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Structural identification of sedimentary C_{21} and C_{22} highly branched isoprenoid alkanes

Jaap S. Sinninghe Damsté [a,*], M. Baas [a], Jan A.J. Geenevasen [b], Fabien Kenig [c]

[a] *Department of Marine Biogeochemistry and Toxicology, Royal Netherlands Institute for Sea Research (NIOZ), P.O. Box 59, 1790 AB Den Burg, The Netherlands*
[b] *University of Amsterdam, van't Hoff Institute for Molecular Science, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands*
[c] *Department of Earth and Environmental Sciences, University of Illinois at Chicago, Chicago, IL 60607-7059, USA*

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Abstract

C_{21} and C_{22} highly branched isoprenoid (HBI) alkanes occurring in high relative abundance in lagoonal sediments of Abu Dhabi have been unambiguously identified as 2,6,10-trimethyl-7-(3-methylpentyl)dodecane and 3,7,11-trimethyl-6-(3-methylpentyl)tridecane, respectively, using NMR spectroscopy. A second C_{21} HBI isomer is tentatively identified as 3,7,11-trimethyl-6-(3-methylbutyl)tridecane, on the basis of comparison of its mass spectral fragmentation with those of fully identified HBIs. The structures of these three components are formed by extension of the "parent" C_{20} HBI alkane, first identified in Rozel Point Oil by Yon et al. [Yon, D.A., Maxwell, J.R., Ryback G., 1982. 2,6,10-trimethyl-7-(3-methylbutyl)dodecane, a novel sedimentary biological marker. Tetrahedron Letters 23, 2143–2146], by one or two carbon atom(s), respectively, at the terminal carbon atom of one or two of the T-branches. In contrast to the C_{20} HBI alkane present in the oil, ^{13}C NMR spectroscopy did not indicate the presence of diastereoisomers for the C_{21} and C_{22} HBIs in sediments, indicating stereospecific enzyme-controlled biosynthesis of these components by an unidentified biological source. The stable carbon isotopic compositions of the major C_{20}–C_{22} HBIs are identical, suggesting a common biological source.

1. Introduction

Highly branched isoprenoid (HBI) alkenes and alkanes were first identified in sediments and petroleum (Rowland and Robson, 1990; Yon et al., 1982). The C_{25} (**I**; see Fig. 1 for structures) and C_{30} (**II**) HBIs have subsequently been found in diatoms as alkenes with various numbers of double bonds (e.g., Volkman et al., 1994). Recently, it was established that these HBI alkenes occur in only two specific phylogenetic groups of the diatoms (Sinninghe Damsté et al., 2004). A phylogenetic cluster in the centric diatoms comprising only *Rhizosolenia* species produces both C_{25} and C_{30} HBI alkenes, whereas a distinct phylogenetic cluster in the pennate diatoms comprising *Navicula*, *Haslea*, *Pleurosigma* and *Gyrosigma* species are able to biosynthesize C_{25} HBI alkenes. Since Massé et al. (2004) established that the biosynthetic pathway for HBI alkenes in

* Corresponding author. Tel.: +31 222 369550; fax: +31 222 319674.
E-mail address: damste@nioz.nl (J.S. Sinninghe Damsté).

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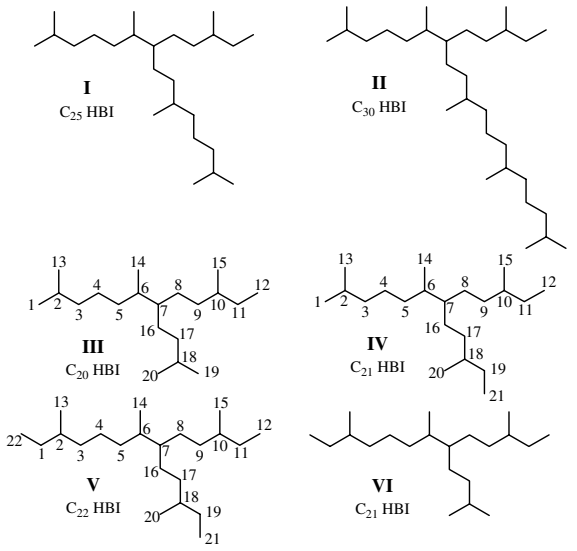

Fig. 1. Structures of HBI alkanes listed in the text. The carbon numbering of the C_{20}–C_{22} HBIs is indicated.

representatives of these phylogenetic clades is fundamentally different, it was concluded that the HBI biosynthetic capability must have evolved independently in the centric and pennate diatoms (Sinninghe Damsté et al., 2004).

Less advanced is our knowledge concerning the C_{20} HBI (**III**), whose structure was established via synthesis after its isolation from Rozel Point Oil (Yon et al., 1982). Subsequently, this component has also been encountered in immature sediments (e.g., Dunlop and Jefferies, 1985; Kenig et al., 1990; Kohnen et al., 1992; Schouten et al., 1997; Smittenberg et al., 2004). Rowland et al. (1985) reported the green alga *Enteromorpha prolifera* as a possible biological source but its presence may have been caused by co-occurring epiphytic algae as indicated by the authors. Since the C_{20} HBI alkane is often observed in sediments with depositional environments characterized by nutrient-rich water column conditions (e.g., Monterey Fm., Schouten et al., 1997; nutrient-rich Norwegian fjord, Smittenberg et al., 2004) and because of the structural resemblance to the C_{25} and C_{30} HBIs, diatoms are still considered to be the most likely biological source for the C_{20} HBI. However, in a study of >120 different marine diatom cultures we have not yet been able to identify the C_{20} HBI alkane or derivatives thereof (Sinninghe Damsté et al., unpublished results). Therefore, its biological source remains enigmatic.

In addition to HBIs comprising a full number of isoprenoid units, sedimentary HBIs with one or two additional carbon atoms have also been found. Dunlop and Jefferies (1985) reported, in addition to the C_{20} HBI alkane (**III**), the tentative identification of C_{21} (**IV**) and C_{22} HBI (**V**) alkanes in lagoonal sediments from Shark Bay (Australia). The same compounds were observed in modern and Holocene lagoonal sediments and microbial mats from Abu Dhabi (UAE; Kenig et al., 1990, 1995). Additionally, Kenig (1991) tentatively identified a second C_{21} HBI isomer (**VI**) in Abu Dhabi lagoonal sediments. Rospondek et al. (1997) and Schefuss et al. (2001) reported C_{26} HBIs in sediments from the Oligocene Menilite Formation (Poland) and Mid-Pleistocene sediments from the upwelling cell in Lüderitz Bay (South Atlantic Ocean, ODP Site 1084), respectively. Again, the biological source(s) for these methylated HBIs are unknown.

In this paper, we report the unambiguous identification of the predominant C_{21} and C_{22} HBI alkanes occurring in the lagoonal sediments from Abu Dhabi following isolation and analysis by NMR spectroscopy. This reveals that the methylation of the C_{20} HBI skeleton occurs at a position different from the one tentatively reported for the C_{25} HBI.

2. Materials and methods

2.1. Extraction and fractionation

Sediment samples from the Abu Dhabi lagoonal system were freeze dried and extracted as described previously (Kenig et al., 1990). Fractions containing the saturated hydrocarbons were obtained using column chromatography following the procedure described in Simons and Kenig (2001).

2.2. Isolation of HBI alkanes

The C_{21} and C_{22} HBI alkanes were isolated from various saturated hydrocarbon fractions using preparative capillary gas chromatography (PCGC) with a low-bleed fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil 5 CB (film thickness 0.52 μm) mounted in a Hewlett–Packard 6890 gas chromatograph in conjunction with a Gerstel preparative fraction collector, similar to that described by Eglinton et al. (1996). The isolated C_{21} and C_{22} HBI alkanes from the various fractions were combined, weighed, and analysed using GC and GC–mass spectrometry (MS) for purity.

2.3. GC and GC–MS

GC was performed using a Fisons Instruments GC 8000 series and a Hewlett–Packard 6890 instrument, both equipped with an on-column injector. A fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil 5 CB (film thickness 0.12 μm) was used with helium as carrier gas. The samples were injected at 70 °C and the oven was programmed to 130 °C at 20 °C/min and then at 4

°C/min to 320 °C, at which it was held for 20 min. GC–MS was performed with a Hewlett–Packard 5890 gas chromatograph interfaced with a VG Autospec Ultima mass spectrometer operating at 70 eV, with a mass range of *m/z* 50–800 and a cycle time of 1.7 s (resolution 1000). The gas chromatograph was equipped with an on-column injection system and the same capillary column as described for GC. The carrier gas was helium. The temperature programme was the same as described for GC.

2.4. Isotopic analysis

The isotopic composition of individual compounds was obtained using GC-combustion-isotope ratio monitoring mass spectrometry following the method described in Kenig et al. (1994).

2.5. NMR analysis

NMR spectroscopy was performed using a Varian Unity Inova 500 spectrometer equipped with an SWBB probe. All experiments were recorded at 300 K in $CDCl_3$. Proton and carbon chemical shifts were referenced to internal $CDCl_3$ (7.24/77.0 ppm).

3. Results and discussion

A previous investigation (Kenig et al., 1990) of the Abu Dhabi lagoonal sediments revealed that the saturated hydrocarbon fraction of those containing seagrass remains was dominated by the C_{20} HBI and two pseudo-homologues, tentatively identified as C_{21} and C_{22} HBIs (cf. Dunlop and Jefferies, 1985). A representative gas chromatogram is given in Fig. 2 and the mass spectra of the components are given in Fig. 3(a) and (b).

In an attempt to confirm the tentative identifications of Dunlop and Jefferies (1985), we isolated the C_{21} and C_{22} HBIs by preparative GC in amounts (ca. 0.3 mg) sufficient for NMR analysis. The purity of the isolated HBIs was >95% as indicated by GC analysis. Analysis using ^1H NMR indicated the presence of seven methyl groups in both components (Table 1), in line with the tentative assignment as HBI components by Dunlop and Jefferies (1985).

Full structural identification was obtained with ^{13}C NMR using an attached proton test (APT) and an distortionless enhancement by polarization transfer [DEPT(90)] experiment to reveal the multiplicity of the carbon atoms (Table 1). These data were compared with those reported for the C_{20} HBI alkane (Yon et al., 1982; Robson, 1987; Table 1). The APT spectrum of the C_{21} HBI alkane **IV** revealed signals for C-1 to C-15 almost identical to those reported for the C_{20} HBI alkane (Table 1). The carbon signals of the third chain (C-16 to C-20) were, however, quite different. They exhibited similarities to those of the 3-methylpentyl chain (C-8 to C-12, C-15) of the C_{20} HBI alkane (Table 1). The characteristic signals of the isopropyl groups were also less pronounced than in the ^1H and ^{13}C NMR spectrum

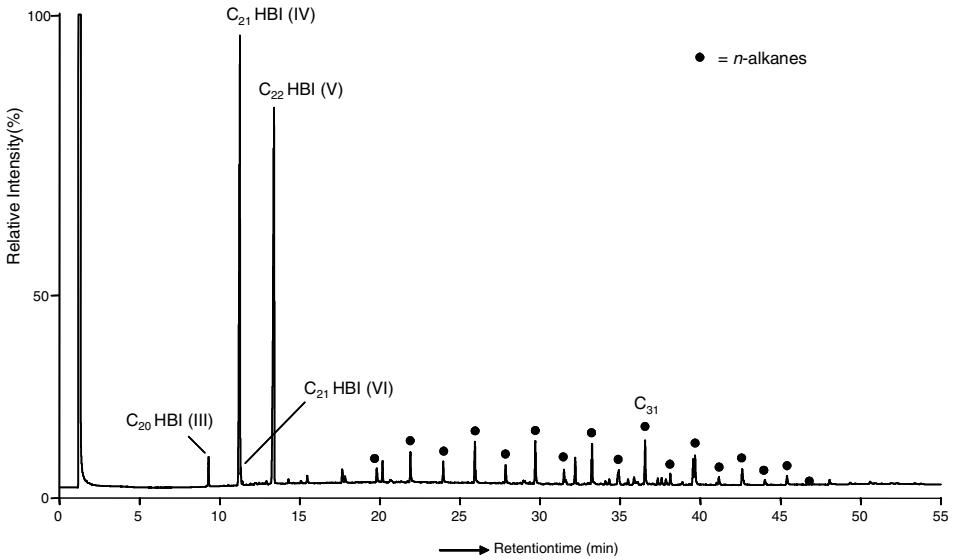

Fig. 2. Gas chromatogram of saturated hydrocarbon fraction of a representative lagoonal sediment sample from Abu Dhabi (sample # 89684). The C_{20}–C_{22} HBI alkanes (**III**–**VI**) and the homologous series of *n*-alkanes are indicated. The sample was collected along the Channel of Mussafah (Site I, 3.2 km), 1.4 m below the Sabkha surface, and is a Holocene lagoonal mud containing sea grass remains (Kenig, 1991), with a TOC of 0.6% and a Rock Eval hydrogen index of 435 mg hydrocarbons/g TOC.

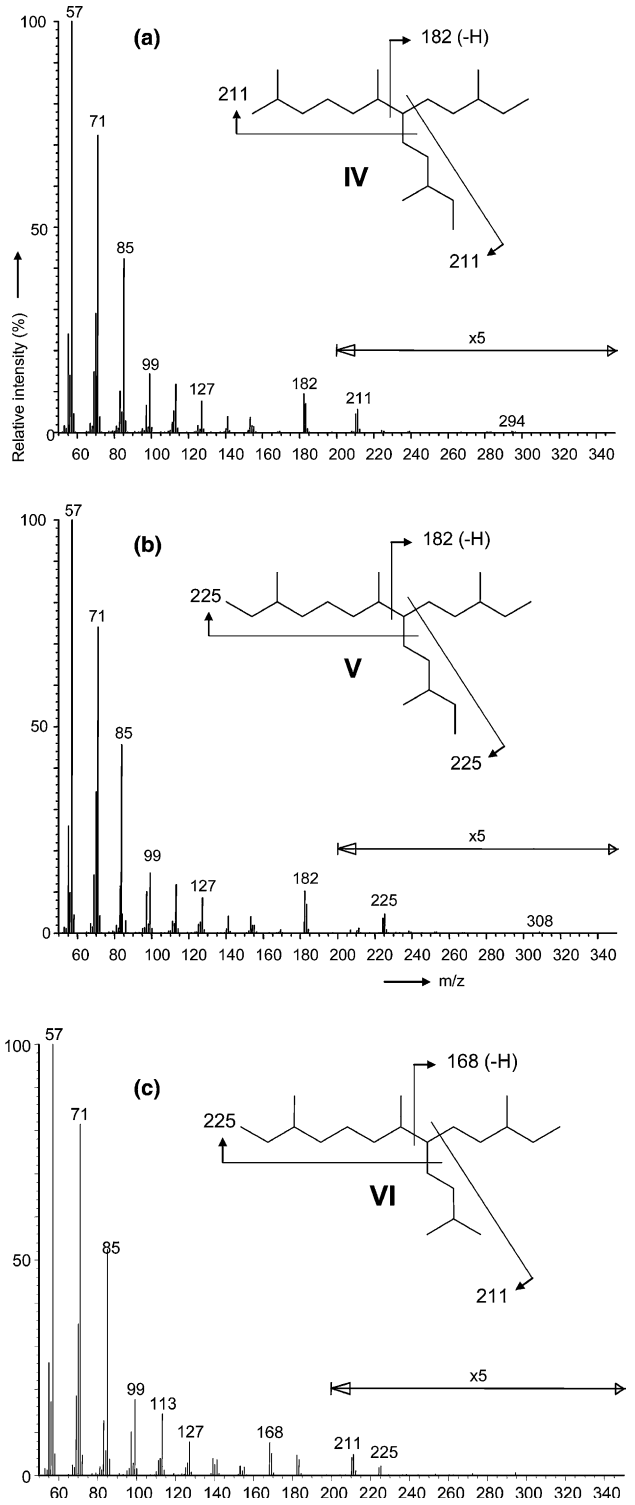

Fig. 3. Mass spectra (subtracted for background) of (a) C_{21} HBI alkane (**IV**), (b) C_{22} HBI alkane (**V**) and (c) tentatively identified C_{21} HBI alkane (**VI**). Putative mass spectral fragmentation patterns are indicated. Note that the spectrum of the C_{21} HBI alkane **VI** is not pure (e.g., the ion at *m/z* 182) since it elutes on the tail of component **IV**.

Table 1
^{13}C and selected ^{1}H NMR data for the C_{20}–C_{22} HBI alkanes

Carbon number[a]	^{13}C, δ (ppm)[b]			^{1}H, δ (ppm)	
	C_{20} HBI[c]	C_{21} HBI (**IV**)	C_{22} HBI (**V**)	C_{21} HBI (**IV**)	C_{22} HBI (**V**)
1	22.53[d] (p)	22.63[d] (p)	29.55 (s)	0.888 (3H, d, J = 6.6 Hz)	
2	27.87[e] (t)	27.96 (t)	34.34 (t)		
3	39.31 (s)	39.38 (s)	36.91 (s)		
4	25.45 (s)	25.54 (s)	25.20 (s)		
5	34.13[f] (s)	34.01 (s)	34.05 (s)		
6	34.77[f] (t)	34.92 (t)	34.91 (t)		
7	42.86[f] (t)	43.15 (t)	43.18 (t)		
8	27.38[g] (s)	27.33 (s)	27.33 (s)		
9	34.88[g] (s)	34.89[h] (s)	34.89[h] (s)		
10	34.34[f] (t)	34.39 (t)	34.38 (t)		
11	29.41[f] (s)	29.37[i] (s)	29.36[i] (s)		
12	11.28 (p)	11.40 (p)	11.39 (p)	0.878 (3H, t, J = 7.4 Hz)	0.878 (3H, t, J = 7.2 Hz)
13	22.66[d,f] (p)	22.71[d] (p)	19.21[j] (p)	0.888 (3H, d, J = 6.6 Hz)	0.865[k] (3H, d, J = 6.3 Hz)
14	15.50[g] (p)	15.83 (p)	15.81 (p)	0.798 (3H, d, J = 7.1 Hz)	0.798 (3H, d, J = 6.9 Hz)
15	19.23[f] (p)	19.38 (p)	19.37[j] (p)	0.871 (3H, d, J = 6.8 Hz)	0.871[k] (3H, d, J = 6.7 Hz)
16	28.67[f] (s)	28.52 (s)	28.51 (s)		
17	37.33[g] (s)	35.04[h] (s)	35.03[h] (s)		
18	28.36[e,g] (t)	34.82 (t)	34.81 (t)		
19	22.53[f] (s)	29.41[i] (s)	29.40[i] (s)		
20	22.66[d,f] (p)	19.38 (p)	19.24[j] (p)	0.871 (3H, d, J = 6.8 Hz)	0.871[k] (3H, d, J = 6.7 Hz)
21	n.a.	11.40 (p)	11.39 (p)	0.878 (3H, t, J = 7.4 Hz)	0.878 (3H, t, J = 7.2 Hz)
22	n.a.	n.a.	11.42 (p)		0.878 (3H, t, J = 7.2 Hz)

[a] Carbon numbering is indicated in Fig. 1.
[b] ^{13}C NMR spectra were measured in $CDCl_3$; p = primary carbon atom, s = secondary carbon atom, t = tertiary carbon atom; these assignments are based on APT (C_{20} HBI) or APT and DEPT(90) spectra.
[c] Originally reported by Yon et al. (1982), also reported by Robson (1987). The latter data are listed here and represent, where indicated, averages of 2 or 4 signals in cases of diastereoisomeric splitting.
[d] Assignments may be interchanged.
[e] Assignments have been interchanged with those reported by Robson (1987) since the signal at 27.38 ppm is not observed in the spectra of the C_{21} and C_{22} HBIs.
[f] Split into two signals.
[g] Split into four signals.
[h–k] Assignments may be interchanged.

of the C_{20} HBI alkane. This clearly established the identity of the C_{21} HBI alkane as 2,6,10-trimethyl-7-(3-methylpentyl)dodecane (**IV**), which can be thought of as a derivative of the C_{20} HBI alkane by the addition of a methyl group at C-19 (or C-20) of the C_{20} HBI alkane.

The APT spectrum of the C_{22} HBI alkane **V** shared similarities with that of the C_{21} HBI alkane **IV**; the signals of carbon atoms C-5 to C-21, with the exception of C-13, were more or less identical to those of the C_{21} HBI alkane (Table 1). The signals of C-1 to C-4 and C-13 were rather different but again showed similarities to those of the carbon atoms of the 3-methylpentyl chain(s) of the C_{20} and C_{21} HBI alkanes. This indicates that the additional carbon atom (C-22) is at C-1 of the C_{21} HBI alkane, thus confirming the structure of this component as 3,7,11-trimethyl-6-(3-methylpentyl)tridecane (**V**).

Both structural assignments are in accordance with the tentative assignments of Dunlop and Jefferies (1985) and consistent with the mass spectral fragmentation patterns indicated in Fig. 3. The mass spectra of the C_{21} and C_{22} HBI alkane (Fig. 3a and b) both show that the characteristic fragment ion of the C_{20} HBI alkane at *m/z* 168 (Yon et al., 1982) due to cleavage of the bond between the tertiary carbon atoms C-6 and C-7 with concomitant hydrogen transfer has shifted to *m/z* 182, consistent with an additional methyl at C-19. The other two characteristic cleavages around C-7 are less intense because they relate to the cleavage of a bond between a tertiary and a secondary carbon atom but provide confirmation of the position of the second additional carbon atom in the C_{22} alkane at C-1.

A second C_{21} HBI alkane isomer was tentatively identified by Kenig (1991) as 3,7,11-trimethyl-6-(3-methylbutyl)tridecane (**VI**). This compound, which elutes just after **IV**, was present in too low a concentration (Fig. 1) to be isolated for analysis by NMR spectroscopy. However, its mass spectral fragmentation pattern (Fig. 3c) is

Table 2
Sample description and the stable carbon isotopic compositions of total organic carbon (TOC), and the C_{20}, C_{21} and C_{22} HBI alkanes

Sample number[a]	Description[a]	$\delta^{13}C$ (‰)[b]			
		TOC	C_{20} HBI	C_{21} HBI	C_{22} HBI
73061	Microbial mat (Holocene)	−12.3	−11.1 ± 0.7 (n = 2)	−9.7 ± 0.4 (n = 2)	−10.8 ± 0.3 (n = 2)
73184	Lagoonal aragonitic mud (Holocene)	n.d.	−10.7 ± 0.8 (n = 2)	−11.9 ± 0.5 (n = 2)	−12.7 ± 0.6 (n = 2)
73039	Lagoonal aragonitic mud (Holocene)	−12.2	−11.9 ± 0.1[c] (n = 3)	−9.2 ± 0.1 (n = 3)	−9.8 ± 0.5 (n = 3)

[a] See Kenig et al. (1990) and Kenig (1991) for exact sample description and location.
[b] Average carbon isotopic composition (‰ vs. VPDB) and standard deviation of replicate analyses. Numbers in parenthesis indicate the number of replicate analyses.
[c] Partial co-elution with pristane affects accuracy.

consistent with the fragmentation pattern of the fully identified HBI alkane and clearly indicates the addition of a methyl group at C-1 of the C_{20} HBI alkane (**III**).

Comparison of the ^{13}C NMR shifts of the C_{21} and C_{22} HBI alkanes with those reported for the C_{20} HBI alkane (Table 1) indicated that only one signal per carbon atom was observed for the C_{21} and C_{22} HBI alkanes, whereas for the C_{20} HBI alkane isolated from the Rozel Point Oil up to four signals per carbon atom were reported (Robson, 1987; Table 1), ascribed to the presence of diastereoisomers. The C_{20} HBI alkane contains three chiral centres and thus can exist in eight diastereoisomeric forms and hence four enatiomeric pairs, explaining the presence of up to four signals for each carbon atom. The fact that we only observe one signal per carbon atom in the ^{13}C NMR spectra of the stereochemically even more complex C_{21} and C_{22} HBI alkanes (with 3 and 4 chiral centres, respectively) makes it plausible that the chiral centres in these components have a fixed (although as yet not established) stereochemistry. This points to direct biosynthesis of the C_{21} and C_{22} HBI alkanes since reduction of corresponding HBI alkenes in the sediment would most likely result in isomerization at chiral centres, although biohydrogenation could result in stereoselective reduction of double bonds. In agreement with this, unsaturated C_{21} and C_{21} HBI alkenes are present in the sediments in either trace amounts relative to the saturated HBIs or they are absent, respectively. Direct biosynthesis by an as yet unidentified organism is thus the likely pathway of formation for the C_{21} and C_{22} HBI alkanes.

The stable carbon isotopic compositions of the C_{20}–C_{22} HBI alkanes in three sediments from the Abu Dhabi lagoon are reported in Table 2. These data reveal that (i) the HBI alkanes are enriched in ^{13}C relative to bulk organic matter and many other biomarkers in the sediments (Kenig et al., 1994) and (ii), more importantly, that the C_{20}–C_{22} HBI alkanes have barely distinguishable carbon isotopic values. This latter point indicates that it is likely that the C_{20} to C_{22} HBI alkanes derive from the same biological source, which is also strongly supported by their close structural relationship. Apparently, methylation of the parent C_{20} HBI alkane skeleton fulfils a specific physiological role but we need to know the source organism of these components before this question can be addressed further.

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