Structural Determination of a Highly Branched C_{25} Sedimentary Isoprenoid Biomarker by NMR Spectroscopy and Mass Spectrometry

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The C_{25} diene 2,6,10,14-tetramethyl-7-(3-methylpent-4-enyl)pentadec-5-ene has been isolated from benthic sediments and characterised by ^1H and ^{13}C NMR spectroscopy together with mass spectrometry.

Polyunsaturated lipids found in sediments and sedimentary rocks have proved to be extremely valuable tools for the determination of palaeoseawater surface temperatures and thus for palaeoclimatic reconstruction.[1–3] Despite this, a complete structural analysis (*i.e.* beyond the parent skeleton)[5–7] of the abundant and widespread polyunsaturated highly branched isoprenoid hydrocarbons found in sediments[4] has been confined to a relatively few examples.[8–10] The recent discovery that these highly branched isoprenoids are biosynthesised by a restricted number of diatomaceous algae[11] is likely to increase their value as 'biomarker' compounds.[12] In this communication, we describe the detailed structural characterisation of a widely occurring highly branched isoprenoid C_{25} diene **1** using NMR spectroscopy and mass spectrometry. Compound **1** is a colourless oil that was isolated from recent benthic sediments of the Caspian Sea by solvent extraction, column chromatography and Argentation TLC.[9] Initial identification of a highly branched C_{25} diene structure came from the EI mass spectrum ($M^+ = 348$), which showed two degrees of unsaturation, together with its low RI 2079 (DB1). Further, the position of the T branch in the isoprenoid chain was located by hydrogenation of **1** to a C_{25} alkane which co-chromatographed (GC) with previously synthesised 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane[6] on three phases (DB1, DB5 and DB-wax). Having established the parent structure, the locations of the two double bonds needed to be established.

Sufficient **1** (*ca.* 5 mg) was obtained from a bulk sediment extraction for ^1H and ^{13}C NMR spectroscopic analysis.† The fully proton decoupled ^{13}C spectrum shows a total of 25 resonances with four alkenic and 21 aliphatic type carbons as expected. Analysis *via* the DEPT sequence reveals the presence of seven methyl, ten methylene, seven methine and a single quaternary carbon (C-6) consistent with structure **1** (amongst others), while the observation of a methylene carbon in the alkenic region (δ 112.3) confirms the presence of a double bond in a terminal position. The ^1H spectrum of **1** consists of resonances associated with alkenic, allylic and aliphatic protons. The most conspicuous of these are the three sets of resonances at δ 5.65, 4.93 and 4.89, which arise from a vinyl ($-C_2H_3$) functionality (Fig. 1). Given the established C_{25} isoprenoid skeleton, it is clear that this double bond must be located at C-24. Proton H-23 appears as a low field seven line multiplet due to a *trans* coupling to H-24a (17 Hz), a *cis* coupling to H-24b (10 Hz) and an additional vicinal coupling to H-22 (8 Hz). This latter coupling to a single allylic H-22 proton is further verification of the assignment of the first double bond. The allylic H-22 proton itself appears as a multiplet at δ 2.08 due to couplings to H-23, H-21 and H-25. This assignment was based upon the following decoupling experiments. Primarily, when the resonance at δ 2.08 was irradiated, the seven line multiplet associated with H-23 collapsed to a doublet of doublets due to the H-24a and H-24b couplings. Similarly, when a reciprocal irradiation was carried out at δ 5.65, one of the couplings at δ 2.08 was eliminated. The irradiation of H-22 also resulted in the transformation of the doublet at δ 0.95 to a singlet, allowing assignment of H-25. The only singlet in the spectrum is found at δ 1.42 and arises due to H-17. The remaining methyl protons [H-18, H-(1,16), H-(15,19)] show couplings to the neighbouring methine protons and appear as doublets in the region δ 0.8–0.9.

Further analysis of the ^1H spectrum demonstrates the presence of an additional alkenic proton (H-5) which appears as a triplet due to an allylic methylene coupling (H-4). The absence of a proton on the remaining alkenic carbon (C-6) is consistent with the observation of a single quaternary carbon in the ^{13}C spectrum. The assignment of the H-4 allylic protons was made by irradiating H-5 and observing the change in multiplicity at δ 1.99 from a quartet to a triplet. Similarly, irradiation at δ 1.99 resulted in the observation of a singlet for the H-5 resonance. At this stage, the structural data described for the second double bond position was consistent with six

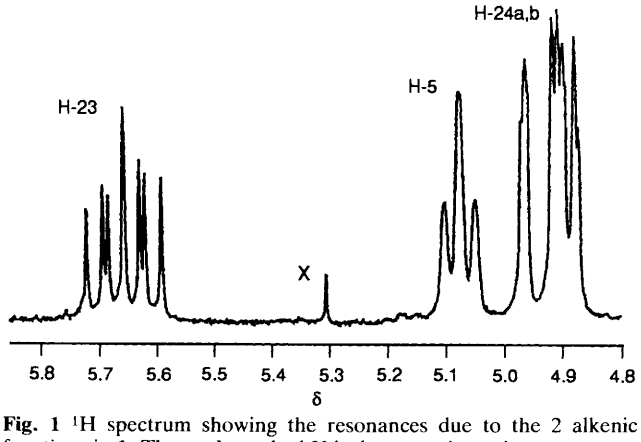


Fig. 1 ^1H spectrum showing the resonances due to the 2 alkenic functions in **1**. The peak marked X is due to an impurity.

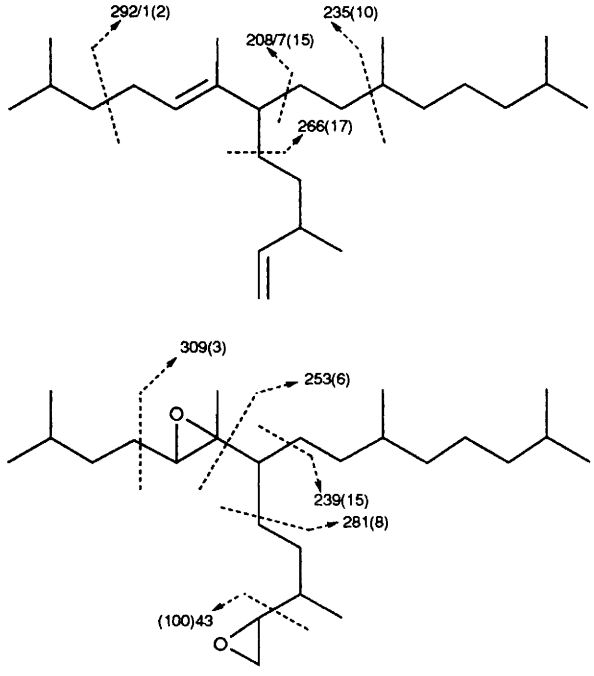
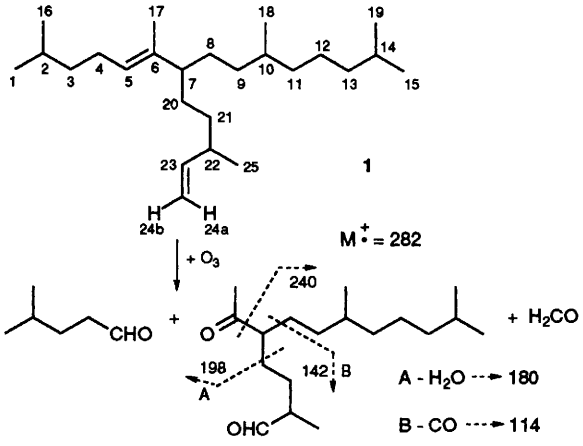


Fig. 2 Fragmentation pathways observed from the mass spectra of **1** and its bis-epoxidation product.



Scheme 1 *Reagents and conditions*: 0.5 mmol dm^{-3} (CH_2Cl_2), O_3 flow rate 5 cm^3 min^{-1}, −70 °C

other C_{25} diene structural isomers. However, by inspection of these, it could be seen that structure **1** was the only isomer that contained a total of four allylic methylene or methine protons (H-22, H-4, H-7) in agreement with the integration of these proton resonances. From this, it is possible to assign a single structure for compound **1**. The allylic proton located at the T-branch (H-7) occurs as a multiplet at δ 1.8 with couplings to H-20 and H-8. The corresponding carbon resonance is shifted to significantly higher frequency (at least 10 ppm, identified *via* ^1H–^{13}C COSY compared with the other sp^3 hybridised carbons.

Additional confirmation of structure **1** has been achieved *via* ozonolysis and epoxidation in conjunction with mass spectrometry. Ozonolysis of **1** yields the aldehyde and ketone products as shown in Scheme 1, while Fig. 2 demonstrates how epoxidation of the parent diene can be monitored conveniently by mass spectrometry.

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Footnotes

† *Selected NMR data* for **1** at 270 MHz for ^1H in $CDCl_3$. ^1H and ^{13}C. Numbering shown in displayed formula. Missing integrations due to overlap. ^1H: δ 5.65 (ddd, 1H, $J_{H23-H24a}$ 17, $J_{H23-H24b}$ 10, $J_{H23-H22}$ 8 Hz, H-23) 4.93 (dd, $J_{H24a-H24b}$ 2 Hz,H-24a), 4.89 (dd, H-24b), 5.08 (t, 1H, J_{H5-H9} 7 Hz, H-5), 2.08 (m, H-22), 1.99 (q, J_{H4-H5} = J_{H-H3} = 7 Hz, H-4), 1.80 (m, 1H, H-7), 1.42 (s, 3H, H-17), 0.95 [d, 3H, $J_{H25-H22}$ = 6.5 Hz, H-25], 0.88 [d, 6H, $J_{H(1,16)-H2}$ = 4.5 Hz, H(1,16)], 0.85 [d, 6H, $J_{H15,19-H14}$ = 4.5 Hz,H(15,19)], 0.82 (d, 3H, $J_{H18-H10}$ = 6.5 Hz, H-18); $^{13}C\{^1H\}$, DEPT and ^1H–^{13}C COSY: δ 145.0 (C-23),136.3 (C-6), 126.3 (C-5), 112.3 (C-24), 49.3 (C-7), 37.8 (C-22), 25.5 (C-4), 22.5–22.7 (C-(1,16,15,19)), 20.5 (C-25), 19.9 (C-18), 11.3 (C-17).

‡ *Conditions for epoxidation*: 37.5 μmol MCPBA, 6.25 μmol **1** in 0.25 cm^3 CH_2Cl_2, 25 °C, 90 h.

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