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STRUCTURES OF SOME BOTRYOCOCCENES: BRANCHED HYDROCARBONS FROM THE B-RACE OF THE GREEN ALGA *BOTRYOCOCCUS BRAUNII*

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Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; botryococcenes; triterpenoid hydrocarbons; structural determination.

Abstract—Nine branched hydrocarbons of the botryococcene type (C_nH_{2n-10} $30 \leqslant n \leqslant 37$) have been isolated from the green alga *Botryococcus braunii*. Hydrocarbon mixtures were recovered from wild algae collected in fresh water lakes or from the same strains growing in laboratory; they were further separated by reversed-phase, and in some cases by normal phase, HPLC. From chemical investigations, GC/MS analyses, ^{1}H and ^{13}C NMR spectroscopy, the structures of four new botryococcenes (one $C_{33}H_{56}$, two $C_{34}H_{58}$ and one $C_{37}H_{64}$) were elucidated.

INTRODUCTION

The analysis of the hydrocarbons produced by several strains of *Botryococcus braunii*, originating from various fresh water lakes and grown in laboratory had shown that this alga includes two races indexed under the same name [1] and of rather similar ultrastructures [2, 3]. Each of them synthesizes a well-defined class of hydrocarbons, *n*-alkadienes and trienes, odd numbered from 23 to 31, for the A race, polyunsaturated and branched hydrocarbons, of general formula C_nH_{2n-10}, $30 \leqslant n \leqslant 37$, termed botryococcenes,‡ for the B race. Therefore, the same strain is unable to yield successively the two types of hydrocarbons, contrary to previous proposals [4].

In spite of a cosmopolitan distribution of *B. braunii* [5] and of the occurrence of massive blooms, very few detailed analyses have been reported on the hydrocarbon composition of wild samples [1, 6, 7]. It does appear that botryococcene mixtures can exhibit a large variability—different molecular mass and numerous isomers—in connection with genetical and physico-chemical factors [1]. Moreover the difficulties encountered in the structural determination of botryococcenes is certainly related to the inability of classical chromatographic techniques to separate on a preparative scale these components from complex mixtures. Among the thirty components up to now identified by GC/MS, only six structures have been determined, including two C_{34} isomers-'botryococcene' (**6A**) [8] (Scheme 1, Table 1) and 'isobotryococcene' (**5A**) [9], one C_{36} compound **8** called 'darwinene' [9] and three compounds, C_{30} (**1**), C_{31} (**2**), C_{32} (**3**), for which an NMR investigation was reported [10].

The structure of four new botryococcenes: one C_{33} **4**, two C_{34} **5B** and **7** and one C_{37} (**9**) is presented here. The determinations are based on evidence drawn from ^{1}H and ^{13}C NMR spectra and from mass spectrometry of the fully hydrogenated botryococcene derivatives and of the products arising from oxidative cleavage. A revised structure for a C_{31} compound is also proposed. The structural data concerning the previously described botryococcenes are included to allow for comparison. The separation of botryococcenes by HPLC has greatly facilitated the structural elucidations.

RESULTS

Strain origins and botryococcene isolations

Botryococcenes were extracted from four samples (A–D, Table 1); A and B were directly collected from nature, C and D correspond to strains growing in the laboratory [1, 10]. In this table are included the compositions of the botryococcene mixtures; the molecular mass was obtained from GC/MS analysis.

Owing to their high concentrations in some samples, three hydrocarbons—**5** (sample B), **4** (sample C) and **3** (sample D)—were analysed by NMR without additional purification other than elution of the crude extracts from an alumina column with hexane; attempts to isolate the other compounds in a pure form on $AgNO_3$–silica gel plates were unsuccessful. Since reversed-phase HPLC has already been shown to be useful for separating various lipids, such as carotenoids [11] or triacylglycerols [12], this technique was tried.§ When botryococcenes differing from one another only by their M_r as in sample D, their separation could be achieved using a reversed-phase C_{18} column (see Experimental). With the analytical system

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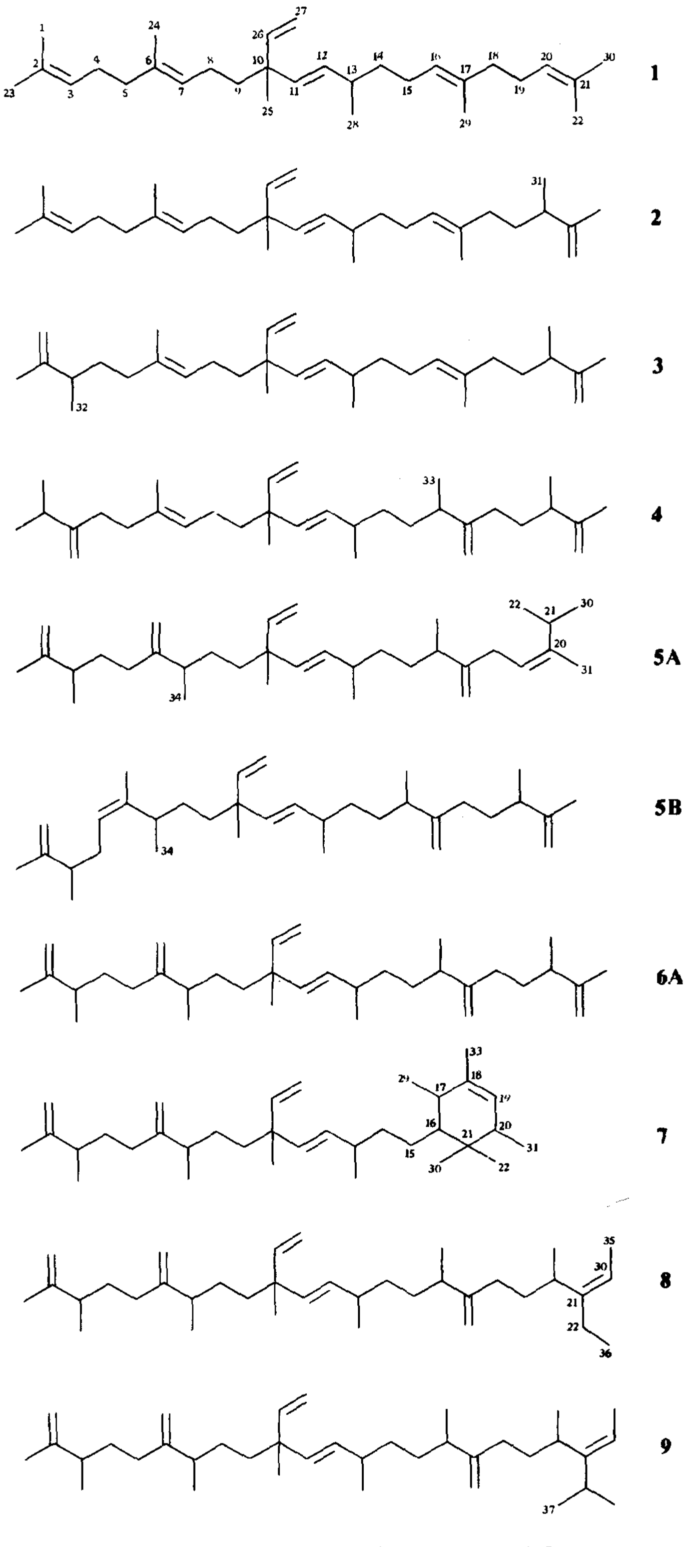
‡'Botryococcene' was the name formerly given to the first discovered C_{34} compound of the series [7]. Taking into consideration the common structural features of all the afore-mentioned compounds, we prefer to name botryococcenes all the hydrocarbons of this family.

§This work was coming to an end when a recent paper showed the advantage of reversed-phase HPLC to separate *n*-alkadienes and trienes produced by the A race of *B. braunii* [13].



Scheme 1. Structures of botryococcenes 1–9.

employed, the total amount of hydrocarbons that could be collected after injection was *ca* 2 mg; repeated injections were necessary to obtain sufficient amounts for detailed analysis.

However, application of the HPLC technique to the most complex mixture (sample A), containing mainly five hydrocarbons including three C_{34} isomers, resulted in a poorly resolved chromatogram. In this case, three eluates were recovered. While the last peak afforded the pure C_{37} botryococcene (9), the first and second peaks

Table 1. Sample origin, GC-MS analyses and compositions of the botryococcene mixtures (% of the whole)

C_nH_{2n-10}			Wild samples		Laboratory cultures*	
			A	B	C	D
		Relative retention time/	Darwin (Australia, November	Martinique-Paquemar (West Indies,	Martinique-La Manzo (West Indies)	Martinique-La Manzo (West Indies)
n	Compound No.	squalene	1981)	May 1982)	strain MLM1	strain MLM3
30	**1**	0.760				7
31	**2**	0.770				10
32	**3**	0.870				83
33	**4**	0.887			86	
34	**5**	0.814	8†	72†		
34	**6**	0.847	19	9		
34		0.870			11	
34	**7**	0.912	15			
36	**8**	1.175	35			
37	**9**	1.263	10			
Other botryococcenes			13	19	3	—

*Culture conditions: see Experimental. From La Manzo lake two strains of botryococcene-producing algae have been isolated with a rather stable botryococcene composition under well defined culture conditions (MLM1, here studied, and MLM2 [1]). A third strain, MLM3, unlike these two last, has shown after growing one year in laboratory, a modification in its botryococcene composition. Presently it yields essentially C_{34} hydrocarbons.

†In spite of identical RR_t, these two compounds have different structures: **5A** and **5B**.

corresponded to the coelutions of **5A** + **6** (two C_{34}) and **7** + **8** (one C_{34} and one C_{36}). In spite of this poor resolution some structural information could be obtained, for it has been previously shown, on one hand, that the two coeluted C_{34} compounds, **5A** and **6**, differ only by double bond isomerism [6, 7, 9] and on the other hand, that in reversed-phase HPLC, double bond location has a negligible influence on the retention times of isomers [14]. Therefore, the hydrocarbon **7** must present some structural features very different from those of the two other C_{34} isomers. The hydrocarbons **7** and **8** were further separated by normal phase HPLC, using the peak shaving-recycle technique [15].

GC/MS

Mass spectra of botryococcenes give little structural information. The use of chemical ionization with ammonia as the reagent gas resulted in $[M+1]^+$ and $[M+18]^+$ peaks. When using electron impact ionization, peaks were observed at m/z $[M]^+$ and $[M-15]^+$ (loss of a methyl group); lower m/z peaks were not characteristic of any one botryococcene.

Previously, it was shown that with the fully saturated form—'botryococcane' C_{34} [16] and 'darwinane' C_{36} [9]—the size of the alkyl groups attached to the quaternary carbon could be inferred from ion doublets of high mass. Eight botryococcanes, **1H**–**5H** and **7H**–**9H**, were recovered after catalytic hydrogenation; **5A**, **5B**, **6A** and **6B** yielded the same saturated hydrocarbon **5H**. Their mass spectra did not show any peaks at m/z $[M]^+$ and $[M-H]^+$. All botryococcanes exhibited series of three doublets in the mass region $m/z > 200$ (Table 2). They could be related to the losses of one ethyl group and two alkyl chains bound to a quaternary carbon. For **1H**, these peaks were at m/z 393, 392 $[M-C_2H_5]^+$, $[M-C_2H_5-H]^+$; 267, 266 $[M-C_{11}H_{23}]^+$, $[M-C_{11}H_{23}-H]^+$; 211, 210 $[M-C_{15}H_{31}]^+$, $[M-C_{15}H_{31}-H]^+$. In the spectra of higher botryococcanes, the shifts of the lowest doublets were indicative of an elongation of the alkyl chains R^1 and R^2 by methylene units (Scheme 2). Thus one CH_2 unit has been incorporated into R^1 in **3H** and **4H** and to R^2 in **2H** and **3H**, two to R^1 in **5H**, **7H**, **8H** and **9H** and to R^2 in **4H**, **5H** and **7H** and four and five, respectively, to R^2 in **8H** and **9H**.

From these data, it also appeared that the botryococcane **7H** did not originate from the reduction of six double bonds as all the other saturated hydrocarbons of this series. Accordingly, the peak $[M-C_2H_5]^+$ was at m/z 447, compared with 449 for its C_{34} homolog **5H**, and the second doublet was also shifted down by two mass units. Furthermore the retention of the fragment at m/z 239, 238 established that **7H** contained a ring moiety in the R^2 group. A fragment at m/z 153, absent in the spectrum of **5H**, and some biosynthetic considerations further developed, suggested the possible existence of a pentamethylated cyclohexyl group.

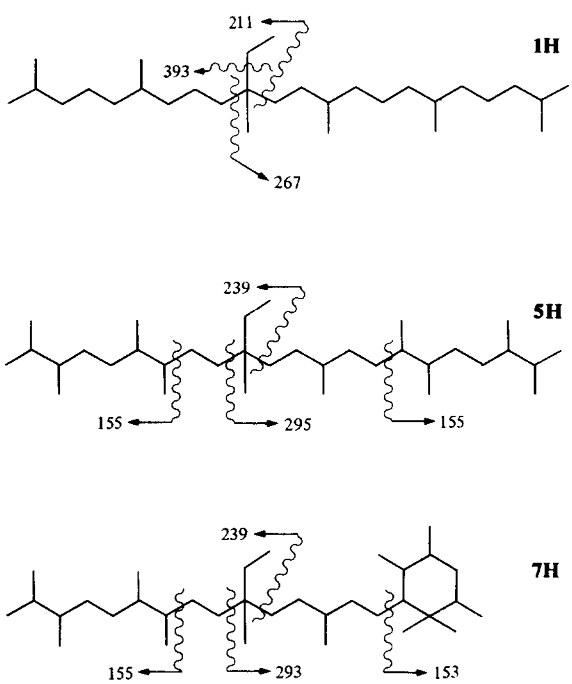
^{1}H NMR

The C_{30} botryococcene **1**, exhibited the simplest spectrum in the series, with eight methyl groups. In the vinylic methyl region, two signals at δ1.56 (four methyls) and 1.65 (two methyls) were consistent with the assignments **10** and **11** as for squalene (Scheme 3). The higher field region showed two signals at δ1.06 (s) and 0.98 (d, $J = 6$ Hz),

Table 2. Ion doublets in botryococcane spectra (electron impact ionization)

Botryococcanes	Ion doublets*
1H $C_{30}H_{62}$ M=422	393, 392 $[M-C_2H_5]^+$; 267, 266 $[M-C_{11}H_{23}]^+$; 211, 210 $[M-C_{15}H_{31}]^+$
2H $C_{31}H_{64}$ M=436	407, 406 $[M-C_2H_5]^+$; 281, 280 $[M-C_{11}H_{23}]^+$; 211, 210 $[M-C_{16}H_{33}]^+$
3H $C_{32}H_{66}$ M=450	421, 420 $[M-C_2H_5]^+$; 281, 280 $[M-C_{12}H_{25}]^+$; 225, 224 $[M-C_{16}H_{33}]^+$
4H $C_{33}H_{68}$ M=464	435, 434 $[M-C_2H_5]^+$; 295, 294 $[M-C_{12}H_{25}]^+$; 225, 224 $[M-C_{17}H_{35}]^+$
5H $C_{34}H_{70}$ M=478	449, 448 $[M-C_2H_5]^+$; 295, 294 $[M-C_{13}H_{27}]^+$; 239, 238 $[M-C_{17}H_{35}]^+$
7H $C_{34}H_{68}$ M=476	447, 446 $[M-C_2H_5]^+$; 293, 292 $[M-C_{13}H_{27}]^+$; 239, 238 $[M-C_{17}H_{33}]^+$
8H $C_{36}H_{74}$ M=506	477, 476 $[M-C_2H_5]^+$; 323, 322 $[M-C_{13}H_{27}]^+$; 239, 238 $[M-C_{19}H_{39}]^+$
9H $C_{37}H_{76}$ M=520	491, 490 $[M-C_2H_5]^+$; 337, 336 $[M-C_{13}H_{27}]^+$; 239, 238 $[M-C_{20}H_{41}]^+$

*The second values correspond to $[M-R-H]^+$.



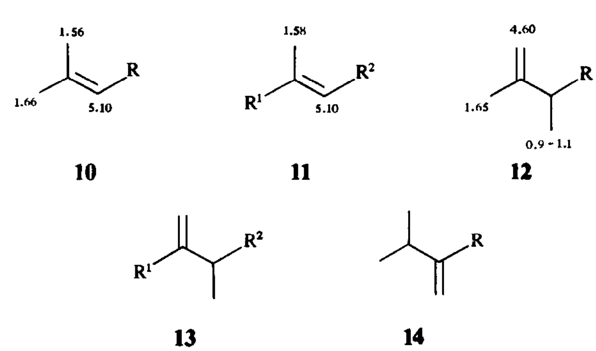
Scheme 2. Main fragmentation of botryococcanes R^1–$C(C_2H_5)(CH_3)$–R^2.

respectively, ascribable to methyl groups borne by quaternary and methine carbons. The spectrum also exhibited two poorly resolved signals at δ2.00 and 1.25, typical of allylic protons (13 protons) and non-allylic protons (four protons). The olefinic region at δ5.10 integrated for two less $RCH_3C{=}CHR'$ groups than squalene. Additional signals at δ4.80, 4.90 and 5.86, one terminal double bond, and at δ5.23, one disubstituted double bond $RCH{=}CHR'$ compensated for this loss.

From the comparison of this spectrum with those of **2**, **3**, **4** and **6**, it appeared that there was a gradual and concomitant collapse of signals at δ5.10 and 1.56 which became complete for **6**. This modification was related to the appearence and regular increase of an exomethylene signal at δ4.60 (**12**, **13**, Scheme 3). On going from C_{30} to C_{34} further peaks in the region 0.9–1.1 were indicative of additional methyl groups. These data established that methylation has successively occurred on the four trisubstituted double bonds of **1** (**10** and **11**, Scheme 3). The comparison of signals at δ1.65, 1.56 and 4.60 showed also that **4**, which has three additional methyl groups, contained an isopropyl terminal group **14**, as commonly encountered for the sterol side chain.

For the three C_{34} isomers, **5B**, **6A** and **7**, the most important differences concerned the exomethylene signals at δ4.60. Intensity measurements established the existence of three, four and two $R_2C{=}CH_2$ groups, respectively. For **5B**, a resonance between δ2.4–2.8 was consistent with a *cis*-α-methine.

The highest botryococcene **9** had a spectrum very similar to that already described for **8** [9]. Both retained a *cis*-α-methine signal at δ2.5–2.8 and three exomethylenes at δ4.60. Survey around δ0.9–1.1 showed for **9** an additional methyl compared with **8**.



Scheme 3. Partial structures and proton chemical shifts.

Table 3. ^{13}C NMR chemical shifts and assignments for botryococcenes

C	1	2	3	4	5B	6A	7	8	9
1	17.68	17.68	109.35	21.87	109.14*	109.51	109.47	109.48	109.51
2	131.21	131.20	150.01	33.10	149.78†	149.88	149.94	149.91	149.93
3	124.36	124.60	40.74	155.95	41.64	41.02	41.03	41.02	41.02
4	26.72	26.76	33.38	33.86	32.86	33.38	33.40	33.39	33.41
5	39.71	39.73	37.52	38.52	123.88	31.64	31.64	31.64	31.59
6	134.65	134.63	134.91	134.71	139.33	154.64*	154.71	154.65	154.66
7	124.67*	124.80*	124.61*	124.84	34.54	40.59†	40.59	40.57	40.57
8	23.11	23.13	23.13	23.13	29.36	30.10	30.11	30.10	30.08
9	41.29	41.36	41.38	41.33	39.53	39.08	39.08	39.07	39.03
10	42.02	42.01	42.01	42.01	41.79	41.82	41.82	41.82	41.82
11	135.78	135.81	135.81	135.61	135.84	135.83	135.59	135.80	135.78
12	133.70	133.70	133.71	133.86	133.69	133.74	133.95	133.74	133.74
13	36.67	36.75	36.77	37.28	37.30	37.30	36.96	37.29	37.30
14	37.36	37.44	37.45	34.99	35.01	35.02	35.01	35.01	35.00
15	25.79	25.83	25.84	33.39	33.38	33.38	32.68	33.39	33.33
16	124.77*	124.70*	124.49*	40.08	40.08	40.09†	40.84	40.03	40.03
17	134.65	134.97	134.91	154.91	154.79	154.90*	28.01	155.05	155.10
18	39.71	37.54	37.52	31.64	31.63	31.64	138.51	32.08	32.23
19	26.72	33.37	33.38	33.45	33.38	33.38	132.41	33.61	33.62
20	124.36	40.74	40.74	41.01	41.00	41.02	38.49	33.86	34.46
21	131.21	150.09	150.01	149.91	149.94†	149.88	34.47	145.01	150.47
22	17.68	109.31	109.35	109.48	109.54*	109.51	19.76*	23.06	28.65
23	25.69	25.68	18.96	21.87	19.39	18.92	18.93	18.92	18.91
24	15.95	15.97	15.97	15.98	19.78	107.36‡	107.33	107.32	107.32
25	23.51	23.58	23.58	23.58	23.59	23.59	23.60	23.59	23.53
26	146.72	146.71	146.70	146.72	146.84	146.90	146.96	146.90	146.90
27	111.06	111.07	111.09	111.07	110.97	110.99	110.94	110.97	110.99
28	21.16	21.14	21.15	21.15	21.18	21.17	21.02	21.15	21.16
29	15.95	15.90	15.97	107.16	107.23	107.22‡	23.30	107.07	106.99
30	25.69	18.97	18.96	18.90	18.87	18.92	29.51	116.51	116.07
31		19.67	19.68	19.79	19.67	19.77	16.10	19.76	19.77
32			19.68	106.17	19.54	19.77	19.72*	19.32	19.37
33				20.19	20.22	20.26§	24.84	20.22*	20.26*
34					17.96	20.41§	20.42	20.41*	20.42*
35								12.96	13.07
36								13.16	25.13
37									24.39

*,†,‡,§Assignments with the same sign could be interchanged for a given compound.

^{13}C *NMR*

The ^{13}C NMR chemical shifts are given in Table 3. The assignments were based upon those for **6**, **8** [8, 9] and squalene [17], from substituent effects [18] and recording off-resonance spectra. The previously published ^{13}C assignments for **2** [10] have been revised in accordance with the mass fragmentation of the botryococcane **2H**.

In all spectra, seven shifts ascribed to carbons of the central unit, C-10–C-13 and C-25–C-27, remained essentially invariant throughout the series. Data concerning compound **7** implied the occurrence of a cyclic structure and in other respects it was the sole hydrocarbon which possessed a second aliphatic quaternary carbon C-21. Some doubts remained as for the assignments of methyl groups attached to the ring (groups 22, 30 and 31 were assigned as axial, equatorial and axial, respectively).

For **4**, the partial structure **14** (Scheme 3) was confirmed on one hand by the chemical shifts of the exomethylene carbons: two resonances at *ca* δ155 ($R_2{}^{13}C{=}CH_2$) and one at *ca* δ150 ($RCH_3{}^{13}C{=}CH_2$) and on the other hand by the presence of a trisubstituted double bond located at C-6–C-7. For **5B**, the *cis*-configuration of the trisubstituted double bond C-5–C-6 was based on the chemical shift of the allylic C-7 atom at δ34.5, which would be more deshielded in a *trans*-configuration (δ39–40). On going from **8** to **9** the deshielding of carbons 21, 22 and 36 by five units was characteristic of a methyl substitution on C-22; C-20 and C-30 were also affected but to a lesser extent, the other resonances remaining essentially unshifted.

Ozonolysis

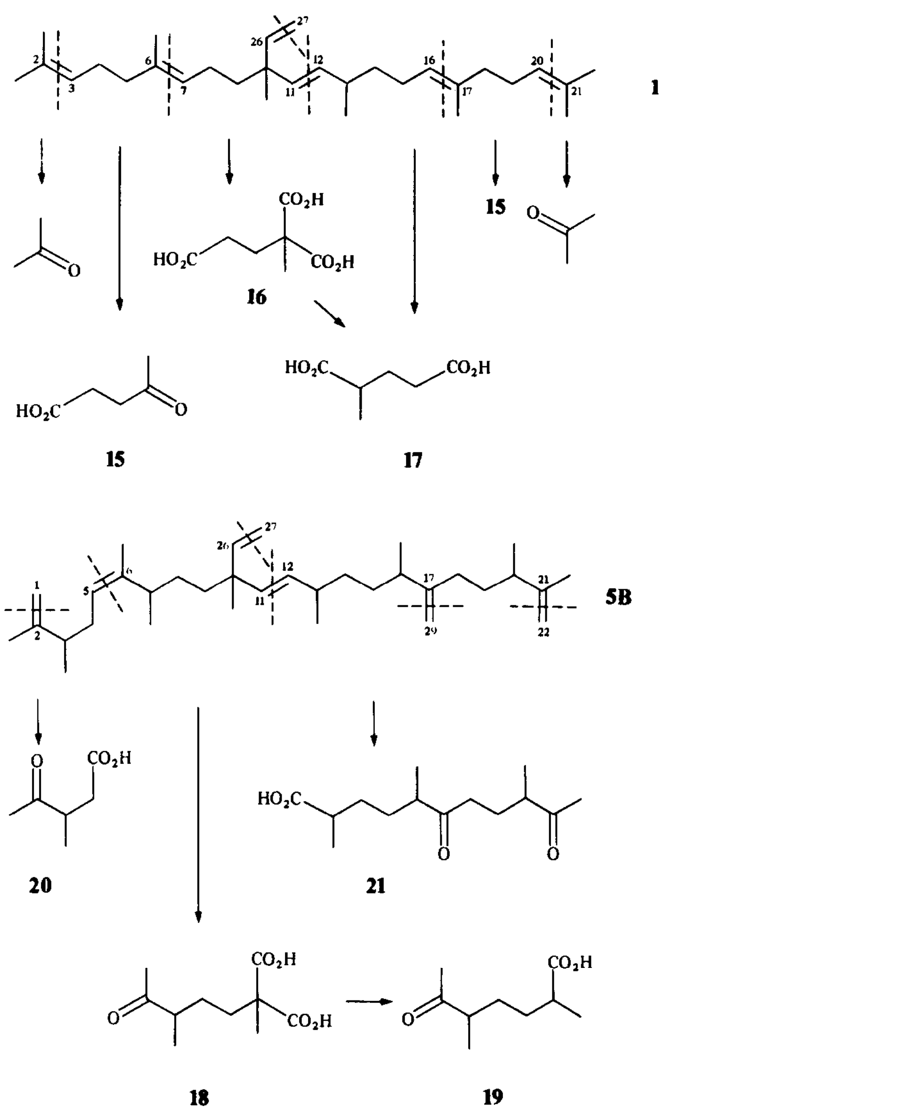
The hydrocarbons **1** and **5B** were submitted to ozonization in order to confirm the double bond positions. The acids derived from oxidative cleavage of the resulting polyozonides were analysed by GC/MS, as their methyl esters (Table 4, Scheme 4).

Under these conditions, **1** afforded only laevulinic acid (**15**) and 2-methyl glutaric acid (**17**). Isolation of these two

Table 4. Mass spectral data of cleavage compounds analysed as methyl esters (relative intensities of main ions in parentheses)

15*	130 $[M]^+$, (1%); 115 ($[M-Me]^+$ (16); 99 $[M-OMe]^+$ (20); 88 $[M-CH_2{=}C{=}O]^+$ (6); 71 $[M-CO_2Me]^+$ (5); 59 (11); 55 (17); 43 (100).
17*	174 $[M]^+$ (0%); 143 $[M-OMe]^+$ (24); 142 (10); 115 $[M-CO_2Me]^+$ (36); 114 (84); 99 (31); 88† (21); 83 (19); 73 $[M-CO_2Me-CH_2{=}C{=}O]$ (41); 59 (59); 56 (28); 55 (100); 43 (25); 42 (19); 41 (29).
19	186 $[M]^+$ (0%); 143 $[M-Ac]^+$ (1); 127 $[M-CO_2Me]^+$ (5); 126 (7); 115 $[M-Ac-CO]^+$ (2); 88† $[M-C_6H_{10}O]^+$ (19); 74 (16); 59 (28); 56 (31); 55 (88); 43 (100); 42 (28); 41 (38).
20	144 $[M]^+$ (2%); 129 $[M-Me]^+$ (2); 113 $[M-OMe]^+$ (10); 112 (7); 102 $[M-CH_2{=}C{=}O]^+$ (11); 87 $[M-CH_2{=}C{=}O-Me]^+$ (33); 85 $[M-CO_2Me]^+$ (8); 74 (7); 69 (7); 59 (28); 55 (11); 43 (100).
21	270 $[M]^+$ (0%); 143 $[M-C_7H_{11}O_2]^+$ (4); 115 (5); 114 (10); 88† (100); 83 (17); 59 (29); 57 (18); 56 (32); 55 (46); 45 (52); 43 (39); 41 (56).

*Mass spectra of ethyl laevulinate and 2-methyl methyl glutarate have been previously reported [19, 20].
†McLafferty re-arrangement.



Scheme 4. Botryococcene ozonolysis. Oxidative decomposition of the polyozonides.

products in *ca* equal amounts suggested that 2-methyl glutaric acid (**17**) originated both directly from the cleavage of the double bonds C-11–C-12 and C-16–C-17 and from the fast decarboxylation of a triacid **16** formed by cleavage of the double bonds C-6–C-7, C-11–C-12 and C-26–C-27.

A similar degradation of **5B** provided essentially three compounds. They were tentatively identified by GC/MS as two keto-acids **19** (presumably derived from a keto-diacid **18**) and **20**, and a diketo-acid **21** previously isolated from a permanganate–periodate oxidation of **6** [8].

DISCUSSION

On the basis of the analytical data, structures **1–9** are proposed; our results confirm the previously published structures for **6** and **8** [8, 9]. As earlier hypothesized, they suggest a terpenoid origin for botryococcenes [8]. Moreover recent developments on the biosynthesis of these compounds suggest that the C_{30} botryococcene should act as precursor of all the higher metabolites of this series [21]. In this respect, monomethylations of the supposed precursor **1** should occur in most cases on carbons 3, 7, 26 and 20: other positions should also be involved: 18 for **7**, 22 and 30 for **8** and **9**. As it is well documented for the sterol side chain [22] the proton lost during alkylation originates in the chain (as for compound **2**, alkylation on C-20) or in the introduced methyl (as for compound **4**, alkylation on C-3). These observations suggest that each botryococcene should derive from its lower homologue of similar structure, through methylation, as outlined for the following homogeneous series: **1** → **2** → **3** → → **6A** → → **8** → **9**. The accumulation of one or more botryococcenes in the alga might be an indication of a regulation of the methylation system in connection with genetic and physicochemical factors.

Scheme 5 is proposed to explain the probable origin of **7**, the sole partially cyclized metabolite of this series. In a first step, the methylation on C-20 could be the starter of the cyclization, the resulting cyclohexyl cation leading then to the olefin **22**, which would be in turn methylated.

All these structures illustrate the natural disposition of *B. braunii* (B race) to perform methylations and so to give highly methylated triterpenes. This aptitude may not be restricted to botryococcenes, in so far as a monomethylated derivative of squalene has been identified in an extract of this alga [10]; to our knowledge no alkylation has ever been observed before squalene cyclization.

From a geochemical point of view, the C_{34} hydrocarbons **5A**, **5B** and **6**, appear to be of some interest. If we consider that these hydrocarbons constitute on the whole, a noticeable amount of the oily material produced by *B. braunii* (B race) in some fresh water lakes, as in Darwin: 30% [6], Paquemar: 80% [1] and Oakmere: 100% [7], we can apprehend their involvement in the formation of their common fully saturated derivative identified in a crude oil [16]. Owing to the cosmopolitan distribution of *B. braunii* and its implication in petroleum genesis [23], the discovery in crude oils of other botryococcanes—C_{30} to C_{37}—is to be expected.

EXPERIMENTAL

B. braunii samples. The origin of the algae, their isolation and their culture conditions (unaerated for C, aerated by sterile air + 1% CO_2 for D), have already been described [1, 10].

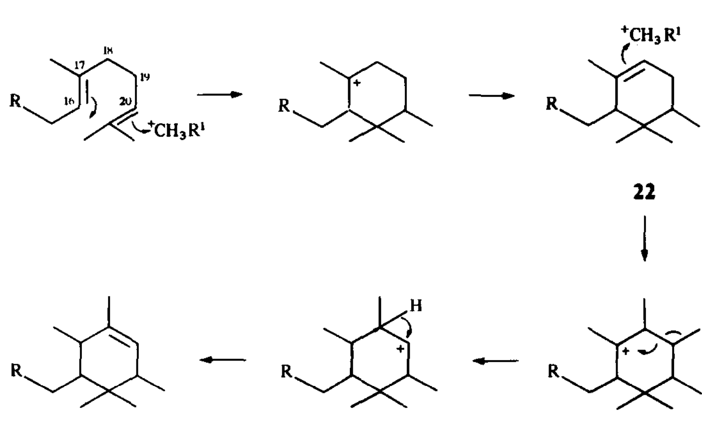
Hydrocarbons were extracted with hexane from algae dried under vacuum at 60°; they were further purified on an alumina column [1].

HPLC. Reversed-phase separations were performed on an instrument equipped with a 7000 psi inj valve with a 20 μl loop. The detector was a differential refractometer (RI), thermostated at 25°. The reversed-phase column was a Resolve 5 μ spherical C_{18} Waters (two 150 × 3.9 mm). Botryococcene samples were injected as solns in Me_2CO (20 μl, 10% in solvent). Mobile phase (dist. in glass; filtered through an AP 25 Millipore prefilter): Me_2CO–MeCN (2:3), flow rate 90 ml/hr. R_ts (min): **1**: 5.5, **2**: 6, **3**: 7, **4**: 8, **5A** and **6A**: 7.8, **7** and **8**: 10, **9**: 11. Successive injections of samples were carried out and the compounds collected.

Normal phase HPLC was carried out using the peak shaving recycle technique [15] on a prep. apparatus equipped with two cartridge of Prep PAK™-500 silica; detection by RI. Dist.-in-glass hexane was used as mobile phase.

GC/MS. Hydrocarbons and their derivatives were characterized using $CI(NH_3)$ and EI. The apparatus was equipped with a fused silica column; 25 m, WCOT SE 52. The following column temps were used. Botryococcenes; prog 220–260° at 2°/min, botryococcanes: 260°, Me esters: 100°.

^1H (60 MHz) and ^{13}C (25.17 MHz) NMR spectra were obtained from $CDCl_3$ solns using TMS as int. ref. The ^{13}C FT NMR spectra were recorded using the following typical pulse conditions: pulse width, 20 μsec, flip angle of *ca* 43°; acquisition time, 0.8 sec; pulse delay, 0.4 sec; spectral width, 5000 Hz. The operating conditions gave a digital resolution of 1.5 Hz, and the precision of the results, relative to TMS, was ± 0.06 ppm.



7

Scheme 5. Proposed mechanism for the biosynthesis of 7.

Hydrogenation. Botryococcenes were reduced with H_2 (catalyst: rhodium 5% on charcoal) in hexane as solvent. After 18 hr at 100° (15 atm. pres.), the catalyst was removed by centrifugation and the solvent eliminated under vacuum.

Oxidative cleavage of botryococcenes **1** *and* **5B**. Botryococcenes (100 mg) dissolved in CH_2Cl_2 (3 ml) were ozonised at −65° (30 mg O_3/l. air; 40 l./hr) until the characteristic blue colour of ozone persisted. CH_2Cl_2 was then removed at room temp. with a stream of N_2 and the products decomposed by refluxing with H_2O_2 30% (1.5 ml) and HCO_2H (3 ml) for 1 hr. The resulting acid mixtures were continuously extracted with hot toluene, then esterified with dry MeOH–HCl and further extracted with Et_2O.

The polyozonide of **1** was submitted to a reductive degradation by refluxing with H_2O. Me_2CO was isolated through distillation, as its 2,4-dinitrophenylhydrazone. Perhaps because of the great instability of the complex polyozonide, attempts to isolate the formaldehyde derivative were unsuccessful (C-26–C-27 cleavage).

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