On the stability of various highly branched isoprenoid (HBI) lipids in stored sediments and sediment extracts.

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ABSTRACT

The stability of various highly branched isoprenoid (HBI) lipids in sediments and sediment extracts stored under different conditions has been investigated over a period of about two years. Our data reveal an increased tendency for tri-unsaturated HBIs to undergo degradation compared to mono- and di-unsaturated counterparts, with sediments stored in plastic bags and exposed to light also showing a greater degradation extent than for samples stored in glass vials or those kept in the dark or at lower temperature. The composition of sediment extracts stored as dry material or as solutions, and at room temperature and in the fridge, exhibited no clear trends, but the extremely high variability in concentrations suggests that analysis of such stored extracts will likely lead to anomalous outcomes.

Keywords: highly branched isoprenoid; HBI; degradation; IP_{25}

1. Introduction

Highly branched isoprenoid (HBI) alkenes are unusual lipids made by a relatively small number of diatom genera (Volkman et al., 1994; Belt et al., 2000; Brown et al., 2014). The structures of numerous HBI alkenes have now been reported, with most common sedimentary forms containing a C_{25} skeleton (Fig. 1) with between one and three double bonds (DB) (e.g., Belt et al., 2000, 2007). Associations between certain HBIs and individual diatom species (or genera), together with some environmental control over HBI occurrence and distribution highlights their potential as environmental markers in paleo records. For example, the HBI monoene I (IP_{25}; Fig. 1) represents a useful biomarker proxy for seasonal sea ice conditions in the Arctic (Belt et al., 2007), a structurally similar HBI diene (II) has been proposed as a sea ice proxy for the Antarctic (Massé et al., 2011), while a HBI triene (III) has been associated with the retreating ice edge in both polar regions (Collins et al., 2013; Belt et al., 2015).

The quality of the outcomes from such studies relies on the accurate identification and quantification of individual HBIs, which has been made possible by the previous reporting of key chromatographic and mass spectral data (e.g., Johns et al., 1999; Belt et al., 2000; Brown et al., 2014). In a previous inter-laboratory investigation of IP_{25} in Arctic sediments, however, a number of analytical pitfalls were identified, which provided the basis for proposing a standard operating procedure for future studies (Belt et al., 2014). However, that study was conducted on carefully stored (-20°C) sediment material, with no consideration given to the potential impacts of variable storage conditions, either for sediments or sediment extracts. In practice, archived sediments are not

always stored under such well-controlled conditions, especially if their intended use did not require these, as might be the case for mineralogical analysis. Similarly, sediment extracts are often fractionated into sub-fractions containing analytes of immediate interest, while other sub-fractions might be stored for (potential) future use. This approach, however, raises further questions about longer-term analyte integrity. A few years ago, we believed that as the number of paleoenvironmental studies based on HBI lipids would likely grow, so would the importance of establishing the integrity of archived samples, either as sediments or sediment extracts. We therefore decided to investigate the long-term stability of various HBIs in sediments and extracts, aiming to replicate the most likely scenarios for storage conditions pertinent to each sample type. The outcomes of this investigation are presented here.

2. Experimental

2.1 Sediments and sediment extracts

Sediment material was collected from Barrow Strait in the Canadian Arctic Archipelago (Belt et al., 2007). Although we cannot account for any HBI degradation that may have occurred between the collection date and the start of the investigation, the sediment was stored at low temperature (-20°C) throughout, and frequent extraction of such material for analytical control purposes in other investigations (see Belt et al., 2015 for details) has shown no major indication of change in composition. Sediment was homogenised and sub-samples stored in glass vials and plastic bags, with different combinations of temperature and light/dark. Sub-samples were occasionally shaken by hand and analysed every 1-6 months for 24 months. Potential variability in analytical

procedures was monitored by co-extraction and analysis of further sediment sub-samples kept at low temperature (-20°C). At the beginning of the study, sub-samples of one extract were stored dry and in solution (hexane), each at room T and at ca. 5°C. Sediments were extracted using the method of Belt et al. (2015).

2.3 Analysis of extracts

All HBI-containing extracts were analysed using gas chromatography–mass spectrometry (GC–MS) in total ion current (TIC) and single ion monitoring modes with an Agilent 7890A 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., 2015). Identification of individual HBIs was based on characteristic chromatographic (RI) and mass spectral data (Johns et al., 1999; Belt et al., 2000, 2007). Quantification was achieved by dividing integrated GC–MS peak areas of individual HBIs by the corresponding area of the internal standard (9-octyl-heptadec-8-ene; 9-OHD), adjusting these ratios according to instrumental response factors, and normalising to the amount of sediment extracted (Belt et al., 2015). Temporal changes in the concentrations of all HBIs were calculated as percentage differences relative to values at the beginning of the investigation. We report mean values from duplicate determinations.

3. Results and discussion

The principal HBI biomarkers in the extracts obtained from stored sediments were identified as IP_{25}, diene II and trienes III and IV on the basis of their GC–MS characteristics (Johns et al., 1999; Belt et al., 2000, 2007). The concentration of IP_{25} in different samples showed some temporal variation,

although the majority of these were relatively small and represented both depletions and apparent enhancements (Fig. 1). We attribute such small changes (± 10%) to reflect a combination of the inherent analytical variability (typically ca. 7%; Belt et al., 2014) and the heterogeneous exposure of the sediments to the ambient conditions (temperature, air, container material). As such, we only consider values of greater than ca. 10% to be indicative of significant change. Based on this benchmark, IP_{25} concentration showed no major change when sediments were stored in glass vials at room temperature or 5°C and in the dark, although there was evidence for a small degree of degradation for samples stored in plastic bags (both temperatures) or in glass vials in the light. The most substantial changes, however, were observed for samples stored in plastic bags at room temperature and exposed to light, with depletions of up to ca. 40% (Fig. 1a). Similar observations were made for diene II (Fig. 1b), with a slightly higher depletion (compared to IP_{25}) for sediment in plastic bags left in the laboratory (i.e. room T, light). Depletions in trienes III and IV (Fig. 1c,d) were also most noticeable for samples within plastic bags kept in the open laboratory, but the impact of light exposure on these HBIs was also evident for samples stored in glass vials. Smaller losses of trienes III and IV could be observed for samples kept in the dark, although these were slightly larger for samples stored at room T, and generally greater than the changes seen for IP_{25} or diene II. The tendency for HBIs with three DB (i.e. III and IV) to undergo greater degradation compared to IP_{25} and diene II is especially clear from the 24-month data alone (Fig. 1e) and is also consistent with the previously demonstrated order of reactivity of these HBIs towards photo- and auto-oxidation reactions (Rontani et al., 2011, 2014).

The degradation state of the samples, more generally, could be observed through inspection of the TIC chromatograms, with the occurrence of unresolved complex mixtures for samples stored in plastic bags, in particular (Fig. 2).

The concentrations of all four HBIs in stored sediment extracts exhibited much greater variation compared to the sediments, with no clear trends identifiable for any individual HBI or storage method. In some cases, depletions of ca. 100% indicated near-complete degradation, while apparent enhancements of up to ca. 250% suggest greater degradation of the internal standard (9-OHD) relative to the HBIs. Potentially, this could have been investigated further through use of a more inert internal standard (e.g., a saturated alkane such as 7-hexylnonadecane often used in the quantification of IP_{25} (Belt et al., 2014)), but this was not included as part of the experimental protocol adopted in this study. All changes to HBI concentrations were, however, extremely inconsistent.

4. Conclusions

The stability of various HBIs in archived sediments and sediment extracts shows some dependence on storage conditions, with the extent of degradation also dependent on the degree of unsaturation of individual HBI lipids. Our data highlight the need to carefully consider the potential impacts of storage conditions on long-term HBI stability, with increased degradation of more unsaturated homologues in sediments, and all components in sediment extracts, identified as being especially important.

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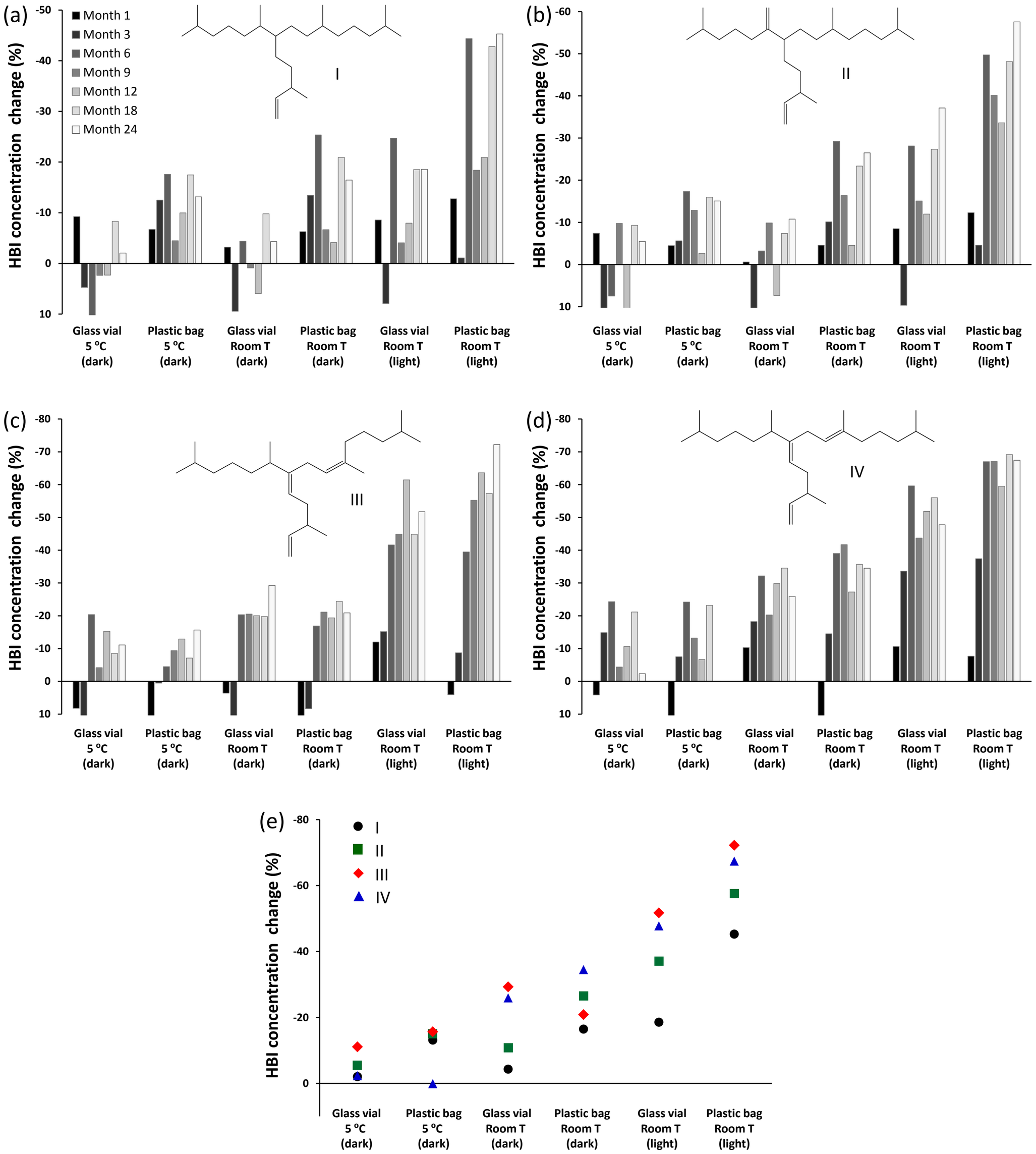
Figures

Figure 1. (a-d) Temporal changes (%) in concentration of individual HBI lipids in sediments stored under various conditions. (e) % change after 24 months. A general direction of ‘poorer’ storage conditions can be seen from left to right.

Figure 2. Partial TIC chromatograms of non-polar extracts obtained from sediments stored under various conditions for 24 months.

Figure



Figure

Figure 2: