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Novel monocyclic sester- and triterpenoids from the marine diatom, *Rhizosolenia setigera*

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Abstract—The structures of two polyunsaturated monocyclic triterpenes have been elucidated using NMR spectroscopy following their isolation from the common marine diatom, *Rhizosolenia setigera*. The structure of a related monocyclic sesterterpene is proposed on the basis of mass spectral comparisons with the two monocyclic triterpenes.
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Over the past few years, we[1,2] and others[3] have reported on the structures of a series of polyunsaturated acyclic sester- (C_{25}) and triterpenoids (C_{30}) biosynthesised by the commonly occurring marine diatom, *Rhizosolenia setigera*. The structures of these highly branched isoprenoid (HBI) rhizenes (e.g. **1**–**4**, Fig. 1) together with their distributions have been shown to be strongly dependent on the strain investigated,[1–4] and more recently, the physiological status of the species, as measured by its position within a life cycle.[5] In all

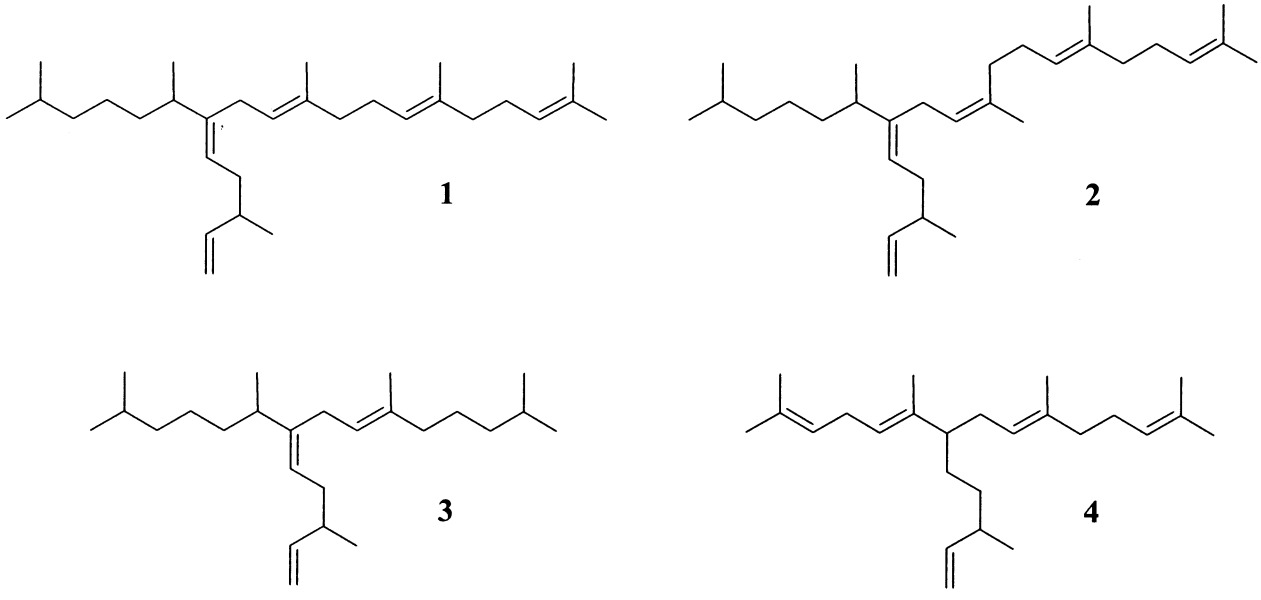

Figure 1. Structures of acyclic C_{25} and C_{30} HBI rhizenes characterised previously.

Keywords: triterpenes; sesterterpenes; diatoms; *Rhizosolenia setigera*; hydrocarbons; NMR spectroscopy; rhizenes.
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cases, verification of the HBI carbon skeletons has been achieved by exhaustive hydrogenation to the respective C_{25} and C_{30} alkanes followed by co-chromatography and mass spectral comparison with authentic standards. For some strains however, this approach has also revealed the presence of a small number of related compounds, which have not yielded fully saturated, acyclic C_{25} or C_{30} hydrocarbons following hydrogenation. Instead, compounds possessing one or two Double Bond Equivalents (DBEs) have been formed even after prolonged hydrogenation.[4,6] Until now, these isomers have been presumed to either contain double bonds resistant to hydrogenation, cyclic moieties, or both. In order to distinguish between these possibilities, we decided to determine the structures of these polyenes by growing large-scale cultures of selected strains of *R. setigera* followed by isolation, purification and analysis (NMR) of individual compounds according to methods described previously.[1]

Large-scale culturing of *R. setigera* (CCMP 1694) followed by freeze drying of filtered cells and solvent extraction (chloroform/methanol), yielded a total organic extract which was further fractionated using column chromatography (SiO_2/hexane). GC–MS analysis of this hydrocarbon fraction revealed the presence of heneicosa-3,6,9,12,15,18-hexaene (n-$C_{21:6}$) together with a series of C_{25} and C_{30} polyenes. While the chromatographic and mass spectral properties of some of these sester- and triterpenoid hydrocarbons corresponded to acyclic compounds characterised previously (e.g. **1**–**4**), three further species, corresponding to the aforementioned unknowns, were also present. Mixtures of C_{25} polyenes were subsequently separated from the C_{30} homologues using further column chromatography (SiO_2/hexane), and these were fractionated into individual isomers using a modified preparative Ag^+-HPLC method.[6] As a result, two novel triterpenes (**5**: 10 mg; **6**: 3 mg) and a sesterterpene (**8**: <0.1 mg) were obtained with >95% purity (GC).

GC–MS analysis of the most abundant of these new polyenes (triterpene **5**) indicated a compound with 5 DBEs (RI $2548_{HP\text{-}1}$; M^+ 412) whose MS fragmentation was extremely different when compared with that of acyclic pentaenes **1** and **2**. Thus, while the mass spectra of **1** and **2** featured ions at m/z 299 and 367, the mass spectrum of triterpene A was dominated by ions at m/z 231, 259 and a particularly intense ion at m/z 315. The ^{13}C NMR spectrum of **5** (Table 1) revealed 29 magnetically inequivalent ^{13}C nuclei consistent with a structure possessing virtually no symmetry. In addition, the occurrence of eight (and not ten) resonances in the high frequency region of the spectrum confirmed the presence of four double bonds only for this compound. As such, it was concluded that the remaining DBE must arise from a cyclic moiety. By further examination of the high frequency region of the ^1H and ^{13}C NMR spectra of **5** (Tables 1 and 2), the presence of a vinyl group (C15–C16), a 1,1-disubstituted (C27–C28) and two trisubstituted (C6–C7, C22–C23) double bonds could also be elucidated (see structure **7**, Fig. 2 for numbering scheme). In the low frequency region, the

Table 1. ^{13}C NMR data (100 MHz, $CDCl_3$) for monocyclic triterpenes **5** and **6**

^{13}C chemical shift (ppm)		^{13}C number
5	**6**	
22.7	25.8	1
28.0	131.1	2
38.8	124.5	3
25.9	26.9	4
40.1	39.8	5
135.2	134.8	6
124.1	124.4	7
26.7	26.8	8
37.9	37.8	9
46.6	46.6	10
42.6	42.6	11
22.6	22.6	12
38.1	38.2	13
37.9	37.8	14
145.1	145.1	15
112.3	112.2	16
22.7	17.8	17
16.0	16.1	18
21.1	21.1	19
20.3	20.2	20
52.2	52.2	21
126.0	126.0	22
136.0	136.0	23
40.3	40.3	24
28.8	28.8	25
51.9	51.9	26
148.1	148.1	27
109.3	109.3	28
20.0	20.0	29
16.7	16.7	30

DEPT spectrum was used to determine the multiplicities of the individual aliphatic ^{13}C nuclei ($6CH_3$, $10CH_2$, 4CH) which also included a single quaternary carbon atom (C10). Having established these features, the remaining structural elucidation of **5** (Fig. 2) was determined using detailed analysis of various 2-D (HMQC, HMBC, COSY) and NOE difference spectra. For example, the HMBC spectrum showed 2- and 3-bond correlations between H19–C21, H28–C26 and H21–C10,11,19,22,23,26,27, which established the 3,4-disubstitution of the cyclohexene moiety. In addition, strong correlations between H21 and each of H22 and H26 (COSY) confirmed the C26–C21–C22 connectivity. However, although the apparent H21–H26 coupling constant ($J=10$ Hz) could readily be explained by a di-axial (and *anti*) orientation, a much smaller coupling would have been expected for H21–H22 in a half chair conformation. Rather, a triplet multiplicity was observed for H21 (at 270 and 400 MHz and in 3 different solvents), suggesting equal (or almost equal) coupling between H21 and H22,26. This was confirmed by homonuclear decoupling of H22, which resulted in the formation of a doublet for H21 (J (H21–H26) = 10.4 Hz), whilst a similar irradiation of H26 also yielded a doublet, this time with a slightly smaller coupling constant (J (H21–H22) = 9.4 Hz). Since the

Table 2. ^{1}H NMR data (400 MHz, $CDCl_3$) for monocyclic triterpenes **5** and **6**

5		**6**	
^{1}H chemical shift (multiplicity, coupling constant, integration)	^{1}H number	^{1}H chemical shift (multiplicity, coupling constant, integration)	^{1}H number
5.68 (ddd, J=17, 10, 7 Hz, 1H)	15	5.68 (ddd, J=17, 10, 7 Hz, 1H)	15
5.08 (t, J=7 Hz, 1H)	7	5.08 (m, 2H)	3, 7
4.9 (m, 3H)	16, 22	4.9 (m, 3H)	16, 22
4.6 (m, 2H)	28	4.6 (m, 2H)	28
2.4 (m, 1H)	26	2.4 (m, 1H)	26
2.26 (t, J=10 Hz, 1H)	21	2.26 (t, J=10 Hz, 1H)	21
1.9–2.1 (m, 7H)	5, 8, 14, 24	1.9–2.1 (m, 9H)	4, 5, 8, 14, 24
1.82, 1.42 (m, 2H)	25	1.82, 1.42 (m, 2H)	25
1.65 (s, 3H)	29	1.65–1.65 (5×s, 15H)	1, 17, 18, 29, 30
1.56, 1.57 (2×s, 6H)	18, 30	1.1–1.5 (m, 8H)	9, 11, 12, 13
1.1–1.5 (m, 13H)	2, 3, 4, 9, 11, 12, 13	0.96 (d, J=7 Hz, 3H)	20
0.96 (d, J=7 Hz, 3H)	20	0.79 (s, 3H)	19
0.85 (d, J=7 Hz, 6H)	1, 17		
0.79 (s, 3H)	19		

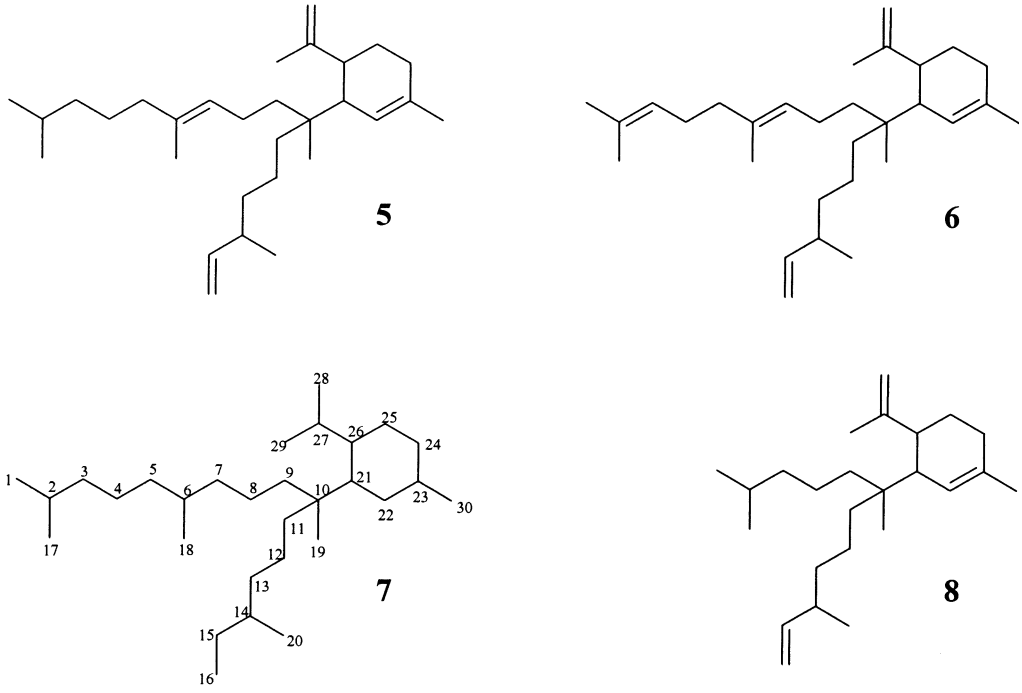

Figure 2. Structures of C_{25} and C_{30} monocyclic hydrocarbons identified in the current study.

H21–H22 coupling would be expected to be substantially smaller than this (typically 1–5 Hz for a dihedral angle of 60–90° in a half chair), the observed coupling leads us to an alternative conformation for **5** which places both C25 and C26 above (or below) the C21–C24 plane in a twist boat. As such, none of the substituents can be considered as truly axial or equatorial, but H21, H22 and H26 can become virtually eclipsed with dihedral angles of ca. 15–20°, consistent with the observed ^{1}H couplings. This was further corroborated by the observation of positive NOEs between H21, 22, 25, 26.

GC–MS analysis of the less abundant triterpene **6** (RI $2579_{HP\text{-}1}$) suggested a compound with a structure closely related to that of **5**, but with an additional double bond. Thus, the mass spectrum of **6** (M^+ 410) was very similar to that of **5**, with a mass unit difference of 2 Da for the majority of the ions. The presence of an additional double bond was confirmed by analysis of the ^{1}H and ^{13}C NMR spectra obtained for this compound (Tables 1 and 2) which revealed the presence of a trisubstituted double bond in a terminal position (C2–C3). The remaining chemical shift assignments were made via further NMR analysis and

by comparisons with assignments made for **5** previously.

In order to explain the hydrogenation behaviour of compounds such as **5** and **6**, each of these two monocyclic triterpenes were hydrogenated ($PtO_2 \cdot 2H_2O$/hexane) over various time intervals, with aliquots analysed by GC–MS and NMR spectroscopy. For each of **5** and **6**, relatively short periods of hydrogenation (4 h) yielded a common intermediate with 2 DBEs (RI 2549; M^+ 418). The double bond in this intermediate, whose position (C22–C23) was confirmed by ^1H (δ 4.92, d, $J = 9.4$ Hz, H-22) and ^{13}C (δ 135.3 (C-23) and 127.5 (C-22)) NMR spectroscopy, could only be hydrogenated further following more prolonged reaction, with ca. 50% conversion to the parent C_{30} alkane **7** after 10 h.

Although the previously uncharacterised sesterterpene **8** was not present in sufficient quantities (< 0.1 mg) to allow for complete analysis by NMR spectroscopy, its mass spectrum exhibited consistent similarities with those obtained for triterpenes **5** and **6** but with a difference of 68 Da between the majority of the ions. Since this corresponds to a structural difference of an isopentenyl moiety, the most likely structure of sesterterpene C is **8**.

Previous reports of monocyclic triterpenes (and sesterterpenes) are extremely rare. Instead, the majority of monocyclic terpenes are monoterpenes (e.g. limonene, menthol, camphor) and a limited number of diterpenes (e.g. vitamin A). In contrast, triterpenes are generally tetracyclic (e.g. the ubiquitous steroids) or pentacyclic, including the bacteria-derived hopanes.[7] The few examples of monocyclic triterpenoids include achilleol A, obtained from *Achillea odorata*,[8] and the hydrocarbon 11,15-dihydro-Δ^{13}-10,14-cyclosqualene, isolated from sediments of Lake Cadagno.[9] In the context of the latter, numerous other examples of sedimentary triterpenoid hydrocarbons have been reported without structural identification. On the basis of their hydrogenation behaviour and mass spectral characteristics, these have been postulated as being either acyclic compounds with hydrogenation-resistant double bonds, or mono- (or di-) cyclic.[10–14] Now that the structures of some monocyclic tri- and sesterterpenoids have been elucidated, a more rigorous analysis of these geochemical reports can be made.

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