Pergamon

Tetrahedron Letters 42 (2001) 5583–5585

TETRAHEDRON LETTERS

Structural characterisation of C_{30} highly branched isoprenoid alkenes (rhizenes) in the marine diatom *Rhizosolenia setigera*

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

[a]*Petroleum and Environmental Geochemistry Group, Department of Environmental Sciences, University of Plymouth, Drake Circus, Plymouth PL4 8AA, Devon, UK*

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

Received 17 April 2001; accepted 14 June 2001

Abstract—Highly branched isoprenoid C_{30} penta- and hexaenes have been isolated from the marine diatom *Rhizosolenia setigera* and characterised by NMR spectroscopy.

C_{20}, C_{25} and C_{30} highly branched isoprenoid (HBI) alkenes are widely occurring secondary metabolites that are routinely found in a range of geochemical settings ranging from recent sediments to ancient oils (see Fig. 1 for parent structures).[1] In a number of recent reports, we and others have reported on source organisms for the C_{25} and C_{30} alkenes, though to date, a primary producer for the C_{20} analogues remains elusive.[2–5] Structures of various C_{25} HBIs have been determined using NMR spectroscopy following large scale culture of the source diatoms *Haslea ostrearia* (and related species),[6–12] *Pleurosigma intermedium*[4,5] and *Rhizosolenia setigera*.[3] These compounds exist in a number of isomeric forms exhibiting both geometric and configurational isomerism.[4–6,10,11] In addition, their degrees of unsaturation range from two to six for alkenes isolated from diatoms, while monoenes and the saturated hydrocarbons also exist in sediments and oils.[1] However, there have been no reports on the structures of the pseudo-homologous C_{30} compounds (rhizenes) other than their unsaturation.[2] Here, we describe the structural elucidation of C_{30} HBI penta- and hexaenes ($C_{30:5}$ and $C_{30:6}$) following large scale culture of the marine diatom *R. setigera* (Fig. 2).

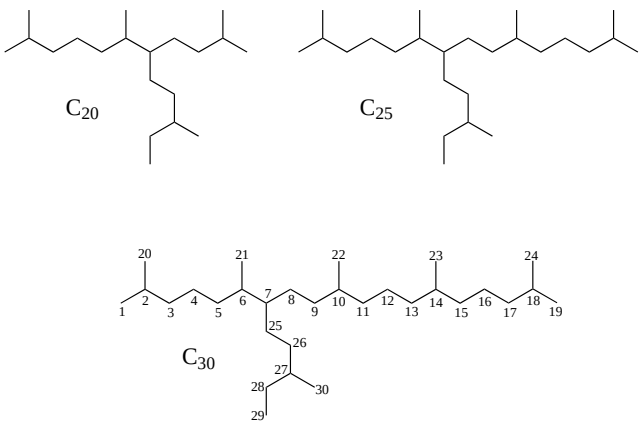

Figure 1. Parent carbon skeletons of C_{20}, C_{25} and C_{30} highly branched isoprenoids.

R. setigera was isolated from Le Croisic, France and cultured in 4×60 L tanks containing underground saltwater enriched with $NaNO_3$ (8 mg ml^{-1}) and Guillard's medium[13] (f/2, 0.2 ml l^{-1}) at 16–18°C. After centrifuging, the concentrated biomass was freeze-dried and extracted with hexane to yield a non-polar fraction. This was then saponified with KOH/MeOH to remove triglyceride esters and then re-extracted into hexane. Analysis of this non-saponifiable lipid fraction by GC and GC–MS revealed the presence of heneicosa-3,6,9,12,15,18-hexaene (n-$C_{21:6}$) together with two pairs of compounds whose chromatographic (retention indices[14]) and mass spectral properties (M^+ 412, 410) were consistent with C_{30} rhizenes possessing five and six double bonds. Separation of these components was achieved using column chromatography (SiO_2/hexane) to yield two $C_{30:5}$ (21 mg) and two $C_{30:6}$ (10 mg) alkenes.

Keywords: highly branched isoprenoids; alkenes; diatoms; *Rhizosolenia setigera*; NMR spectroscopy; rhizenes.

* Corresponding author. Tel.: +44 (0)1752 233042; fax: +44 (0)1752 233035; e-mail: sbelt@plymouth.ac.uk

0040-4039/01/$ - see front matter
PII: S0040-4039(01)01063-2

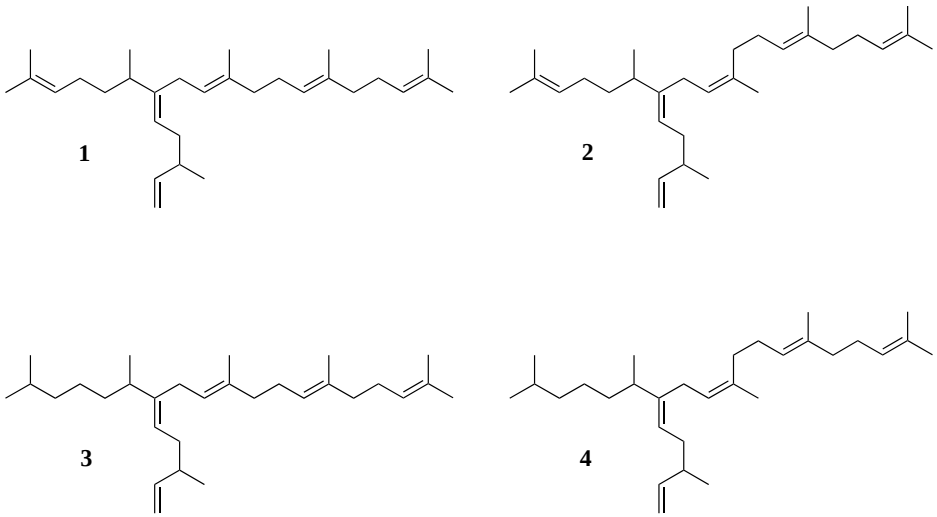

Figure 2. Structures of C_{30} penta- and hexaenes isolated from *R. setigera*.

Examination of the ^{1}H, ^{13}C and DEPT NMR spectra of the two $C_{30:6}$ HBI alkenes revealed the presence of a vinyl moiety and five tri-substituted double bonds. Two of the tri-substituted double bonds could be located at the terminal positions of the main alkyl chain as resonances due to isopropyl groups were absent. A further double bond could be located at the C-7 branch point (see Fig. 1 for numbering scheme) due to characteristic ^{13}C resonances for this quaternary carbon (δ 142.6 and 142.3 ppm) and the absence of any peak at ca. 45–50 ppm, which is diagnostic when HBI alkene isomers are saturated at this position.[3–12] The remaining two tri-substituted double bonds were positioned at C9–C10 and C13–C14 since the H-8 protons (and only H-8) were found to be di-allylic (δ 2.6 ppm). No evidence could be found for the presence of positional isomers, a feature which is commonly observed for C_{25} HBI alkenes from *Haslea* sp.[6–12] However, the presence of two hexaenes could be explained in terms of geometric (and not configurational) isomerism. Thus, unique ^{1}H and ^{13}C resonances were detected for the methyl group at C-22,[14] indicating that the geometric isomerism exists at C9–C10 in an analogous manner to that observed for the pseudo-homologous C_{25} tri-, tetra- and pentaenes isolated from *P. intermedium*.[4,5] Pairs of resonances with significant differences in frequency could be assigned to C-7, C-8 and C-9,[14] verifying that this isomerism was at C9–C10 rather than at C13–C14. Further, by comparison of the relative intensities of the ^{1}H and ^{13}C resonances with those of the peaks corresponding to the two $C_{30:6}$ HBIs in the total ion current (TIC) chromatogram, the GC elution order could be determined (viz. *Z* before *E*), which is the same as that observed for the related C_{25} alkenes.[4,5]

The structures of the co-occurring pentaenes ($C_{30:5}$) were also examined by ^{1}H and ^{13}C NMR spectroscopy and not surprisingly many of the spectral features were extremely similar to those found for the $C_{30:6}$ alkenes. Thus, resonances were consistent with a vinyl moiety and four tri-substituted double bonds with one of these occurring at the C7–C25 branch point.[14] *E*/*Z* isomerism was also verified as being present at C9–C10, though somewhat surprisingly the major isomer was found to be *Z* ($Z/E = 1.5$ cf. 0.9 for **2**/**1**). The only significant spectral differences consisted of resonances attributable to a single isopropyl group confirming that one end of the main hydrocarbon chain was saturated. Despite the extreme structural similarities and expected spectrosopic features for isomers possessing unsaturation at either end of the principal carbon chain (viz. C2–C3 and C17–C18), an unambiguous assignment could be made by careful examination of the ^{13}C spectrum. For example, for the pseudo-homologous $C_{25:3}$, $C_{25:4}$ and $C_{25:5}$ alkenes (eight isomers due to positional and geometric isomerism) the frequency for C-6 is always significantly higher ($\Delta\delta = 0.3$–0.4 ppm) when C2–C3 is unsaturated (ca. δ 34.3 ppm).[4,5] Since C-6 was found to resonate at 34.3 ppm, with the corresponding resonance for the C_{30} hexaenes (which is unsaturated at C2–C3) occurring at 33.9 ppm, C2–C3 must be saturated at this position. An analogous trend was observed for C-7 with chemical shifts to higher frequency when C2–C3 is saturated (irrespective of whether C9–C10 is *E* or *Z*).

The structures of the two pairs of rhizenes ($C_{30:5}$ and $C_{30:6}$) are therefore established as **1**–**4**. The double bond positions and stereochemistry are equivalent to those established for some C_{25} alkenes found in other strains of *R. setigera*[15] and *P. intermedium*.[4,5] Although the biosynthesis or function of the rhizenes is as yet unknown, isotope ratio (δ ^{13}C) measurements suggest that they may be derived from different (and temperature dependent) pools of isopentenyl pyrophosphate (IPP) within the cells.[15] Now that the structures of the C_{30} alkenes have been established, their biosynthesis and function can be examined in more detail.

Acknowledgements

This work was supported by the University of Plymouth for studentships to W.G.A. and G.M., the British Council (ALLIANCE program), the Region des Pays de la Loire, France and the Royal Society of Chemistry, UK for a JWT Jones Travelling Fellowship to S.T.B.

References

1. Rowland, S. J.; Robson, J. N. *Mar. Environ. Res.* **1990**, *30*, 191–216.
2. Volkman, J. K.; Barratt, S. M.; Dunstan, G. A. *Org. Geochem.* **1994**, *21*, 407–413.
3. Sinninghe Damsté, J. S.; Schouten, S.; Rijpstra, W. I. C.; Hopmans, E. C.; Peletier, M. H.; Gieskes, W. W. C.; Geenevasen, J. A. J. *Org. Geochem.* **1999**, *30*, 95–100.
4. Belt, S. T.; Allard, G.; Robert, J.-M.; Massé, G.; Rowland, S. J. *Chem. Commun.* **2000**, 501–502.
5. Belt, S. T.; Allard, G.; Robert, J.-M.; Massé, G.; Rowland, S. J. *Geochim. Cosmochim. Acta* **2000**, *64*, 3839–3851.
6. Belt, S. T.; Cooke, D. A.; Robert, J.-M.; Rowland, S. J. *Tetrahedron Lett.* **1996**, *37*, 4755–4758.
7. Wraige, E. J.; Belt, S. T.; Lewis, C. A.; Cooke, D. A.; Robert, J.-M.; Massé, G.; Rowland, S. J. *Org. Geochem.* **1997**, *27*, 497–505.
8. Wraige, E. J.; Belt, S. T.; Johns, L.; Massé, G.; Robert, J.-M.; Rowland, S. J. *Org. Geochem.* **1998**, *28*, 855–859.
9. Wraige, E. J.; Belt, S. T.; Johns, L.; Massé, G.; Robert, J.-M.; Rowland, S. J. *Phytochemistry* **1999**, *15*, 69–73.
10. Johns, L.; Wraige, E. J.; Belt, S. T.; Lewis, C. A.; Massé, G.; Robert, J.-M.; Rowland, S. J. *Org. Geochem.* **1999**, *30*, 1471–1475.
11. Johns, L.; Belt, S. T.; Lewis, C. A.; Rowland, S. J.; Massé, G.; Robert, J.-M.; König, W. *Phytochemistry* **2000**, *53*, 607–611.
12. Allard, W. G.; Belt, S. T.; Massé, G.; Naumann, R.; Robert, J.-M.; Rowland, S. *Phytochemistry* **2001**, *56*, 795–800.
13. Guillard, R. R. L. In *Culture of Marine Invertebrate Animals*; Smith, W. L.; Chanley, M. H., Eds. Culture of phytoplankton for feeding marine invertebrates; Plenum Press: New York, 1975; pp. 26–60.
14. Retention indices were measured using HP-1 and HP-5 stationary phases. HP-1: 2596, **1**; 2545, **2**; 2558, **3**; 2505, **4**. HP-5: 2617, **1**; 2565, **2**; 2574, **3**; 2519, **4**. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL EX-270 NMR spectrometer in $CDCl_3$ with chemical shifts referenced to residual $CHCl_3$ (7.24 ppm) and $^{13}CDCl_3$ (77.0 ppm), respectively. **1**–**2**: ^{1}H (δ/ppm): 5.71 (m, 1H, H-28), 5.07 (m, 5H, H-3,9,13,17,25), 4.92 and 4.88 (2×m, 2H, H-29), 2.60 (m, 3H, 6,8), 1.8–2.2 (m, 13H, H-4,11,12,15,16,26,27), 1.7 (s, H-22, **2**), 1.66 (s, 6H, H-1,19), 1.57, 1.54 (2×s, H-20,23,24,22, **1**), 1.30 (m, 2H, H-5), 0.95 (m, 6H, H-21,30). ^{13}C (δ/ppm): 144.5 (C-28), 142.6 (C-7, **2**), 142.3 (C-7, **1**), 135.7 (C-10), 135.1 (C-14, **2**), 134.9 (C-14, **1**), 131.3, 131.2 (C-2,18), 124.9, 124.3, 124.2, 124.1 (C3,13,17), 123.8 (C-9, **2**), 123.1 (C-9, **1**), 123.0, 122.9 (C-25), 112.2 (C-29), 39.8, 39.7 (C-11, **1**,15), 38.22, 38.17 (C-27), 35.1 (C-5), 34.3 (C-26), 33.9 (C-6), 31.8 (C-11, **2**), 29.1 (C-8, **1**), 28.8 (C-8, **2**), 26.7, 26.5, 26.4 (C-4,12,16), 25.7 (C-1,19), 23.5 (C-22, **2**), 19.5, 19.4 (C-21,30), 17.68, 17.65 (C-20,24), 15.96, 15.92 (C-23), 15.8 (C-22, **1**). **3**–**4**: ^{1}H (δ/ppm): 5.73 (m, 1H, H-28), 5.09 (m, 4H, H-9,13,17,25), 4.93 and 4.88 (2×m, 2H, H-29), 2.60 (m, 3H, 6,8), 1.9–2.2 (m, 11H, H-11,12,15,16,26,27), 1.71 (s, H-22, **4**), 1.66 (s, 3H, H-19), 1.58, 1.56, 1.53 (3×s, H-23,24,22, **3**), 1.50 (m, 1H, H-2), 1.04–1.30 (m, 6H, H-3,4,5), 0.96, 0.94 (2×d, $J=6.6$ Hz, 6H, H-21,30), 0.84 (d, $J=6.6$ Hz, 6H, H-1,20). ^{13}C (δ/ppm): 144.6 (C-28), 143.0 (C-7, **4**), 142.7 (C-7, **3**), 135.6 (C-10), 135.1 (C-14, **4**), 134.9 (C-14, **3**), 131.3 (C-18), 124.4, 124.3, 124.2 (C-13,17), 124.0 (C-9, **4**), 123.3 (C-9, **3**), 122.9 (C-25, **4**), 122.7 (C-25, **3**), 112.1 (C-29), 39.8 (C-11, **3**), 39.7 (C-15), 39.3 (C-3), 38.2 (C-27), 35.3 (C-5), 34.4 (C-26), 34.3 (C-6), 31.8 (C-11, **4**), 29.2 (C-8, **3**), 28.9 (C-8, **4**), 27.9 (C-2), 26.8, 26.7, 26.6 (C-12,16), 25.7 (C19), 25.7 (C-4), 23.5 (C-22, **4**), 22.6 (C-1,20), 19.6, 19.5 (C-21,30), 17.7 (C-24), 15.9 (C-23), 15.8 (C-22, **3**).
15. Rowland, S. J.; Allard, W. G.; Belt, S. T.; Massé, G.; Robert, J.-M.; Blackburn, S.; Frampton, D.; Revill, A. T.; Volkman, J. K. *Phytochemistry* **2001**, submitted.