

Structural Determination of a Highly Branched C₂₅ Sedimentary Isoprenoid Biomarker by NMR Spectroscopy and Mass Spectrometry

Simon T. Belt, David A. Cooke, Simon J. Hird and Steve Rowland

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

The C₂₅ 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 ¹³C 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 sediments⁴ 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 algae¹¹ 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 C₂₅ 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 C₂₅ 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 C₂₅ alkane which co-chromatographed (GC) with previously synthesised 2,6,10,14-tetramethyl-7-(3-methylpentyl)pentadecane⁶ 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 ¹³C NMR spectroscopic analysis.[†] The fully proton decoupled ¹³C 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 (–C₂H₃) functionality (Fig. 1). Given the established C₂₅

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 ¹³C 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

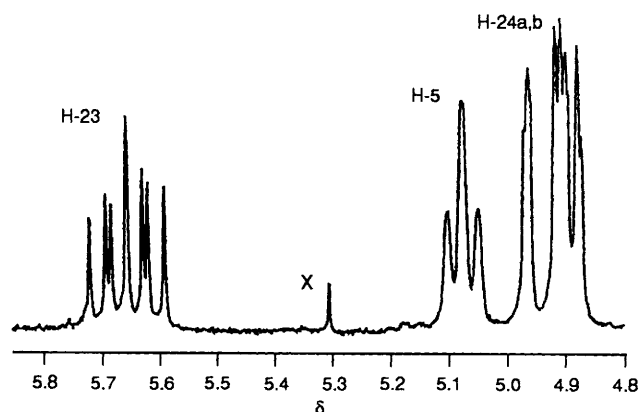


Fig. 1 ¹H spectrum showing the resonances due to the 2 alkenic functions in **1**. The peak marked X is due to an impurity.

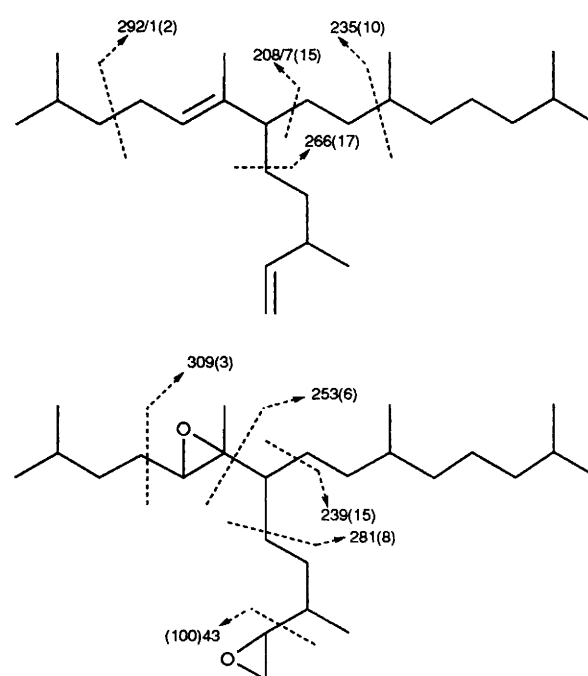
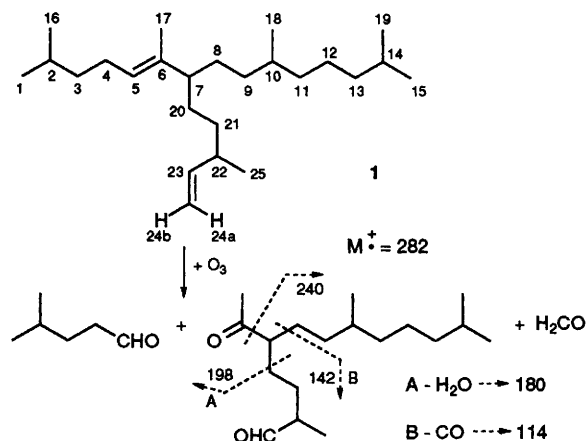


Fig. 2 Fragmentation pathways observed from the mass spectra of **1** and its bis-epoxidation product.



Scheme 1 Reagents and conditions: 0.5 mmol dm⁻³ (CH₂Cl₂), O₃ flow rate 5 cm³ min⁻¹, -70 °C

other C₂₅ 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-¹³C COSY compared with the other sp³ 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 CDCl₃. ¹H and ¹³C. 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); ¹³C{¹H}, DEPT and ¹H-¹³C 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 μ mol MCPBA, 6.25 μ mol **1** in 0.25 cm³ CH₂Cl₂, 25 °C, 90 h.

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