ORGANIC LETTERS

2000
Vol. 2, No. 9
1271−1274

7,11-Cyclobotryococca-5,12,26-triene, a Novel Botryococcene-Related Hydrocarbon Occurring in Natural Environments

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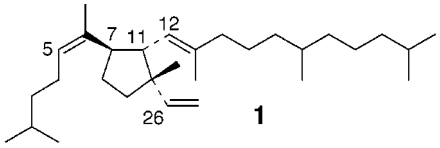
Received February 21, 2000

ABSTRACT



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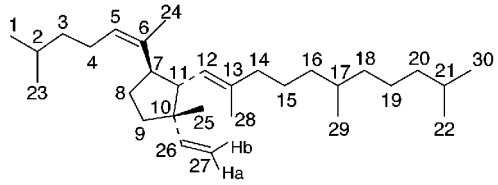
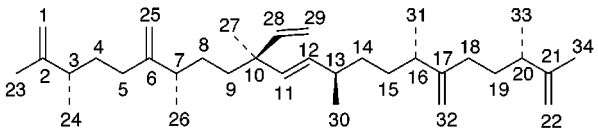
With the unambiguous structural characterization of 7,11-cyclobotryococca-5,12,26-triene 1 by NMR, a novel C_{30} botryococcene-related hydrocarbon skeleton bearing a midchain five-membered ring has been identified in organic-rich sediments from Lake Cadagno (Switzerland). This compound may originate from the green alga *Botryococcus braunii* (race B) widespread in recent and past environments. Alternatively, it may also be a yet unreported lipid of green sulfur photosynthetic bacteria (*Chlorobiaceae*) or a microbial transformation product of the farnesol produced by the latter.

The green alga *Botryococcus braunii*, widespread in freshwater, brackish, and saline environments, significantly contributes to the formation of oil-bearing rocks and boghead coals due to its ability to produce high quantities of hydrocarbons (up to 86% dry weight).[1,2] *B. braunii* algae can be subdivided into three races on the basis of the type of hydrocarbons biosynthesized: race A produces long chain *n*-alkadienes and *n*-alkatrienes, and race L and race B produce lycopadiene and botryococcene hydrocarbons, respectively. Since the first elucidation of the hydrocarbon skeleton of a C_{34} homologue from the botryococcene series,[2] numerous aliphatic (e.g., compound **2**) and monocyclic botryococcene derivatives with the generic structure C_nH_{2n-10} ($30 \leq n \leq 36$) have been identified in the living alga.[3]



7,11-cyclobotryococca-5,12,26-triene **1**



C_{34} botryococcene **2**

Surprisingly, despite the wide abundance of the living alga in recent ecosystems and the ubiquitous presence of microfossil remains of *B. braunii* in recent and past organic-rich sediments, there are only a few studies reporting on the occurrence of botryococcene hydrocarbons among sedimentary lipids,[4] and there is only one example in the literature in which monocyclic polyunsaturated botryococcene deriva-

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(1) Brown, A. C.; Knights, B. A.; Conway, E. *Phytochemistry* **1969**, *8*, 543−547.
(2) Maxwell, J. R.; Douglas, A. G.; Eglinton, G.; McCormock, A. *Phytochemistry* **1968**, *7*, 2157−2171.

10.1021/ol0056980 CCC: $19.00 © 2000 American Chemical Society
Published on Web 04/14/2000

tives (i.e., sacredicene A and B) have been unambiguously identified in recent lacustrine sediments from Kenya.[4a,b]

We report here on the identification of a monocyclic C_{30} botryococcene derivative (compound **1**) bearing a midchain five-membered ring. This compound was isolated from the solvent extract (solvent extraction: CH_3OH/CH_2Cl_2 1:2) of a recent lacustrine sediment (Lake Cadagno, Switzerland[5]) and unambiguously characterized by NMR studies.

GC-MS investigation of the apolar hydrocarbon fraction obtained by silica gel liquid chromatography (eluent hexane/CH_2Cl_2 8:2; 3% yield relative to the extract after removal of native sulfur) of the solvent extract of a recent sulfur- and organic-rich sediment revealed the presence of several C_{30} polyunsaturated hydrocarbons of unknown structure. Among them, one compound showed a molecular ion at *m/z* 414 indicative of a C_{30} hydrocarbon skeleton bearing four unsaturations (Figure 1a). Since no further structural information could be obtained from the fragmentation pattern in mass spectrometry, isolation of compound **1** by reversed phase HPLC was undertaken.[6] Catalytic hydrogenation (H_2/PtO_2, AcOEt, reflux, 3 h) of an aliquot of the purified compound yielded one major isomeric pair of compounds characterized by a molecular ion at *m/z* 418 ($C_{30}H_{58}$)[7] and one minor pair of isomers with a molecular ion at *m/z* 420 ($C_{30}H_{60}$; Figure 1b). When catalytic hydrogenation was carried out under more drastic conditions (H_2/PtO_2, AcOEt/AcOH 1:2, reflux, 3 h), the concentration of the latter increased markedly, indicating most likely a monocyclic C_{30} hydrocarbon skeleton.

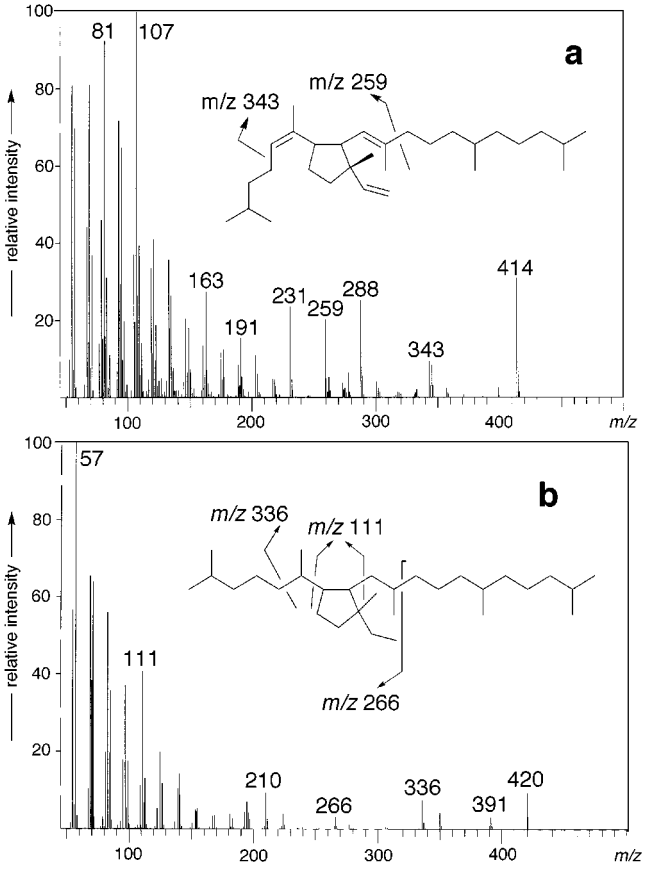

Figure 1. Mass spectra of 7,11-cyclobotryococca-5,12,26-triene **1** (a) and of the saturated hydrocarbon recovered upon catalytic hydrogenation of compound **1** (b).

Finally, sufficient amounts (ca. 500 μg) of pure compound **1** have been isolated, enabling unambiguous structural identification by 1D and 2D heteronuclear (HMBC, HSQC) and homonuclear (COSY, NOESY) NMR studies as C_{30} 7,11-cyclobotryococca-5, 12, 26-triene **1**. Given the presence of eight methyl, eleven methylene, eight methine, and three quaternary atoms, the structure could be established by means of the HMBC and $^1H-^1H$ COSY experiments (Table 1).

Table 1. ^1H (500 MHz) and ^{13}C (125.8 MHz) NMR Data for 7,11-Cyclobotryococca-5,12,26-triene **1** ($CDCl_3$, 293 K)

C	δ ^{13}C, ppm	δ ^1H, ppm	C	δ ^{13}C, ppm	δ ^1H, ppm
[a]1	22.52	0.861	16	36.41	1.21 1.03
[b]2	27.49	1.51	17	32.67	1.35
[c]3	39.63	1.13	18	37.35	1.23 1.04
[d]4	25.29	1.9−2.0	[d]19	25.25	1.30−1.35
5	126.63	5.09	[c]20	39.36	1.13
6	135.68		[b]21	27.97	1.51
7	44.71	2.92	[a]22	22.61	0.860
8	27.20	1.76 1.53	[a]23	22.65	0.856
9	37.53	1.85 1.54	24	18.93	1.60
10	49.54		25	25.66	1.04
11	52.90	2.40	26	144.10	5.90
12	124.67	4.78	27	111.29	4.99 (H_a) 4.93 (H_b)
13	136.79		28	16.33	1.57
14	40.35	1.94	29	19.66	0.83
15	25.79	1.25−1.40	[a]30	22.67	0.853

[a−d] Values may be interchanged.

The *R**,*S**,*R** relative configuration of the chiral centers at C-7, C-10, and C-11 could be assigned given the presence of NOE effects between H-11/25-CH_3 and H-7/H-26. The

(3) See, for example: (a) Cox, R. E.; Burlingame, A. L.; Wilson, D. M.; Eglinton, G.; Maxwell, J. R. *J. Chem. Soc., Chem. Commun.* **1973**, 284−285. (b) Metzger, P.; Casadevall, E.; Pouet, M. J.; Pouet, Y. *Phytochemistry* **1985**, *24*, 2995−3002. (c) David, M.; Metzger, P.; Casadevall, E. *Phytochemistry* **1988**, *27*, 2863−2867. (d) Huang, Z.; Poulter, C. D. *J. Org. Chem.* **1988**, *53*, 5390−5392. (e) Galbraith M. N.; Hillen, L. W.; Wake, L. V. *Phytochemistry* **1983**, *22*, 1441−1443. (f) Murakami, M.; Nakano, H.; Yamaguchi, K.; Konosu, S.; Nakayama, O.; Matsumoto, Y.; Iwamoto, H. *Phytochemistry* **1988**, *27*, 455−457.

(4) See, for example: (a) Huang, Y.; Murray, M.; Eglinton, G. *Tetrahedron Lett.* **1995**, *36*, 5973−5976. (b) Huang, Y.; Murray, M.; Metzger, P.; Eglinton, G. *Tetrahedron* **1996**, *52*, 6973−6982. (c) Moldowan, J. M.; Seifert, W. K. *J. Chem. Soc., Chem. Commun.* **1980**, 912−914. (d) Grice, K.; Schouten, S.; Nissenbaum, A.; Charrach, J.; Sinninghe Damsté, J. S. *Org. Geochem.* **1998**, *28*, 195−216.

(5) Details on the biogeochemistry of Lake Cadagno are provided in, e.g., Putschew, A.; Scholz-Böttcher, B. M.; Rullkötter, J. *Org. Geochem.* **1996**, *25*, 379−390.

(6) Subfractions enriched in compound **1** were obtained by silica gel liquid chromatography (eluent hexane) and were further subjected to two successive reverse-phase HPLC purification steps (DuPont Zorbax ODS, 5 μm, 70 Å; acetone/CH_3OH 1:1; acetone/CH_3OH 3:7). A final cleanup step using thin-layer chromatography over $AgNO_3$-impregnated (10%) silica gel (eluent hexane/CH_2Cl_2 1:1) yielded pure compound **1**.

presence of a NOE effect between H-12/H-14 indicates a Δ^{12} *E* double bond, whereas the NOE effect observed between H-5/24-CH_3 shows a Δ^5 *Z* double bond.

This identification represents the first report of a C_{30} botryococcene hydrocarbon bearing a midchain five-membered ring. Similar to sacredicenes A and B,[4a,b] only three double bonds are present, in contrast to botryococcene hydrocarbons found in *B. braunii* race B, including the most easily reducible Δ^{26} exomethylenic double bond. Huang et al. proposed that the sedimentary sacredicenes A and B might represent original biosynthetic products.[4a] Alternatively, they suggested that these compounds could be formed by the partial reduction of the double bonds of more functionalized precursors via microbiologically induced processes.[4b] Regarding the mode of formation of 7,11-cyclobotryococca-5,12,26-triene **1**, a slight splitting of the 29-CH_3 signal in the ^{1}H NMR spectrum (δ ^{1}H = 0.826 and 0.828 ppm for the major and minor signal, respectively) suggests that two diastereomers are present in a 9/1 ratio, indicating that the reduction of double bonds proceeded enzymatically rather than via abiotic diagenetic processes. In the latter case indeed, a nonspecific reduction would be expected with the subsequent formation of diastereomers in a ca. 1:1 ratio.

In this connection, Requejo et al. reported on the presence in an estuarine sediment (Narragansett Bay, RI) of an unknown C_{30} polyunsaturated hydrocarbon with a mass spectrum close (but not identical) to the mass spectrum of 7,11-cyclobotryococca-5,12,26-triene **1** (Figure 1a).[8] Upon catalytic hydrogenation (H_2/PtO_2, hexane, 25 °C), they obtained two isomers characterized by exactly the same mass spectra as those of the partially hydrogenated derivatives of compound **1** (M^+ 418).[7] They proposed a bicyclic hydrocarbon skeleton for this unknown C_{30} unsaturated hydrocarbon. On the basis of the results of our hydrogenation, it is, however, likely that their compound has the same hydrocarbon skeleton as 7,11-cyclobotryococca-5,12,26-triene **1**, the slight variations observed in the respective mass spectral fragmentation pattern being possibly due to differences in the location of the double bonds.

Concerning its possible biosynthesis, it appears that, similar to acyclic botryococcene derivatives (e.g., compound **2**), compound **1** may be formed from presqualene diphosphate **3**, as illustrated in Figure 2.[9] However, unlike the former case, in which the Δ^{12} double bond is implicated in the formation of the botryococcene hydrocarbon skeleton, the Δ^7 double bond would be involved in the formation of the five-membered ring moiety from compound **1**. It is also noteworthy that the relative configurations of the C-10 and C-11 centers of compound **1** (i.e., CH_3-25 and H-11 on the same side of the cyclopentane moiety) suggest that the cyclization and cyclopropane ring opening steps occurring

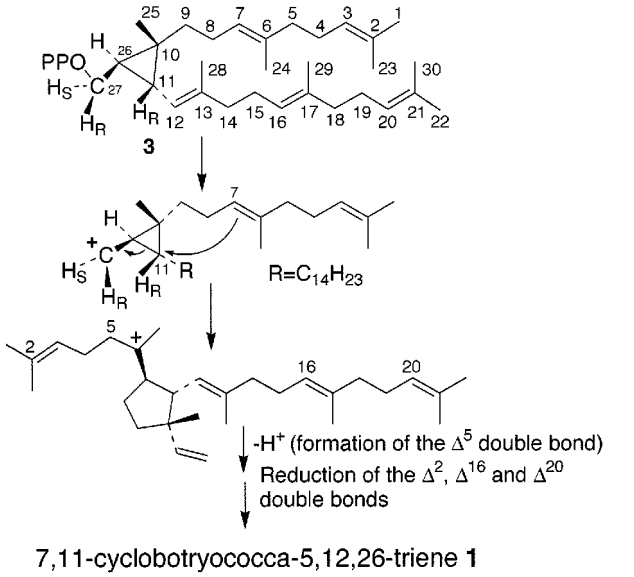

Figure 2. Possible mode of formation of 7,11-cyclobotryococca-5,12,26-triene **1** from presqualene diphosphate **3**.

on presqualene precursor **3** proceed via a concerted mechanism.

Regarding the potential biological precursors of 7,11-cyclobotryococca-5,12,26-triene **1**, *B. braunii* alga can be proposed on the basis of structural grounds. This would be in good agreement with the report of the occurrence of *B. braunii* among the microalgal community from Lake Cadagno.[10] However, C_{32}−C_{36} botryococcene hydrocarbons are usually predominant over lower homologues in both living algae and sedimentary organic matter,[2−4] and it is therefore surprising that only a C_{30} compound was detected in Lake Cadagno. The stable carbon isotopic composition[11] measured for 7,11-cyclobotryococca-5,12,26-triene **1** (δ ^{13}C = −27‰) was found to be enriched in ^{13}C relative to compounds of algal origin (δ ^{13}C between −32‰ and −35‰) in the sediment from Lake Cadagno. This value is close to the values determined for compounds from green sulfur photosynthetic bacteria (i.e., *Chlorobiaceae*[12]), including farnesol, the latter being the major alcohol esterifying the bacteriochlorophylls *c*, *d*, and *e* from *Chlorobiaceae*.[13] Alternative to a biosynthetic origin from *B. braunii*, it can therefore be envisaged that 7,11-cyclobotryococca-5,12,26-triene **1** identi-

(7) MS data (EI, 70 eV) of the partially hydrogenated 7,11-cyclobotryococca-5,12,26-triene derivative (major isomer): 418 (M^+, $C_{30}H_{58}$, 21%), 389 (5), 361 (1), 333 (10), 305 (4), 263 (4), 235 (13), 193 (100), 137 (18), 123 (41), 83 (73), 69 (75).

(8) Requejo, A. G.; Quinn J. G. *Geochim. Cosmochim. Acta* **1983**, *47*, 1075−1090.

(9) Huang, Z.; Poulter, C. D. *J. Am. Chem. Soc.* **1989**, *111*, 2713−2715. Poulter, C. D. *Acc. Chem. Res.* **1990**, *23*, 70−77.

(10) Bachmann, H. *Schweiz. Z. Hydrol.* **1928**, *5*, 1−120.

(11) The stable carbon isotopic composition (δ ^{13}C) of lipids depends on the δ ^{13}C value of the carbon source(s) and on the biosynthetic pathway used by living organisms for carbon fixation (e.g., de Leeuw, J. W.; Frewin, N. L.; van Bergen, P. F.; Sinninghe Damsté, J. S.; Collinson, M. E. In Marine palaeoenvironmental analysis from fossils. Bosence, D. W. J., Allison, P. A., Eds. *Geol. Soc. Spec. Publ.* **1995**, *83*, 43−71). Recent advances in determining the stable carbon isotopic composition of individual components by gas chromatography−isotope ratio monitoring mass spectrometry (GC-irmMS) give the possibility of establishing links between sedimentary lipids and their biological precursors. δ ^{13}C = $10^3[(R_x - R_s)/R_s)]$, where R = ^{13}C/^{12}C, x = sample, s = PDB standard, and R_s = 0.0112372.

(12) *Chlorobiaceae* are strict anaerobes thriving in the anoxic photic zone of the water column from Lake Cadagno (see: Schaeffer, P.; Adam, P.; Wehrung, P.; Albrecht, P. *Tetrahedron Lett.* **1997**, *38*, 4921−4924).

fied in Lake Cadagno may represent a yet unexpected constituent of *Chlorobiaceae* or may be formed by an anaerobic microorganism re-utilizing the farnesol produced by *Chlorobiaceae*.

(13) Brock, T. D.; Madigan, M. T. In *Biology of microorganisms*; Prentice Hall: Englewood Cliffs, NJ, 1988; pp 552−559.

Acknowledgment. We thank E. Mastio and P. Wehrung for the GC-MS and GC-irmMS analyses, R. Graff for the NMR measurements, and Dr. J. Blanzat for his contribution to the sampling of the sediments from Lake Cadagno.

OL0056980