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Configurations of polyunsaturated sesterterpenoids from the diatom, *Haslea ostrearia*

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Abstract

The partial configurations of C_{25} isoprenoid alkenes isolated from the diatom *Haslea ostrearia* Gaillon (Simonsen) have been established. A combination of NMR spectroscopy studies of the alkenes with chiral shift reagents in conjunction with soluble silver β-diketonate complexes and enantioselective gas chromatography of oxidation products of the alkenes was used. Unexpected differences in highly branched isoprenoid isomer configurations were observed between different laboratory cultures of the alga.

Keywords: *Haslea ostrearia*; Bacillariophyceae; Microalgae; Diatoms; Isoprenoid alkenes; Highly branched isoprenoids C_{25} alkenes; Configuration; Stereochemistry

1. Introduction

Recently, we reported the production of a suite of C_{25} highly branched isoprenoid (HBI) polyenes by bacteria-free cultures of the diatom, *Haslea ostrearia* (Gaillon) Simonsen grown in the presence of three antibiotics (Wraige, Johns, Belt, Massé, Robert & Rowland, 1999). This confirmed and extended the findings of an earlier study of a non-axenic culture (Volkman, Barrett & Dunstan, 1994). Similar HBI alkenes (e.g. I–IV) have been found in contemporary marine environments worldwide and some HBIs have been used as chemical fossils of diatom inputs to sediments and petroleum (reviewed in Ref. (Rowland & Robson, 1990)). The biochemical function of the compounds is unknown, but it has been suggested that they may be membrane lipids (Ourisson & Nakatani, 1994). Whilst the primary structures of alkenes I–IV have been determined (e.g. Belt, Cooke, Robert & Rowland, 1996; Johns et al., 1999; Wraige et al., 1999) the configurations at the stereocentres have not been reported. For the diunsaturated hydrocarbons (e.g. I), these stereocentres are situated at C-7, C-10 and C-22, for the tri- to pentaunsaturated compounds at C-7 and C-22 (e.g. II–IV) and for the hexaenes (e.g. V) only at C-7.

The relative non-functionality and non-rigidity of the alkenes obviates the use of some conventional methods of determining the absolute or relative configurations (e.g. by nOe NMR methods) and has required a combination of degradative and spectroscopic studies. The results indicate an unusual variability in the configuration at the C-22 stereocentre, whilst fixed configurations occur at the other centres.

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2. Results and discussion

H. ostrearia is a large, pennate diatom which produces a range of di- to heptaunsaturated sesterterpenoid HBIs (e.g. I–IV). To determine the configurations of the HBI alkenes at the C-7, C-10 and C-22 stereocentres, we isolated milligram quantities of each of a pure diene I and triene II from cultures of the diatom and hydrogenated each alkene to the known alkane (Robson & Rowland, 1986). Each sample of alkane was then oxidised by chromium trioxide/glacial acetic acid (Yon, Maxwell & Ryback, 1982) to mixtures containing 3,7-dimethyloctanoic (DMO) acid, 3-methylpentanoic acid (MP) and 4-methylhexanoic acid (MH) Fig. 1. The configuration at C-10 in I is preserved in position 3- of DMO and that of C-22 in I and II is preserved in positions 3- and 4- of MP and MH, respectively.

The acids were converted to the methyl esters and then examined by enantioselective GC (König, Lutz & Wenz, 1988) on cyclodextrin stationary phases under conditions that completely resolved racemates of the methyl esters. Racemic MP was purchased from commercial sources, racemic MH was synthesised by Arndt–Eistert chain elongation of MP, whilst DMO was obtained by hydrogenation of commercial 3*RS*-citronellic acid (3*RS*, 7-dimethyloct-6-enoic acid). The elution order of each of the acids was *R* before *S*, established by co-chromatography. For example, 3*S*-MP was obtained by Arndt–Eistert chain elongation of commercial 2*S*-methylbutanoic acid (MB) and 3*R*-DMO from hydrogenation of commercial 3*R*-citronellic acid. The configuration of DMO from oxidation of the hydrogenated diene I was predominantly *S* in the single sample examined, corresponding to a 10-*S* configuration for I. Surprisingly, and in contrast, either *R* or 1:1 *RS* configurations were observed for the 3-MP and 4-MH acids from II, obtained from randomly sampled large-scale (440L) cultures of the diatom, thus revealing that 22-*S* and 1:1 22-*RS* configurations occurred in the samples of parent triene II. This apparent non-stereospecificity is unusual.

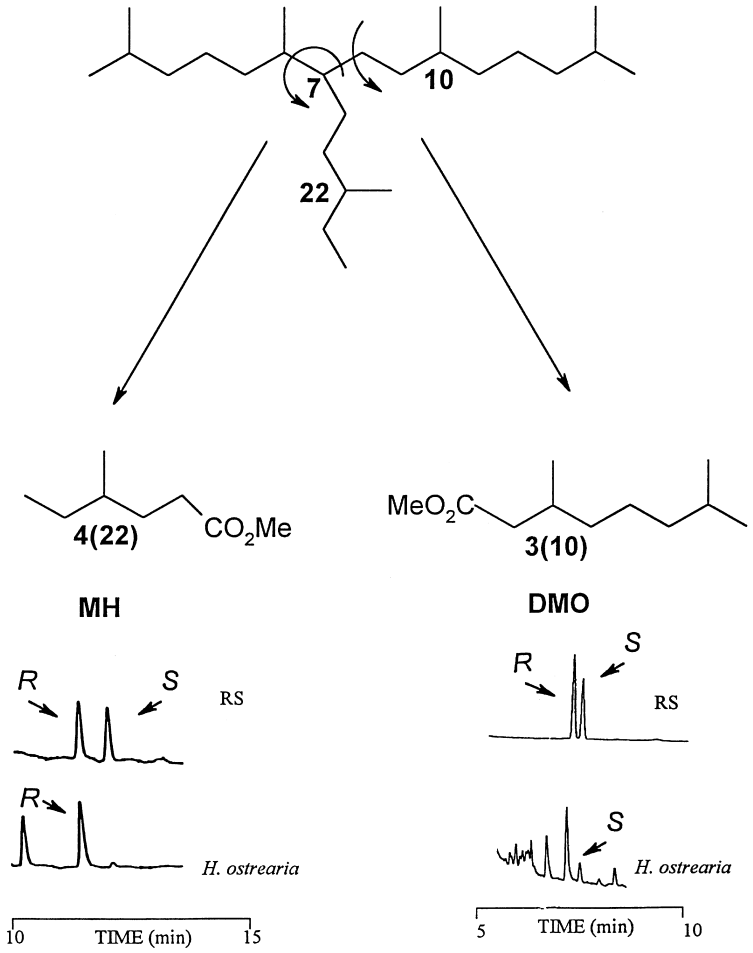


Fig. 1. Enantioselective (cyclodextrin) gas chromatography of HBI alkane oxidation products. Chromatographic conditions: (MH) octakis (6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin (50% in polysiloxane OV 1701, wt./wt.) 50°C, isothermal, hydrogen carrier gas, 0.5 bar inlet pressure; (DMO) 2,6-*O*-dimethyl-3-*O*-pentyl-β-cyclodextrin, (50% in OV 1701, wt./wt.) 95°C hydrogen carrier gas, 0.5 bar inlet pressure.

The remaining chiral centre in I, II and indeed III–V, is at C-7, which is not amenable to study by the foregoing oxidative approach. However, this could be partly de-lineated by examination of 22-*S* and 22-*RS* samples of II by ^{13}C NMR using chiral shift reagents in conjunction with a soluble silver β-diketonate complex, using a refinement of a previous method (Wenzel & Sievers, 1982). In order to test the validity of the method, two model compounds were first investigated. Thus, all the individual ^{13}C resonances of myrcene (VII), which contains mono-, di- and tri-substituted alkene moieties (*cf* II), were shifted ($\Delta\delta = -11.2$ to -1.3 ppm) upon addition of praseodymium tris[3-heptafluoropropylhydroxymethylene)-(+)-camphorate/Ag^I 6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionate ($Pr(hfc)_3$/Ag(fod)) to the solution. This verified that coordination of the shift reagent to all three double bonds via the silver β-diketonate had taken place. In the absence of the Ag^I β-diketonate, no resonance shifts were observed, as expected. Similar resonance changes were observed when 3*RS*-methylpent-1-ene (VIII) was employed as the model substrate ($\Delta\delta = -16.6$ to -0.8 ppm). Further, the influence of the chiral shift reagent resulted in spectroscopic separation of the two enantiomers of VIII ($\Delta\delta(RS) = 0.1$ to 0.3 ppm). Thus, both the dynamic coordination of the shift reagent to a tri-unsaturated alkene and separation of alkene enantiomers were verified. When the method was applied to a 22-*RS* sample of II, coordination of the shift reagent was confirmed by the observation of shifts of similar magnitude ($\Delta\delta = -14.2$ to -3.6 ppm) to those seen for the two model compounds. However, no further *splitting* of resonances was observed, suggesting that C-7 must be homochiral (*R* or *S*) and not racemic (*RS*). Finally, $Pr(hfc)_3$/Ag(fod) also complexed to the 22-*S* isomer of II ($\Delta\delta = -2.5$ to -2.1 ppm), and again no enantiomeric splitting was observed, again suggesting homochirality at C-7. In the absence of any chiral shift reagent, the $\Delta\delta$ between resonances observed for diastereomeric 22*RS* alkene II was of the same order (0.1–0.4 ppm) as that observed for the induced resonance shifts caused by complexation of the shift reagent with the 3*RS*-methylpent-1-ene.

We acknowledge that it is possible that the resonances of the isomers of the HBI alkene were still not separated under these conditions, but we consider it unlikely given the results of our experiments with model compounds such as myrcene and methylpentene. The 7 (*R* or *S*), 10*S*, 22*S* and 22*RS* configurations reported above were established by these somewhat laborious methods for three samples of I and II from two different diatom cultures. However, non-enantioselective GC (Carbowax) revealed that 22*RS* diastereomers of these authenticated compounds could be separated and that the elution order was *S* before *R* (Fig. 2). GC studies of alkenes from numerous *Haslea* cultures then confirmed the variability in the configuration at C-22 in I–III with *S*, *R* and *RS* configurations produced in HBIs isolated from randomly sampled cultures (Fig. 2). Whilst these findings require further investigation, the stereochemical studies may aid elucidation of the biosynthetic pathways to the sesterterpenoids. For instance, others have suggested (Ourisson & Nakatani, 1994) that a plausible precursor to the alkenes is the diphosphate ester of a C_{25} pentaunsaturated alcohol (e.g. VI). Whilst other precursors are possible, it is interesting that loss of diphosphate from VI or other similar esters, would produce hexaene V and progressive saturation would yield IV–I, all of which we have identified previously in *H. ostrearia* (Belt et al., 1996; Johns et al., 1999; Wraige et al., 1999). Saturation at C-22 would give rise to 22*RS* (non-stereospecific), or by stereospecific reduction to *S* or *R* isomers (IV) whereas reduction of the 9(10) bond in II to I would involve a stereospecific step leading to the 10*S* configuration reported above. In comparison with stereospecific saturation of other acyclic isoprenoids, the configuration at C-10 in I (viz corresponding to a 3*S* configuration of the tetrahydrogeranyl group) is contrasts with the observed *R* stereochemistry of the C-3, (and 7, 11 positions) in phytanyl (3,7,11,15-tetramethylhexadecyl-) moieties of the gly-

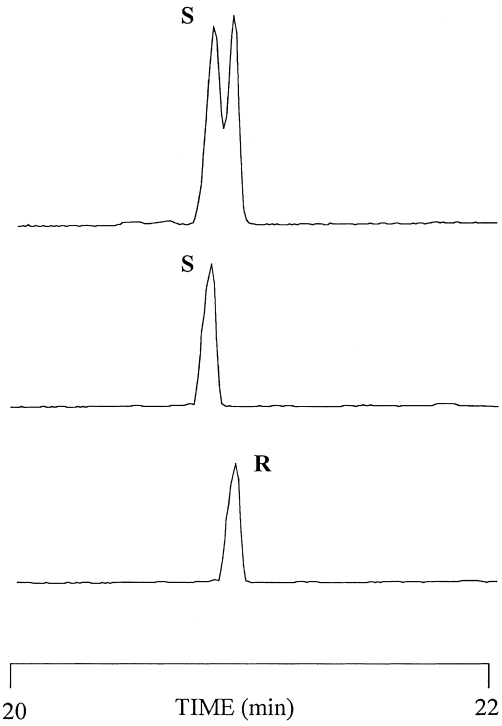
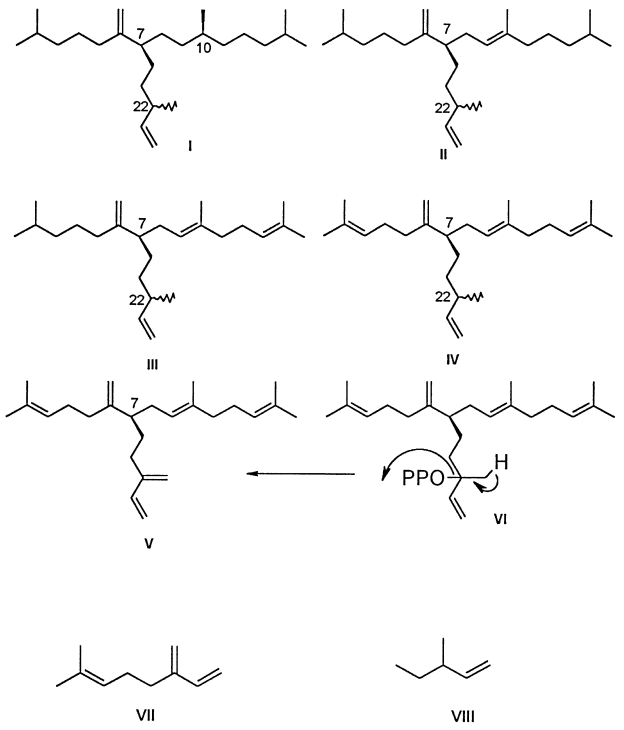


Fig. 2. Partial gas chromatogram (carbowax) indicating separation of C-22 diastereomers of HBI triene (II) isolated from different cultures of *Haslea ostrearia*. Chromatographic conditions are given in the text.

ceryl ethers of numerous prokaryotes (Danson, Hough & Hunt, 1992) and with the C-3*R* (and 7*R*) configurations of 3,7,11-trimethyldodecanol (hexahydrofarnesol) found in the free lipids of methanogenic bacteria (Risatti, Rowland, Yon & Maxwell, 1984). However, we believe that the preferred C-7 configuration which we have tentatively demonstrated in I–V is without precedent, since the unusual coupling of C_{10} and C_{15} units in these HBIs appears to be rare.



3. Experimental

3.1. Algal cultures

H. ostrearia was isolated from oyster ponds of the Bay of Bourgneuf (France) and cultured in an outdoor 440L facility as described previously (Belt et al., 1996). Samples for hydrocarbon analysis were obtained by centrifugation.

3.2. Hydrocarbon extraction, isolation and oxidation

Alkenes I and II (2–5 mg) were isolated by extraction of the centrifuged algal paste with hexane, aided by ultrasonication (45 min, Kerry Pulsatron HB172) followed by column chromatography on silica and elution with hexane. Diene I typically eluted with ca 1 column volume of hexane and triene II with 5–8 column volumes. In a typical oxidation procedure, the alkene (2 mg) was then hydrogenated (hexane, 3 h, $PtO_2{\cdot}H_2O$, 10 mg) and the resultant alkane (2 mg) characterised by gas chromatography–mass spectrometry (GC–MS) and oxidized (2 h) by addition of a solution of CrO_3 (6 mg) in glacial acetic acid (10 ml; 70°C). Products were extracted with dichloromethane, hydrolysed (10% KOH:MeOH wt./wt.) acidified (pH 1; HCl) and the extracted acids converted to methyl esters (BF_3/MeOH).

3.3. Synthesis of chiral acids

Arndt–Eistert extension of 2*S*-MB and 3*RS*-MP was achieved by addition of thionyl chloride (5 ml) to the acid (50 mg) and reflux (30 min). An ethereal solution of diazomethane (ca 20 mg ml^{-1}) was slowly added to the acid chloride dissolved in diethyl ether (2 ml; overnight). Excess diazomethane was removed and the diazoketone was dissolved in hot anhydrous methanol (20 ml). A suspension of Ag_2O (0.5 g) in methanol (10 ml) was added dropwise with continuous stirring. The mixture was refluxed (2 h), allowed to cool and the methanol gently decanted. The residue was washed three times with diethyl ether (5 ml). The washings were combined with the methanol and the solvent removed. The acids were esterified (BF_3/MeOH). For preparation of DMO, 3,7-dimethyloct-6-enoic acid (100 mg) was hydrogenated (hexane; $PtO_2{\cdot}H_2O$, 10 mg, 60 min).

3.4. NMR spectroscopy

Optimal conditions for NMR chiral shift reagent (CSR) measurements in $CDCl_3$ ($^{13}C = 67.8$ MHz) were [Substrate] = 0.08–0.1 M, [Substrate]:[CSR]:[Ag(fod)] = 1:3:3 at 10°C.

3.5. Chromatography

Alkene fractions were examined by GC–MS performed using a Hewlett Packard 5890 series II gas chromatograph coupled to a Hewlett Packard 5970 mass selective detector fitted with a 30 m (0.25 mm i.d.) fused silica capillary column (carbowax stationary phase). Auto-splitless injection and helium carrier gas were used. The gas chromatograph oven temperature was programmed from 50–200°C at 6°C min^{-1} and held at the final temperature for 10 min. Mass spectrometer operating conditions were: ion source temperature 250°C and 70 eV ionisation energy. Spectra (35–500 Da) were collected using Hewlett Packard Chemstation™ software. Esters of acids were examined by GC on 25 m × 0.25 mm fused silica columns coated with either octakis(6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin for MP, MH or heptakis(2,6-di-*O*-methyl-3-*O*-pentyl)-β-cyclodextrin (50% in polysiloxane OV

1701, wt./wt.) for DMO (hydrogen carrier, 0.5 bar inlet pressure, 50°C (MP, MH) or 95°C (DMO).

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