ChemComm

Important sedimentary sesterterpenoids from the diatom *Pleurosigma intermedium*

Simon T. Belt,*[a] Guy Allard,[a] Guillaume Massé,[ab] Jean-Michel Robert[b] and Steve Rowland[a]

[a] *Petroleum and Environmental Geochemistry Group, Department of Environmental Sciences, University of Plymouth, Drake Circus, Plymouth, UK PL4 8AA. E-mail: sbelt@plymouth.ac.uk*

[b] *ISOMer, Faculté des Sciences et des Techniques, Université de Nantes, 2 Rue de la Houssinière, 44027 Nantes Cedex 3, France*

Received (in Liverpool, UK) 8th December 1999, Accepted 16th February 2000

The new sesterterpenoids (5*E*,8*E*/*Z*)-3,9,13-trimethyl-6-(1,5-dimethylhex-4-enyl)tetradeca-1,5,8,12-tetraene have been isolated from the diatom *Pleurosigma intermedium* and characterised structurally by NMR spectroscopy.

Highly branched isoprenoid (HBI) C_{25} and C_{30} hydrocarbons are ubiquitous chemicals found in environmental matrices ranging from recent sediments to ancient oils.[1] The parent structures of these geochemicals (**1** and **2**, Fig. 1) were established by synthesis[1,2] during the 1980s, but it was not until 1994, that Volkman *et al.*[3] reported their occurrence in the diatoms *Haslea ostrearia* (C_{25}) and *Rhizosolenia setigera* (C_{30}) thus revealing the only known source organisms for these compounds. In our own studies, we have reported on the structures of the compounds in *H. ostrearia*, together with descriptions of some of the controls governing their unsaturation.[4–7] The most common HBIs possess between two and six double bonds, though pentaenes and hexaenes are relatively rare in *H. ostrearia*[5] (*e.g.* structures **3** and **4**, Fig. 1). In a recent report, *Rhizosolenia setigera* was shown to produce a C_{25} HBI pentaene rather than C_{30} compounds,[8] but to date, no further species or genus of diatoms have been shown to produce this class of widespread organic geochemicals. In terms of stereochemistry, HBIs exist as single geometric isomers in all cases (*E* where appropriate), though they are often reported as mixtures of configurational diastereoisomers.[4,9,10]

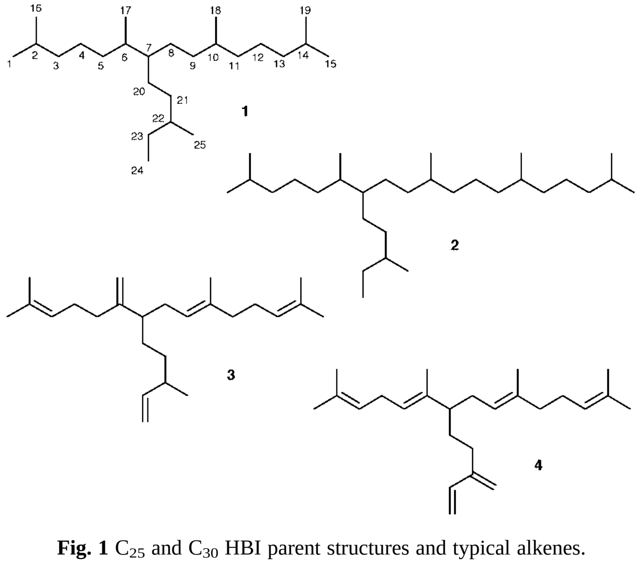

Fig. 1 C_{25} and C_{30} HBI parent structures and typical alkenes.

Here, we report the identification of an entirely different genus of diatom which biosynthesises C_{25} HBIs of a previously unreported structural type, which nonetheless appears to be common in sediments.

Pleurosigma intermedium is a benthic diatom measuring *ca.* 130 × 20 μm that is commonly found in estuarine muds. After large scale (400 L) culture of this species (Bay of Bourgneuf, France) followed by harvesting, centrifugation and freeze drying, we isolated a lipid fraction by extraction with hexane. Subsequent saponification (to remove triglyceride esters of fatty acids) and extraction (hexane) yielded a hydrocarbon fraction that was analysed by GC and GC–MS. Analysis of these chromatograms revealed the presence of n-$C_{21:5}$, n-$C_{21:6}$, squalene and eight compounds possessing related but different properties to previously reported HBIs.[4,5,7] Thus, hydrogenation of this mixture resulted in the formation of a single compound having identical GC–MS characteristics[1] to that of the authentic C_{25} alkane **1**. Following purification by column chromatography (SiO_2/hexane) and re-examination by GC–MS, the two major components (> 55%) of the hydrocarbon fraction were assigned as new HBI pentaenes ($C_{25:5}$, M^+ 342, RI 2126,2172 $_{\text{HP-5}}$; 2112,2159 $_{\text{HP-1}}$, relative abundance 1.6 : 1). The mass spectra of the two compounds were virtually identical, suggesting that that they were stereoisomers rather than positional isomers. Examination of the ^1H and ^{13}C NMR spectra† revealed the presence of four trisubstituted alkene moieties, together with a vinyl group, a structural feature common to all known HBIs. For all of these previously reported HBIs, the main branch point is at C-7, probably as the result of a biosynthetic coupling of geranyl and farnesyl type precursors.[9] However, for these new sesterterpenoids isolated from *Pleurosigma intermedium*, C-7 is unsaturated with a double bond between C-7 and C-20 (note: the numbering scheme for NMR assignments is shown for parent alkane **1**). The determination of this double bond position (C7–C20) and of the other trisubstituted double bonds was established using 2-D NMR methods (COSY, HMQC, HMBC) resulting in structures **5** and **6** (Fig. 2), namely 3,9,13-trimethyl-6-(1,5-dimethylhex-4-enyl)tetradeca-1,5,8,12-tetraene. Of particular note is the absence of any ^{13}C resonances in the 40–50 ppm region indicative of saturated, branched positions (C-7) observed for all previously reported HBIs.[4–10] Instead, alkenic C-7 resonates at δ 142.8 and 142.4 for **6** and **5**, respectively. To date, we have not been successful in separating the two $C_{25:5}$ isomers using further chromatography including argentation TLC. However, both spectroscopic (^{13}C NMR) and chromatographic (GC) separations of these compounds are most consistent with the presence of two geometric isomers (C9–C10). The mixed double bond stereochemistry of C9–C10 (and not C7–C20) was determined by careful examination of the NMR data. Specifi-

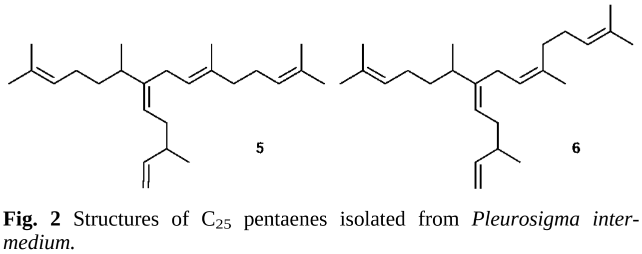

Fig. 2 Structures of C_{25} pentaenes isolated from *Pleurosigma intermedium.*

DOI: 10.1039/a909670a

cally, unique ^{1}H and ^{13}C (CH_3) resonances at δ 1.55, 1.69 (H-18) and δ 15.8, 23.4 (C-18) were observed for **5** and **6** (with **6** being the major isomer). Two further ^{13}C resonances for C-11, each of which correlated with the corresponding H-18 protons in the HMBC spectrum were also observed. Since the alternative position for geometric isomerism (C7–C20) does not possess a CH_3 substituent, the position of *E/Z* isomerism is limited to C9–C10. In order to determine the stereochemistry of the C7–C20 double bond, NOE data were obtained for **5** and **6**. Significantly, NOEs were observed between H-6 and H-20, indicating an *E* configuration. The remaining six HBIs consisted of two trienes ($C_{25:3}$) and four tetraenes ($C_{25:4}$) on the basis of their mass spectra (M^+ 346 and 344, respectively), though insufficient quantities of these compounds were present in the culture to allow for comprehensive NMR analysis.

HBIs in general have been proposed as potential biomarkers or palaeoenvironmental indicators.[11] However, to date, the environmental record of these compounds does not correlate well with those compounds structurally characterised from previous large scale cultures of diatoms including *Haslea ostrearia* and *Rhizosolenia setigera*.[4–10] The reason for this may be that HBIs from these two species undergo relatively mild diagenetic reactions (*e.g.* alkene isomerisation) but since the products of laboratory simulations of these processes do not produce the sedimentary isomers,[12] a more attractive explanation is that other diatoms such as *Pleurosigma intermedium* are the producers of many of the common sedimentary HBIs. To address this more fully, we now intend to isolate and characterise all of the HBIs produced by *Pleurosigma intermedium* under different culture conditions and to compare the mass spectra and chromatographic data (retention indices) of these with the numerous geochemical reports.[13] A preliminary investigation of this type indicates that two of the C_{25} pentaenes (RI 2124, 2169 $_{\text{DB-5}}$) reported from the Todos os Santos Bay, Brazil,[14] are HBIs **5** and **6**.

We would like to acknowledge the British Council (ALLIANCE program) and the University of Plymouth for funding (G. A. and G. M.).

Notes and references

† Selected NMR data for **5** and **6** at 270 and 400 MHz for ^{1}H in $CDCl_3$. (numbering shown for parent alkane **1**). ^{1}H, COSY: δ 5.74 (ddd, 1H, *J* 17.5, 10.0, 7.0 Hz, H-23), 5.10 (m, 4H, H-3,9,13,20), 4.93 (m, 2H, H-24), 2.58 (m, 3H, H-6,8), 2.01 (m, 9H, H-4,11,12,21,22), 1.69 (s, 3H, H-18, **6**), 1.65 (s, 6H, H-1,15), 1.58 (s, 3H, H-16/19), 1.55 (s, 6H, H-16/19,18, **5**), 0.95 (d, 3H, *J* 6.5 Hz, H-25), 0.94, 0.93 (2 × d, 3H, *J* 6.5 Hz, H-17).

^{13}C, DEPT, HMQC, HMBC: δ 144.5 (C-23), 142.8 (C-7, **6**), 142.4 (C-7, **5**), 135.6 (C-10), 131.4, 131.2, 131.1 (C-2,14), 124.9, 124.4, 123.9, 123.3, 123.0, 122.9, (C-3,9,13,20), 112.1 (C-24), 39.8 (C-11, **5**), 38.2 (C-22), 35.2 (C-5), 34.4 (C-21), 33.9 (C-6), 31.8 (C-11, **6**) 29.2 (C-8, **5**), 28.9 (C-8, **6**), 26.7, 26.6, 26.4 (C-4,12), 25.7 (C-1,15), 23.4 (C-18, **6**), 19.5 (C-25), 19.4 (C-17), 17.6 (C-16,19), 15.8 (C-18, **5**).

1 J. N. Robson and S. J. Rowland, *Nature*, 1986, **324**, 561.
2 J. N. Robson and S. J. Rowland, *Tetrahedron Lett.*, 1988, **29**, 3837.
3 J. K. Volkman, S. M. Barratt and G. A. Dunstan, *Org. Geochem.*, 1994, **21**, 407.
4 S. T. Belt, D. A. Cooke, J.-M. Robert and S. J. Rowland, *Tetrahedron Lett.*, 1996, **37**, 4755.
5 E. J. Wraige, S. T. Belt, C. A. Lewis, D. A. Cooke, J.- M. Robert, G. Massé and S. J. Rowland, *Org. Geochem.*, 1997, **27**, 497.
6 E. J. Wraige, S. T. Belt, L Johns, G. Massé, J.-M. Robert and S. J. Rowland, *Org. Geochem.*, 1998, **28**, 855.
7 E. J. Wraige, S. T. Belt, L. Johns, G. Massé, J.-M. Robert and S. J. Rowland., *Phytochemistry*, 1999, **15**, 69.
8 J. S. Sinninghe Damsté, W. I. C. Rijpstra, S. Schouten, H. Peletier, M. J. E. C. van der Maarel and W. W. C. Gieskes, *Org. Geochem.*, 1999, **30**, 95.
9 L. Johns, E. J. Wraige, S. T. Belt, C. A. Lewis, G. Massé, J.-M. Robert and S. J. Rowland, *Org. Geochem.*, 1999, **30**, 1471.
10 L. Johns, S. T. Belt, C. A. Lewis, S. J. Rowland, G. Massé, J.-M. Robert and W. König, *Phytochemistry*, 2000, **53**, 607.
11 D. A. Yon, G. Ryback and J. R. Maxwell, *Tetrahedron Lett.*, 1982, **23**, 2143.
12 S. T. Belt, G. Allard, J. Rintatalo, L. Johns, A. C. T. van Duin and S. J Rowland, *Geochim. Cosmochim. Acta*, 1999, submitted.
13 S. J. Rowland and J. N. Robson , *Mar. Environ. Res.*, 1990, **30**, 191.
14 C. Porte, D. Barceló, T. M. Tavares, V. C. Rocha and J. Albaigés, *Arch. Environ. Contam. Toxicol.*, 1990, **19**, 263.

Communication a909670a