only present as relatively minor species. The

main HBIs in picked cells of *R. polydactyla f. polydactyla* and *R. hebetata f. semispina* were III, V and VII, although their relative concentrations were somewhat different to those of the same lipids in the total sample, with a much more even distribution in the picked cells (Figs. 3b and 4b,c). On the other hand, some other C_{25} HBIs (e.g., IV, VI and VIII) were either absent or below the limit of detection. Unfortunately, cells of the most abundant species (*P. lineola*) were too small and difficult to remove from the glass vial walls to enable their isolation and lipid analysis. The total C_{25} HBI concentration was estimated to be ca. 7, 3 and 2 pg/cell for *R. setigera, R. hebetata f. semispina* and *R. polydactyla f. polydactyla*, respectively.

3.3. C_{25} HBIs in western Svalbard and South Atlantic surface sediments

Previously, HBI III has been reported in 27 surface sediments from western Svalbard with concentration in the range 0.27–8.78 ng/g (Smik and Belt, 2017). Here, we re-examined the GC–MS chromatograms from this previous study and identified tri-unsaturated IV as the major HBI in most cases, together with III, as reported previously, and IX as an additional minor component. In contrast, of the more unsaturated HBIs, only V could be identified, and this was only present in a few extracts and in very low relative amounts (ca. 1%; Fig. 5a). For the seven surface sediments from the South Atlantic, III was the most abundant HBI, with a concentration range of 6–250 ng/g. Similar to the western Svalbard sediments, HBI

trienes IV and IX could also be quantified, but only trace amounts of HBI V were detected (Fig. 5b).

4. Discussion

Despite the common occurrence of C_{25} HBIs in sediments (e.g., Rowland and Robson, 1990; Belt et al., 2000; Sinninghe Damsté et al., 2004; Belt and Müller, 2013), relatively few studies have reported on the presence of these lipids in their native marine or lacustrine settings, either in mixed phytoplankton assemblages or in individual taxa. Exceptionally, IP_{25} and $IPSO_{25}$ have been identified in individual and mixed assemblages of Arctic and Antarctic sea ice diatoms (Nichols et al., 1988; Belt et al., 2007, 2013, 2016; Brown et al., 2011, 2014b), di- through to penta-unsaturated C_{25} HBIs have been reported in a small number of Antarctic phytoplankton samples (Massé et al., 2011; Smik et al., 2016a), and some further di- and tri-unsaturated C_{25} HBIs were also observed in *Pseudosolenia calcar-avis* isolated from surface waters of the south-eastern Baltic Sea (Kaiser et al., 2016). IP_{25} and some other HBIs have also been reported in sinking particles following the release of sympagic algae from melting sea ice in the Arctic (Brown et al., 2016; Rontani et al., 2016). As such, our identification of a range of C_{25} HBIs in phytoplankton samples from polar (western Svalbard) and sub-polar (South Atlantic) locations adds to the growing reports of these biomarkers in their source environments and we believe it to be the first example from individual taxa isolated from Arctic or South Atlantic pelagic settings.

With respect to the individual HBI-producing diatoms described in the current study, our findings represent the first report of HBIs in *R. polydactyla f. polydactyla* and *R. hebetata f. semispina,* although the occurrence of C_{25} and C_{30} HBIs within *R. setigera* is well known (Volkman et al., 1994; Sinninghe Damsté et al., 1999; 2004; Belt et al., 2001a, 2002; Rowland et al., 2001; Massé et al., 2004) and some HBIs have also been identified in *R. fallax*, *R. shrubshrolei* and *R. pungens* (Sinninghe Damsté et al., 2004). The absence of any C_{30} HBIs is also intriguing given their biosynthesis by *R. setigera* in most laboratory cultures (Volkman et al., 1994; Belt et al., 2001a, 2002; Rowland et al., 2001). On the other hand, C_{30} HBIs were also absent in cultures of *R. setigera* isolated from the east coast of the USA (Sinninghe Damsté et al., 1999), although this strain was additionally unusual in that it produced only one (penta-unsaturated) C_{25} HBI and with a double bond at C5/6 compared to C7/20, which is a more common characteristic of C_{25} and C_{30} HBIs in other strains of *R. setigera* (Belt et al., 2001a, 2002; Rowland et al., 2001). However, even within the C_{30} HBI-producing strains, the presence and distribution of the C_{25} counterparts exhibit notable differences. For example, Volkman et al. (1994) first reported the occurrence of several C_{30} HBIs (but no C_{25} HBIs) in an Australian strain (CS-62) of *R. setigera*, and Belt et al. (2001a) reported similar findings for a further strain (Nantes 99) isolated from northern France. In contrast, Rowland et al. (2001) detected both C_{25} (including III–VI identified here) and C_{30} HBIs in an Australian strain of *R. setigera* (CS 389/A), while Belt et al. (2002) showed subsequently that their distribution was strongly

influenced by life cycle characteristics, with the biosynthesis of C_{25} HBIs, in particular, being stimulated during the sexual reproduction or auxosporulation stage. In any case, the absence of C_{30} HBIs in our mixed phytoplankton and individual *Rhizosolenia* diatoms isolated from natural surface waters may potentially explain the relatively small number of reports of these biomarkers in marine sediments, at least compared to their C_{25} pseudo-homologues (Rowland and Robson, 1990; Belt et al., 2000; Sinninghe Damsté et al., 2004; Belt and Müller, 2013). On the other hand, the identification of desmosterol as the major sterol in the *R. setigera*-rich V12 sample is consistent with previous findings from laboratory cultures (Barrett et al., 1995; Massé et al., 2004; Rampen et al., 2010). Similarly, the presence of 22-dehydrocholesterol as the major sterol in sample E103 from the South Atlantic is consistent with the occurrence of *Pseudo-nitzschia lineola* as the most abundant diatom. Thus, although we are not aware of any investigations into the sterol content of *P. lineola* in culture, Rampen et al. (2010) identified 22-dehydrocholesterol as the major sterol in *P. seriata.*

In addition to the variability in HBI composition within *Rhizosolenia* diatoms, the type, concentration and distribution of individual isomers identified in V12, E103 and picked cells from both of these mixed algal assemblages, exhibit some parallels with HBI content in other diatoms, even in those of diverse (phylogenetically) genera. For example, the co-occurrence of III–VIII found here in centric *Rhizosolenia* diatoms has been reported previously in laboratory cultures of the pennate diatom *Pleurosigma intermedium* (Belt et al., 2000), which is also

capable of biosynthesising IX (Brown and Belt, 2016). Furthermore, our estimates of cellular (total) HBI concentrations (ca. 2–6 pg/cell) are typical of those reported previously in laboratory cultures of HBI-producing diatoms (Volkman et al., 1994; Rowland et al., 2001; Massé et al., 2004; Belt et al., 2013; Brown et al., 2014a; Kaiser et al., 2016) and individual species isolated from natural ice-algal assemblages (Brown et al., 2014b; Belt et al., 2016).

For all three *Rhizosolenia* species, we note, in particular, the presence of a tri-unsaturated C_{25} HBI (HBI III) that has been proposed as a potential proxy for ice-edge pelagic conditions in both the Arctic and the Antarctic (Collins et al., 2013; Belt et al., 2015; Smik et al., 2016a,b; Ribeiro et al., 2017). Given the near-ubiquity of *Rhizosolenia* spp. in marine phytoplankton worldwide, including the Arctic and Antarctic (Priddle and Fryxell, 1985; Priddle *et al.,* 1990; Scott and Thomas, 2005), it seems likely that the *Rhizosolenia* species identified here contribute to the sedimentary budget of HBI III in certain polar and sub-polar environments. Previously, HBI III has been reported in surface and down-core sediments from western Svalbard (Cabedo-Sanz and Belt, 2016; Smik et al., 2017) and we also identified it in each of the surface sediments from the South Atlantic as part of the current study, so a combination of our new and previous findings suggest that *Rhizosolenia* spp. are likely sources. However, since only a single sample was collected from each region, and these were both from ice-free surface waters during spring/summer months, the results from the current study do not really add to the evidence described previously for the use of HBI III as a proxy for ice-edge

conditions in the Arctic and the Antarctic (Collins et al., 2013; Belt et al., 2015; Smik et al., 2016a,b; Ribeiro et al., 2017). Further, our study does not reveal whether HBI III (or other HBIs) might be biosynthesised by other diatoms in these regions that bloom during other intervals. An examination of a greater number of diatom species is therefore required before the contribution from *Rhizosolenia* spp. in polar and sub-polar environments can be fully evaluated.

For both western Svalbard and the South Atlantic study regions, the sedimentary HBI distributions differ, however, from those found in the filtered water samples or individual diatom taxa. Specifically, while the tetra- and penta-unsaturated HBIs V and VII were present as the major components in the samples of filtered water and picked diatoms from both regions (Fig. 3), HBI trienes (III, IV and IX) were the most significant constituents of the surface sediments, with only V as the other quantifiable HBI, and in very low amounts (Fig. 5). We offer three possible explanations for these differences.

First, the snapshot nature of our phytoplankton sampling likely limits the extent to which the corresponding HBI distributions parallel those that reflect accumulation over seasonal or annual timeframes that are pertinent to sediments. As described earlier, there may be further diatoms in these regions that biosynthesise HBIs during different seasons, such that sedimentary distributions may better reflect the collective contribution resulting from seasonal species succession. Thus, additional phytoplanktonic sources of HBIs such as III, IV and IX would likely result in their increased accumulation, relative to HBIs V–VIII, in

sediments. To date, the only other known sources of HBIs III, IV and IX are diatoms belonging to the genus *Pleurosigma* (Belt et al., 2000; Brown and Belt, 2016), but *Pleurosigma* spp. were either absent or only present in extremely low abundances in our samples. However, this does not discount the possibility of HBI production by *Pleurosigma* spp. or other diatoms during different seasons, or by unpicked species in the current samples. Indeed, we note that the distributions of HBIs III, V and VII in *R. polydactyla f. polydactyla* and *R. hebetata f. semispina* (Fig. 4b,c) were slightly different from that in the mixed phytoplankton sample from which they were picked (E103; Fig. 3b), indicating the likely occurrence of additional HBI-producers in the latter. Further, and in contrast to the HBI distributions in the filtered phytoplankton and picked cells from sample V12, the identification of IX and the increased relative abundance of IV compared to III in sediments from western Svalbard (Fig. 5a), indicate that species other than *R. setigera* potentially contribute to the HBI sedimentary budget in this region. On the other hand, the contrasting outcomes between phytoplankton and sedimentary analyses may simply reflect the variability in HBI distribution observed previously in *Rhizosolenia* spp. (Volkman et al., 1994; Sinninghe Damsté et al., 1999; Belt et al., 2001a, 2002; Rowland et al., 2001), with sediment composition indicative of a temporal average of any shorter-term HBI variability within this genus.

Second, the likely increased degradation rates of more unsaturated HBIs such as V–VIII compared to those of HBI trienes (i.e. III, IV and IX) potentially leads to the latter becoming relatively enhanced in sediments. Indeed, although a

direct comparison of the reactivity of HBIs III–IX under environmental conditions has not yet been carried out, in laboratory studies a general increase in reactivity towards photo- and autoxidation processes has been reported for some HBIs containing a larger number of double bonds (Rontani et al., 2011, 2014).

Third, some additional (smaller) HBI-producing diatoms may not have been obtained during water sample collection in the South Atlantic, especially, due to the increased mesh size of the Bongo net employed (100 µm). In any case, the extent to which *Pleurosigma*, or other diatom genera, are additional contributors to the sedimentary budget of HBI III (or other HBIs) will require analysis of a larger number of phytoplankton samples with variable diatom composition. For now, although we were not able to isolate individual cells of the abundant (ca. 50%) *Pseudo-nitzschia lineola* from sample E103, we note that *P. seriata* has been shown previously not to produce HBIs in culture (Sinninghe Damsté et al., 2004).

5. Conclusions

A number of C_{25} HBI alkenes have been identified in natural phytoplankton populations obtained from West Svalbard in the Arctic and north of South Georgia in the South Atlantic (sub-Antarctic), including a tri-unsaturated isomer (HBI III) proposed previously as a potential proxy for seasonal ice-edge conditions in polar and sub-polar settings. From the same samples, picked diatoms belonging to the genus *Rhizosolenia* contained similar distributions of HBIs to those of the mixed phytoplankton assemblages, although they exhibited clear differences to those in

surface sediments from each region and also those reported previously in laboratory cultures of *R. setigera*, with the absence of any C_{30} HBIs being particularly noteworthy. In contrast, the identification of desmosterol as the major sterol in the sample from West Svalbard, containing > 90% *R. setigera*, is consistent with previous investigations into diatom sterol composition. In the future, it will be important to determine whether any other diatoms are capable of producing C_{25} HBIs (especially HBI III) in other polar and sub-polar pelagic settings, and to investigate whether there are any specific environmental controls (e.g., season) over HBI production in order that their potential as palaeoenvironmental proxies can be better understood. Such investigations are currently underway in our laboratories.

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Figures and Tables

Fig. 1. Structures of C_{25} HBI alkenes described in this study.

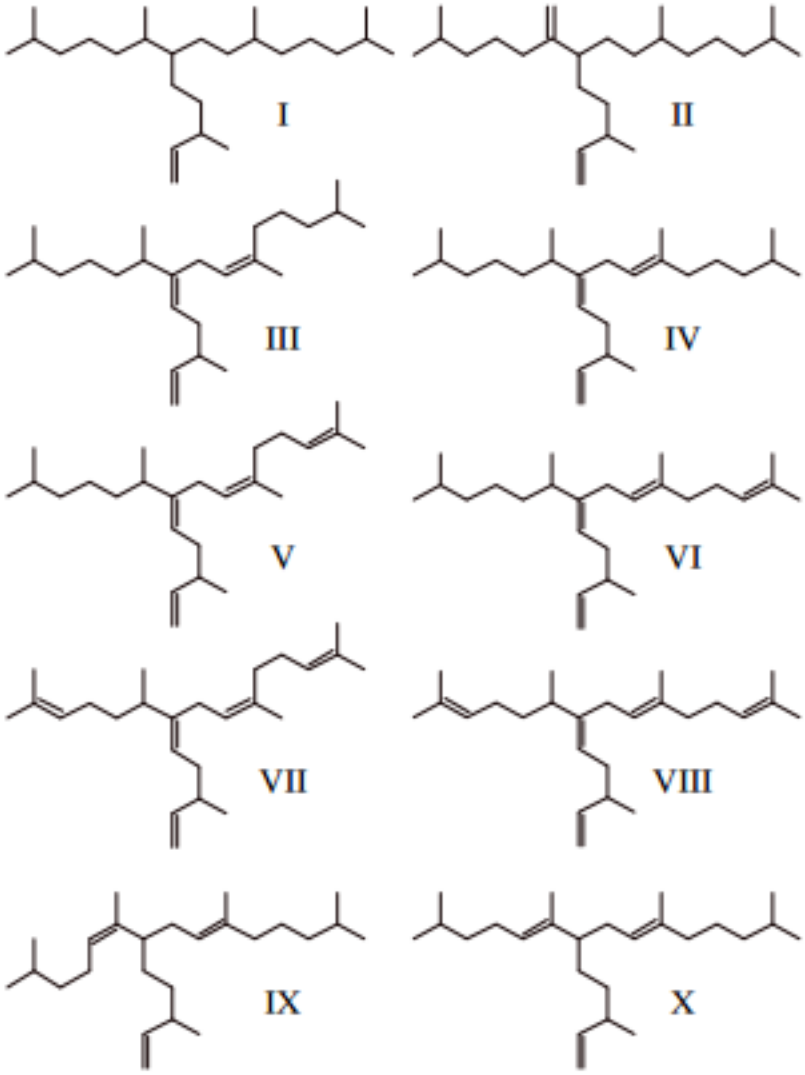
Fig. 2. Map of sampling regions: (a) western Svalbard; (b) South Atlantic. The water sample locations are indicated with a red dot. The locations of surface sediments analysed for HBIs in previous studies (Smik and Belt, 2017) and the current investigation are indicated mainly with black dots. Locations indicated by yellow dots represent the surface sediments for which partial GC–MS data are shown in Fig. 5.

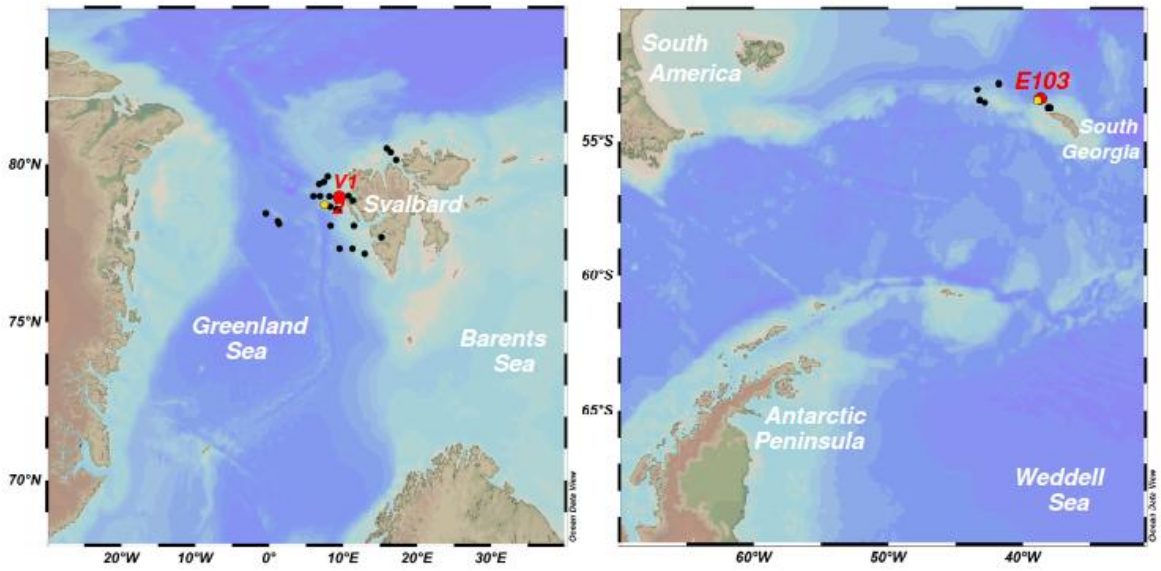
Fig. 3. Partial GC–MS chromatograms (SIM mode) of extracted water samples: (a) V12; (b) E103. In each case, the selected ion corresponds to the molecular ion of C_{25} HBIs with different degrees of unsaturation (*m/z* 346: $C_{25:3}$; *m/z* 344: $C_{25:4}$; *m/z* 342: $C_{25:5}$). Labelled peaks correspond to the structures shown in Fig. 1. Values in parentheses refer to the % contribution of the selected HBI to the total HBI content.

Fig. 4. Partial GC–MS chromatograms of partially purified hexane extracts of picked cells of different diatoms: (a) *R. setigera*; (b) *R. polydactyla f. polydactyla*; (c) *R. hebetata f. semispina*. In each case, the selected ion corresponds to the molecular ion of C_{25} HBIs with different degrees of unsaturation as per Fig. 3. Labelled peaks

correspond to the structures shown in Fig. 1. Values in parentheses refer to the % contribution of the selected HBI to the total HBI content.

Fig. 5. Partial GC–MS chromatograms of partially purified hexane extracts of selected surface sediments: (a) western Svalbard (V12); (b) South Atlantic (E103). In each case, the selected ion corresponds to the molecular ion of C_{25} HBIs with different degrees of unsaturation as per Fig. 3. Labelled peaks correspond to the structures shown in Fig. 1. Values in parentheses refer to the % contribution of the selected HBI to the total HBI content. For consistency with Fig. 3 and 4, the retention time of HBI VII is indicated by a dashed vertical line, although it was below the limit of detection for all sediments.





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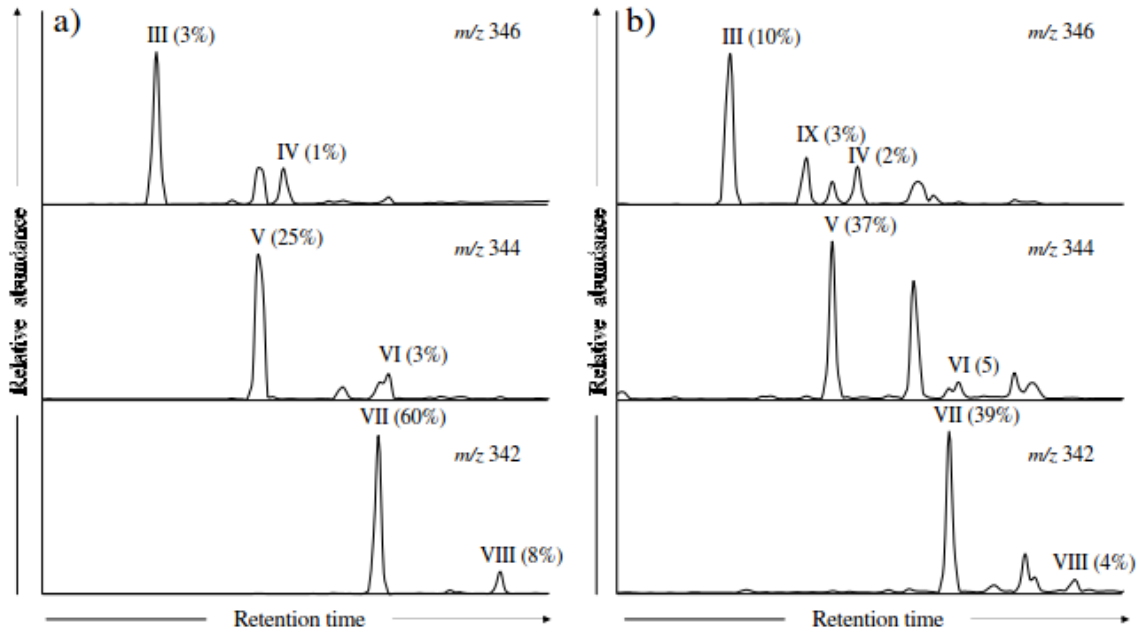

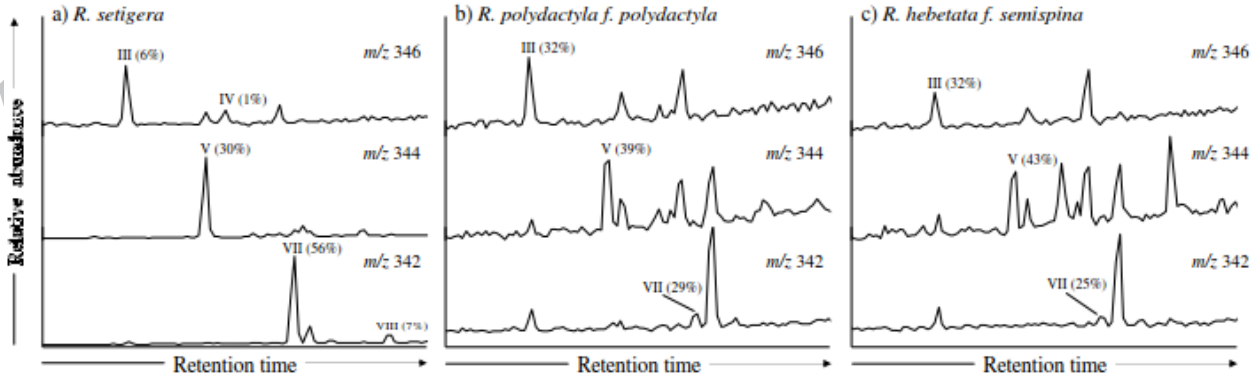

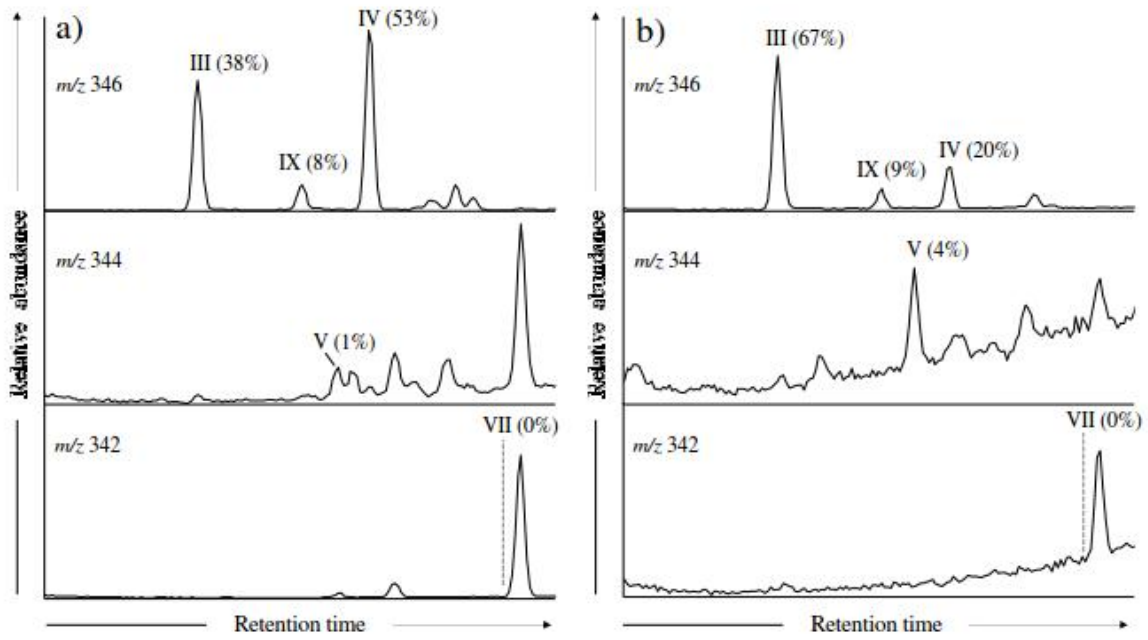

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Highlights

C_{25} HBIs identified in phytoplankton from western Svalbard and the South Atlantic

Sources of C_{25} HBIs identified as three species of *Rhizosolenia*

HBIs include HBI III proposed previously as a possible sea ice-edge proxy

Phytoplankton sterol content consistent with laboratory cultures of major taxa