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8β,9α,10β-Rimuane: A Novel, Optically Active, Tricyclic Hydrocarbon of Algal Origin

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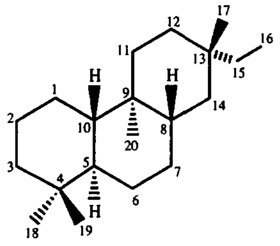
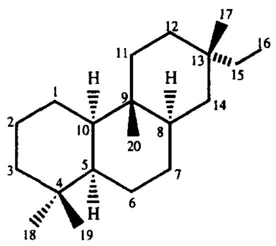
Abstract: The structure of a novel tricyclic diterpane has been determined as 8β,9α,10β-rimuane by NMR spectroscopy and mass spectrometry and an algal origin inferred from its stable carbon isotopic composition. © 1997 Elsevier Science Ltd.

During analyses of the hydrocarbon fractions from the organic extracts of lacustrine sediments from Lake Sokorte Dika, Mt. Marsabit, Kenya, we encountered a novel, dextrorotatory, tricyclic hydrocarbon, which we have identified as 8β,9α,10β-rimuane (**1**).[1] This compound differs stereochemically at C-8, C-9 and C-10 from rimuane (**2**) which has been tentatively found in petroleum,[2] and is thought to be a diagenetically reduced product of rimuene ($C_{20}H_{32}$).[2] Rimuene is a constituent of the conifer *Dacrydium cupressinum* (a species of *Podocarpaceae*),[3-5] but its carbon skeleton can also be formed by acid-catalysed rearrangements of dolabradiene, a constituent of *Thujopsis dolabrata* and *Thuja standishii* (both species of *Cupressaceae*).[6]



(1)



(2)

Lake Sokorte Dika is a small crater lake situated at 1,500 m a.s.l. on the slope of Mt. Marsabit, in the humid montane rain forest. At present, it has a maximum water depth of 1.9 m and pH 7.1 - 8.4. A sediment core, consisting of clayey lake muds and peats, furnishes a sedimentary record of the last 12,000 years (^{14}C dating; Street-Perrott, pers. comm.). About 1.5 m of clayey lake mud exists at the base of the core, with consistently high total organic carbon (TOC) contents (ca. 50 %) and ^{14}C age spanning from ca. 9,300 to 12,000 yr. BP. $\delta^{13}C$ values of TOC vary considerably from -15 ‰ for sediments older than 11,500 yr. BP to -26 ‰ for sediments younger than 10,700 yr. BP, possibly reflecting major changes in the environment. However, the biological source(s) causing such large changes are unknown. Our previous study of ancient (Miocene Clarkia)[7-8] and recent (Sacred Lake, Mt. Kenya)[9-11] lacustrine sediments demonstrated that it was possible to use biomarkers and compound-specific $\delta^{13}C$ analyses to disentangle and attribute the biological origins for the complex mixture of organic compounds present in sediments. For the same purpose, seven sediment samples were solvent-extracted to obtain lipid fractions for molecular and compound specific $\delta^{13}C$ analyses in a similar way as reported previously.[8,11]

The aliphatic hydrocarbon fractions of the sediment extracts, obtained by silica gel column chromatography using hexane as solvent, contain a series of C_{19} to C_{35} *n*-alkanes, varying amounts of triterpenes (mainly botryococcenes and hopenes) and the title compound. The title compound represents a prominent (sometimes the major) peak in the GC chromatograms of the total aliphatic hydrocarbon fractions in all the samples, accounting for from approximately 5 to 20 % of the all the compounds present (concentrations ca. 200 to 500 µg/g dry sediment). GC-MS analyses reveal that the compound has a molecular weight of 276 (accurate mass 276.2821, consistent with a saturated hydrocarbon $C_{20}H_{36}$ with a calculated mass of 276.2817), and a mass spectrum[12] not previously reported in the literature or in the Wiley and NIST mass spectral libraries. The base peak of the mass spectrum, 247 (M^+-29), indicates the presence of an ethyl group on the molecule. This compound was isolated by repeated urea-adduction to remove *n*-alkanes followed by argentatious TLC to remove unsaturated components. The final product has a purity of greater than 95 % (by GC), suitable for NMR characterisation.

Table. NMR chemical shift data for 8β,9α,10β-rimuane (in $CDCl_3$, TMS int. ref.) isolated from sediment of Lake Sokorte Dika, Mt. Marsabit, Kenya.

Carbon No.	DEPT	^1H	^{13}C
1	CH_2	1.71 (eq); 0.89 (ax)	26.03
2	CH_2	1.48 (eq); 1.36 (ax)	22.23
3	CH_2	1.32 (eq); 1.12 (ax)	42.67
4	C	-	33.38
5	CH	1.12 (ax)	45.22
6	CH_2	1.15 (eq); 1.13 (ax)	29.05
7	CH_2	1.74 (eq); 1.04 (ax)	26.93
8	CH	1.16 (ax)	41.24
9	C	-	36.73
10	CH	0.91 (ax)	47.89
11	CH_2	1.54 (eq); 1.09 (ax)	34.81
12	CH_2	1.15 (eq); 1.27 (ax)	32.98
13	C	-	32.92
14	CH_2	0.90 (eq); 1.12 (ax)	40.17
15	CH_2	1.180, 2H, q, 7.6	38.59
16	CH_3	0.810, 3H, t, 7.6	7.69
17	CH_3	0.849, 3H, s	22.37
18	CH_3	0.863, 3H, s	30.90
19	CH_3	0.805, 3H, s	20.32
20	CH_3	0.671, 3H, s	11.72

Proton noise decoupled ^{13}C NMR spectroscopy at 125 MHz (Table) confirmed the presence of twenty carbons, which could be subdivided by a DEPT experiment into five methyls, nine methylenes, three methines and three quaternaries.

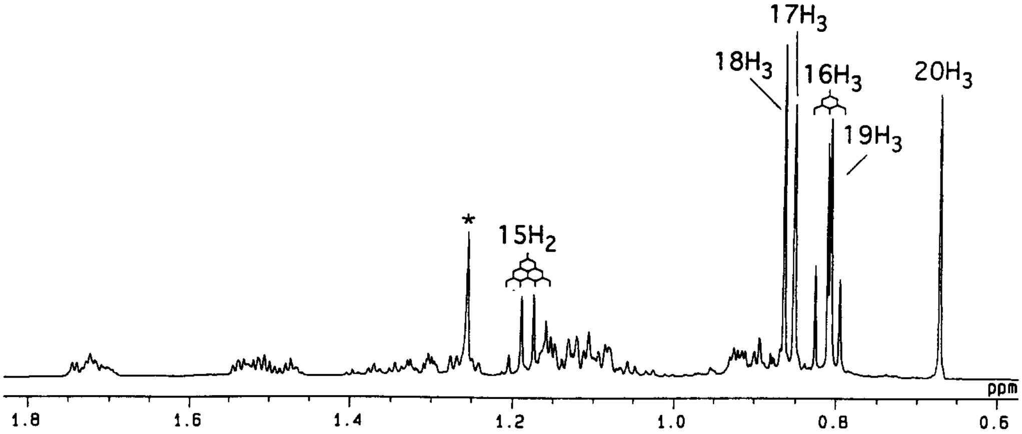


Fig. 1. The 500 MHz ^1H NMR spectrum of 8β,9α,10β-rimuane (**1**). * = impurity

The 500 MHz ^1H NMR spectrum (Fig.1; Table) is characterised by four methyl singlets and an ethyl group (0.810, 3H, t, J= 7.6 Hz, $16H_3$; 1.180, 2H, q, J= 7.6 Hz, $15H_2$). The lack of further coupling (excluding long range effects) to $15H_2$ indicates that the ethyl group is attached to a quaternary carbon. Initial evidence for the assignment of 8β,9α,10β-rimuane (1) came from analysis of a phase-sensitive ^1H-^1H correlation spectrum (PECSY). In particular connectivities were realised from strong long range W couplings from three of the methyl groups ($17H_3$, $19H_3$ and $20H_3$) and from the characteristic cross peaks arising from seven of the possible eight geminal couplings (1axH-1eqH, 2axH-2eqH, 3axH-3eqH, 7axH-7eqH, 11axH-11eqH, 12axH-12eqH and 14axH-14eqH). This experiment indicates the presence of three independent spin systems (ignoring long range effects). One comprises $11H_2$ and $12H_2$ with 11axH displaying a W coupling to $20H_3$ and 12axH a W coupling to $17H_3$ demonstrating that $20H_3$ and $17H_3$ are both axial and are located on opposite faces of the molecule. The second spin system starts from $3H_2$, traces around the A/B ring junction and finishes in the C-ring at $14H_2$. The termini of this spin system are characterised by W couplings: 3axH to $19H_3$ and 14axH to $17H_3$. Interesting observations within this spin system include a strong W coupling between 1eqH and 3eqH and importantly three large couplings (J~13 Hz) to 1axH, which are in accord with a trans A/B ring junction. Similarly the B/C ring junction is also trans shown by three large couplings (J~13 Hz) to 7axH. Within this spin system the similar chemical shifts for 5axH, 6axH and 6eqH presented some initial problems in assignment, which were later established by ^1H-^{13}C correlation techniques. The final spin system is that of the ethyl group, which is located at C-13 from the presence of a W coupling between $15H_2$ and $17H_3$.

Additional structural information was obtained from a long range ^1H-^{13}C correlation experiment (HMBC), which clearly demonstrated the presence of a geminal dimethyl group at C-4 flanked by a methylene carbon (C-3) and a methine carbon (C-5), the presence of a methyl and an ethyl at C-13 flanked by two methylene carbons (C-12 and C-14) and finally the presence of $20H_3$ at C-9 flanked by two methine carbons (C-8 and C-10) and one methylene carbon (C-11). The HMBC data also enabled initial characterisation of the ^{13}C NMR spectrum, which was completed by reference to a one bond ^1H-^{13}C correlation experiment (HSQC), which in itself resolved the uncertainties from the PECSY spectrum regarding the proximity in ^1H chemical shifts of 5axH, 6eqH and 6axH.

Finally a series of nOe difference experiments provided additional information with regard to the trans nature of the ring junctions and the stereochemistry of the methyl groups. Of particular note is irradiation of $20H_3$, which produced clear enhancements of 1axH (dq, J= 3.9 and 12.8 Hz), 1eqH (dt, J= 12.8 and 3.3 Hz), 12axH (dt, J= 4.3 and 13.4 Hz) and 14axH (t, J= 12.5 Hz).

The carbon isotopic compositions of 8β,9α,10β-rimuane (1) in samples with ^{14}C ages older than 11,500 yr BP show extremely heavy $\delta^{13}C$ values[13] of -6 to -8 ‰. The lipids of terrestrial higher plants are alway much lighter than this since they use ambient CO_2 ($\delta^{13}C$ -7 to -8 ‰) as the carbon source and further discriminate against ^{13}C during photosynthesis:[14] typically their lipids are 4 - 5 ‰ (up to 7 - 9 ‰ for *n*-alkanes)[15] more depleted than the bulk biomass.[16] However, algae utilizing dissolved CO_2 can produce a highly ^{13}C enriched biomass when CO_2 is severely limited, and reach values exceeding that of ambient CO_2 when HCO_3^- is used as the carbon source.[17] It can be speculated, therefore, that 8β,9α,10β-rimuane (**1**) is an algal biomarker. Although the nature of the biological precursor to **1** is at present unknown this is the first example of a tricyclic diterpane of algal origin.

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References and Notes

1. Whilst the title compound has an optical rotation of $[\alpha]_D^{26} = +8.2$ (*c* 0.004 $CHCl_3$) we are unable to assign the absolute stereochemistry, hence the enantiomer of (1) is equally likely.
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12. Mass spectrum of the title compound by GC-MS: m/z 54 (27), 69 (25), 81 (26), 97 (34), 109 (35), 123 (35), 137 (20), 151 (26), 165 (10), 177 (11), 191 (12), 247 (100), 261 (10), 276 (14).
13. $\delta^{13}C$ values = $[(^{13}CO_2/^{12}CO_2)_{sample}/(^{13}CO_2/^{12}CO_2)_{standard} - 1] \times 1000$, where standard is the belemnite from the Pee Dee Formation in South Carolina.
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