Identification of 1,6,17,21-Octahydrobotryococcene in a Sediment

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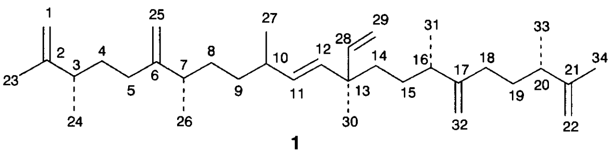
A novel compound, 1,6,17,21-octahydrobotryococcene, apparently derived from the green alga *Botryococcus braunii*, is identified in a sediment from Sacred Lake, Mount Kenya.

The green, colonial, freshwater microalga, *Botryococcus braunii*, is renowned for its capability to produce large quantities (up to 76% of its dry weight[1]) of hydrocarbons. *Botryococcus* is associated with many petroleum-rich deposits[2] and is of interest because of its potential to be a future source of liquid fuel. The B race produces polyunsaturated branched hydrocarbons with general formula C_nH_{2n-10}, $30 \leqslant n \leqslant 37$, collectively termed botryococcenes.[3] The most abundant of these is a C_{34} component, botryococcene, whose structure **1** was first reported by Cox *et al.*[4] It has since been synthesised[5] and its absolute configuration determined.[6] Fully saturated botryococcanes such as **2**, presumably derived from the postgenic reduction of botryococcenes, have been found in several oils or bitumens.[7–9] However, botryococcenes are known for their low resistance to diagenesis since as polyunsaturated compounds they are prone to polymerisation and labile to microbial degradation. For instance, coorongites and torbanites, the fossil remains of *Botryococcus braunii* (referring to race B here) show a complete lack of botryococcenes and botryococcanes.[10,11] In fact, although *Botryococcus* remains are widespread in numerous sediments and kerogens, botryococcanes have only been found in a very limited number of oils and bitumens. This has been an enigma which is further reinforced by the lack in recent sediments containing *Botryococcus* (race B) of any compounds structurally related to botryococcenes.

We report here the unambiguous identification of a novel compound, 1,6,17,21-octahydrobotryococcene **3**, in sediments collected from a tropical freshwater lake, Sacred Lake, which is situated on Mount Kenya, at 2350 m above sea-level in the humid montane rain forest. The sediment horizon (903–904 cm depth) under study has an interpolated ^{14}C age of 14500 yr. BP (last Glacial). Capillary gas chromatography showed a major component comprising more than 60% of the aliphatic hydrocarbon fraction (hexane eluant on Silica gel column) of the solvent extract. This compound was isolated and purified by urea-adduction, $AgNO_3$-impregnated TLC and reversed phase HPLC [C18, 20 cm × 4 mm, Waters; solvent: MeOH–MeCN (4 : 1), 1.5 ml min^{-1}]. The final isolate **3** (2 mg) had a purity of greater than 95% (GC).



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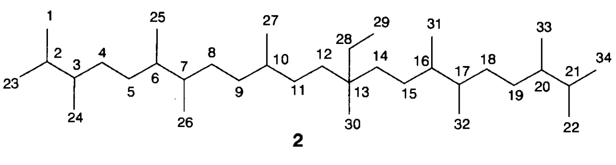

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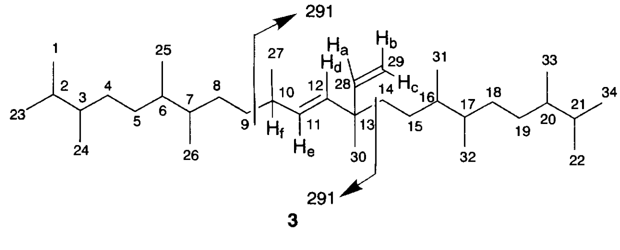

3

The major mass spectral fragments (GC-MS, EI, 70 eV) and intensities (in brackets) of **3** are as follows: *m/z* 57(100), 71(77), 81(90), 95(56), 110(52), 123(41), 137(38), 151(22), 165(11), 207(10), 291(23), 474 (8%). The molecular ion at *m/z* 474 suggests a formula $C_{34}H_{66}$, with two degrees of unsaturation. A characteristic strong ion at *m/z* 291 is derived from the cleavage allylic to the two double bonds (9 and 13), the latter being adjacent to the quaternary carbon. Clearly increased intensities for the ions with a mass difference of 28, representing cleavage at each side of the branch points are observed (*e.g.* 207, 235, 263, 291, 319, 347, 375, 403 and 431; or 250, 278, 306 for another series), characteristic of (methylated) acyclic isoprenoids. The highly branched structure of this C_{34} compound is also suggested by its GC retention time, as it elutes just after C_{28} *n*-alkane.

The olefinic region of the ^1H NMR (400 MHz, $CDCl_3$) of **3** shows five signals, corresponding to the protons on C-28 (H_a: δ 5.775, 1H, dd, J_{ab} = 10.8, J_{ac} = 17.4), C-29 (H_b: δ 4.930, 1H, dd, J_{ba} = 10.8, J_{bc} = 1.6; and H_c: δ 4.908, 1H, dd, J_{ca} = 17.5, J_{cb} = 1.5), C-12 (H_d: δ 5.290, 1H, d, J_{de} = 15.9) and C-11 (H_e: δ 5.131, 1H, dd, J_{ed} = 15.9, J_{ef} = 7.7). The coupling constant J_{de} = 15.9 indicates a trans-double bond at C-11–C-12. The ^1H–^1H COSY spectrum of the compound confirms the above assignments.

Use of ^{13}C NMR, DEPT and ^1H–^{13}C correlation and comparison with literature data[4,11] allowed complete assignment of ^{13}C chemical shifts of the molecule **3**. The ^{13}C NMR (125.1 MHz, $CDCl_3$) showed 34 signals (12 CH_3, 9 CH_2, 12 CH and 1 quaternary carbon by DEPT), all with shifts which agreed with either literature values for similar carbons in botryococcene[4] or C_5–C_9 paraffins,[12] or with calculated values (calculation based on $\delta_C = -2.5 + 9.1\ \alpha_n + 9.4\ \beta_n - 2.5\ \gamma_n + 0.3\ \delta_n + 0.1\ \varepsilon_n + \Sigma S$). The four expected olefinic carbons are observed at δ 110.94 (CH_2), 147.06 (CH), 135.94 (CH) and 133.77 (CH). For all except the quaternary carbon and two others (CH groups), ^1H correlations were observed in a ^1H–^{13}C correlation spectrum at expected ^1H chemical shifts.

In order to confirm further the identification, the compound was hydrogenated (1 atm H_2, PtO_2, EtOAc, 4 h). An authentic sample of C_{34} botryococcane was prepared by hydrogenation of the C_{34} botryococcene (isolated from modern *Botryococcus*). The reduced product and the C_{34} botryococcane have identical mass spectra (confirmed by lit.)[1,7] and coelute on GC (column: HP Ultra-1, capillary fused silica, 17 μm film thickness, 50 m × 0.32 mm).

The 1,6,17,21-octahydrobotryococcene identified in this study shows no evidence of the presence of more than one diastereomer. The ^{13}C NMR spectra shows only 34 signals as expected for the 34 carbons in the molecule. The gas chromatogram shows a single sharp peak, as does its hydrogenated counterpart. However, botryococcane from hydrogenation of algal C_{34} botryococcene displays two partially separated peaks (corresponding to the diastereomers generated at C-6 and C-17), with the sedimentary botryococcane from reduction of **3** coeluting with the first peak.

The occurrence of a single diastereoisomer of 1,6,17,21-octahydrobotryococcene is inconsistent with reduction of the double bonds at the C-1,6,17,21 in C_{34} botryococcene **1** *via* a non-biological hydrogenation in the sediment. Moreover, molecular modelling for the C_{34} botryococcene **1** indicates that the vinyl group at C-28 is highly exposed and would therefore be expected to be reduced prior to the other double bonds, as has been shown by di-imide reduction.[1,4]

Examination of a slightly more polar fraction [hexane–CH_2Cl_2 (6:1) eluant] of the sediment extract indicated the presence of C_{34} botryococcene **1** and several other isomers with the same molecular weight (466), though in much lower abundance than **3**. Since *Botryococcus* is the only known biological source of botryococcene **1**, an input from this alga to the sediment is clearly evidenced.

The stereoisomer specificity of **3** in the sediment may have been directly produced by *Botryococcus* under specific environmental conditions, *i.e.* direct methylation rather than methylenation of a C_{30} precusor. Enzymatic reduction could have occurred specifically at C-1,6,17,21 of botryococcene **1**. Another possibility is the reduction by microbiological processes in the sediment, which are largely unknown.

The compound **3** in Sacred Lake sediment would probably have a higher chance of surviving polymerisation and microbial degradation than botryococcene itself **1**, and may eventually give rise to the fully saturated botryococcane **2** over prolonged geological burial. Hence, even though **1** may be polymerised or degraded in the early stages of diagenesis, the botryococcane **2** in oils and bitumens may be derived from 1,6,17,21-octahydrobotryococcene **3** when it is formed under specific conditions.

By using recent technology (GC-IRMS), we were able to determine the carbon isotopic composition of **3**. The δ value of $-10.3‰$ *vs.* PDB has important relevance to the strong isotopic shifts in bulk organic matter recorded.[13]

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