Pergamon

PII: S0031-9422(96)00563-8

Phytochemistry, Vol. 44, No. 4, pp. 671–678, 1997

0031-9422/97 $17.00+0.00

FOUR POLYMETHYLSQUALENE EPOXIDES AND ONE ACYCLIC TETRATERPENE EPOXIDE FROM *BOTRYOCOCCUS BRAUNII*

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(*Received* 1 *March* 1996)

Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; acyclic triterpenes; methylated squalene epoxides; tetraterpenes; epoxylycopaene; dihydroxylycopaene; absolute configuration; Mosher's (^{1}H) method.

Abstract—New terpene epoxides have been isolated from two strains of the green microalga *Botryococcus braunii*. Polymethylsqualene epoxides C_{32}, C_{33} and C_{34} are 10,11-epoxysqualene derivatives with non-isoprenoid methyls at C-3, C-7, C-18 and (or) C-22; they were isolated from a strain producing large amounts of triterpene hydrocarbons (*B. braunii*, B race). Epoxylycopaene is an acyclic tetraterpene with a *trans* epoxide occurring at C-14, C-15 and a *trans* unsaturation at C-18; it was obtained from a strain characterized by the production of *trans,trans*-lycopadiene, a tetraterpene hydrocarbon (*B. braunii*, L race). The structures were determined by NMR and mass spectrometry. Chemical degradation and application of Mosher ester methodology allowed us to establish the absolute stereochemistry of epoxylycopaene. In addition, 14,15-dihydroxy lycopa-18-ene has been isolated from the L race. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

The green microalga *Botryococcus braunii* has been the subject of numerous chemical analyses, especially with regard to its neutral lipids, several of which exhibit unusual structures [1–5]. While these investigations were focused on the A race, which is chemically defined by the production of *n*-alkadienes and trienes, except for hydrocarbons, little is known about the lipids of the B and L races [6].

The B race of *B. braunii* is characterized by the production of a family of 1′-3 linked triterpenes, C_nH_{2n-10} with $30 \leqslant n \leqslant 37$, named botryococcenes [1, 7–14]. All the higher compounds derive from the parent botryococcene $C_{30}H_{50}$ (**1**), (very likely generated from presqualene diphosphate [15–16]) by successive methylations at C-3, C-7, C-16 and C-20 with *S*-adenosylmethionine [17–18]. In addition to these hydrocarbons (accounting for *ca* 30–40% of the dry weight), squalene and methylated squalenes, such as monomethylsqualene (**2**) [19] and tetramethylsqualene (**3**) [20] are found in low quantities (0.2% of the dry wt). However, Huang and Poulter [20] reported that the concentration of tetramethylsqualene (**3**) could be increased dramatically by supplementing the cultures with methionine and they hypothesized that such an enhancement of squalene methylation could divert squalene away from sterol biosynthesis.

In contrast to the diversity of the hydrocarbons synthesized by the B race, the L race produces lycopadiene (**4**), which is an acyclic tetraterpene exhibiting a lycopane skeleton and comprising two *trans* double bonds in the centre of the molecule [21]. On the basis of the *R* configuration of the four methine stereocentres, it was assumed that phytol could be involved in lycopadiene biosynthesis [21].

We now report on the structures for six new metabolites of *B. braunii*—a series of four epoxides of methylated squalenes (**5**–**8**) isolated from a strain of the B race and epoxylycopaene (**9**) obtained from a strain of the L race. We also report the absolute stereochemistry of compound **9** and the isolation of its dihydroxyderivative compound **10**.

RESULTS AND DISCUSSION

Polymethylsqualene epoxides

The heptane extract of a 3-week-old culture of a Martinique strain (43% of the dry wt) was submitted to column chromatography over alumina. The heptane fraction consisted of a mixture of botryococcenes, constituting 95% of the extract. Elution

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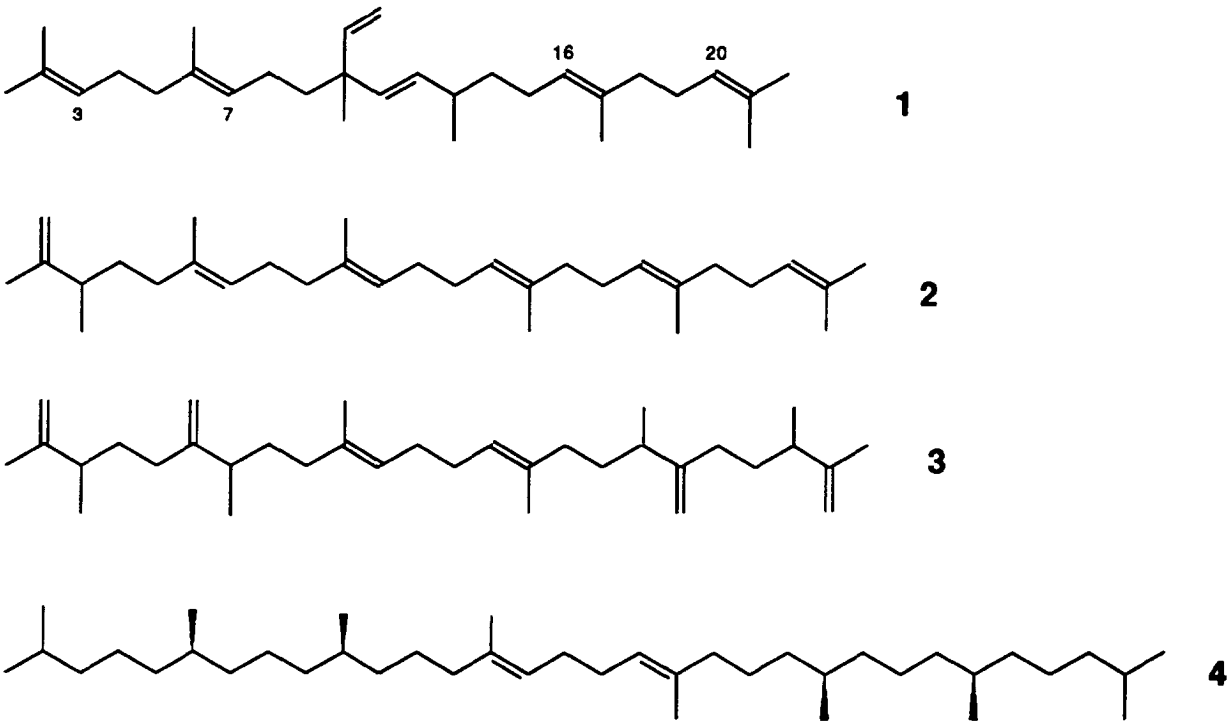


with toluene furnished a small lipid portion (1.8% of the extract) which was further separated by preparative TLC to give several products, including an oily material (26% of the toluene fraction, 0.2% of the dry weight) which exhibited characteristic NMR resonances for a trisubstituted epoxide, i.e. a triplet at δ_H 2.69 and signals for a quaternary carbon at δ_C 61.1 and for a tertiary one at δ_C 63.3. Moreover, a DEPT spectrum revealed that the oil was a mixture of terpenoids, and COSY and HOHAHA spectra allowed the identification of a substructure (Fig. 1) identical to that occurring in 10,11-epoxysqualene. However, no trace of the latter, or of its 2,3- and 6,7-isomers could be detected. In fact, GC-CI (NH_3) mass spectral analysis (Table 1) showed that the oil contained essentially four compounds (**5–8**) with molecular formulae $C_nH_{2n-10}O$, (n = 32, 33, 34) suggesting, along with the ^{1}H NMR data, that they were methylsqualene epoxides; high-resolution EI-mass spectrometry confirmed the molecular formulae.

Purification of this oil by TLC over silica gel–silver nitrate allowed the separation of two subfractions, A and B, each containing two predominant compounds and four minor ones: A (R_f 0.63; **5** and **7**) (33 and 50% of the mixture, respectively) and B (R_f 0.70; **6** and **8**) (36 and 51% of the mixture, respectively). Attempts to obtain substantially greater enrichments in **7** and **8** by HPLC failed; thus NMR analyses were performed on each of the mixtures.

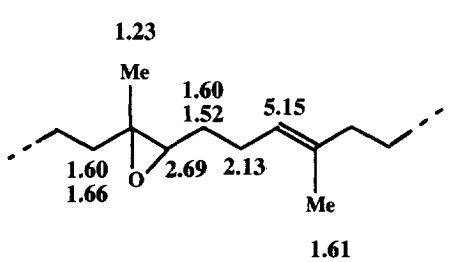
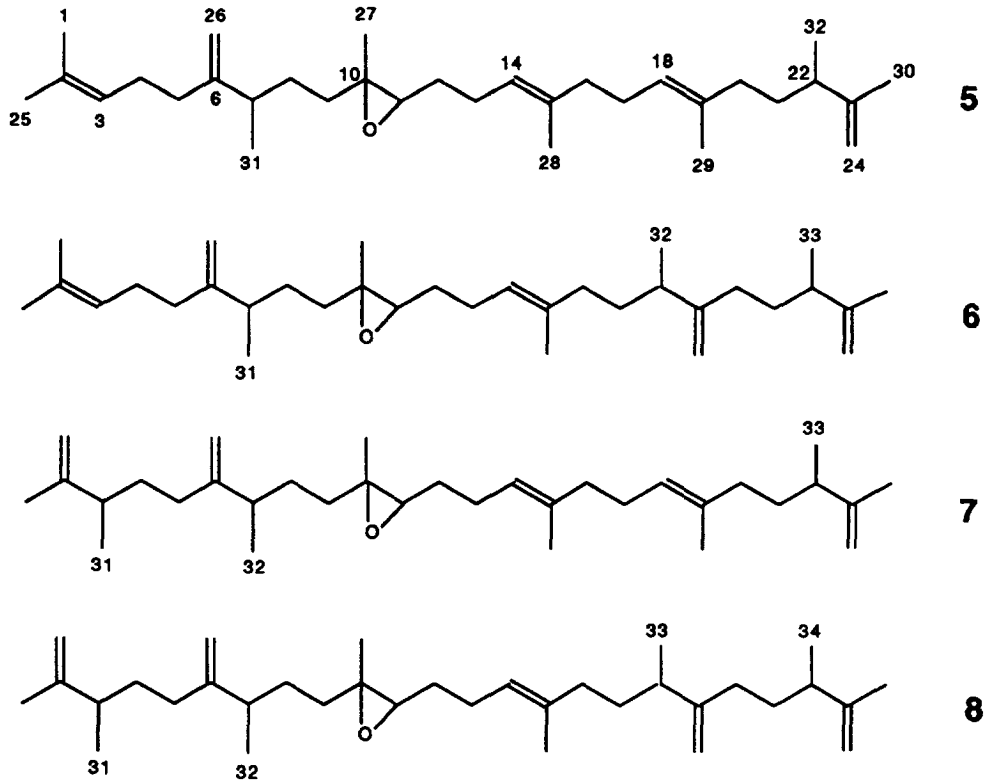


Fig. 1. Partial substructure common to epoxides **5–8**, and relevant ^{1}H NMR data.

The ^{1}H and ^{13}C NMR data (Tables 2 and 3) clearly showed, in addition to the epoxide moiety already mentioned, the presence of several methylated and unmethylated isoprene units, located in terminal and (or) subterminal positions. Thus, in the case of subfraction B, the COSY and HOHAHA spectra, and comparisons of the NMR data with that of **3** [20], established that the dominant compound **8** was a tetramethylsqualene epoxide and that oxidation had occurred at C-10, C-11. The ^{13}C NMR data confirmed this finding. ^{1}H correlations clearly indicated a connectivity between a non-methylated isoprene unit in a terminal position, and a methylated one, in turn connected to the epoxide moiety. This pattern was assumed to belong to the second abundant compound of mixture B, the trimethylsqualene epoxide **6**. The structures of compounds **5** and **7** (mixture A) followed from comparison of the ^{1}H and ^{13}C NMR data with those of **6** and **8**, and ^{1}H–^{1}H COSY.

Confirmation of the epoxide location in the chains of compounds **5–8** was obtained from the mass fragmentation pattern of the trimethylsilyl derivative compounds obtained from the diols produced by acid hydrolysis of A and B. The TMS derivatives exhibited major ions at m/z 191 (**5** and **6**) and at m/z 205 (**7** and **8**) which resulted from the cleavage of the C-10, C-11



bond with subsequent loss of Me_3SiOH in the fragments of lower mass (Fig. 2). These mass data indicated that in the left moieties of the structures, only one isoprene unit was methylated in **5** and **6**, against two in the case of **7** and **8**.

Polymethylated-10,11-epoxysqualenes **5–8**, exhibiting non-isoprenoid methyls at C-3, C-7, C-18 and C-22 are new members of the squalene triterpene family from *B. braunii*. At the end of the active growth phase, they account for 0.2% of the dry biomass, as their parent hydrocarbons.

Epoxylycopaene (**9**)

Extraction of the dry biomass of the Ivory Coast strain with heptane furnished a highly viscous oil (24.8% of dry wt), composed mainly of a rubbery biopolymer [22], the structure of which is under study. The elastic material was removed from the crude extract dissolved in chloroform via precipitation by addition of an equal volume of methanol. The recovered oil (8.7% of the dry biomass) was separated by silica gel CC into five fractions, I–V (see Experimental). Fraction I contained pure lycopadiene (**4**) (7.1% of the extract). Preparative silica gel TLC of fraction II gave an oily compound (0.3% of dry wt) exhibiting a single peak on GC and $[\alpha]_D = -3.5°$. Its molecular formula, $C_{40}H_{78}O$, was established by EI-mass spectrometry. The ^1H and ^{13}C NMR data indicated an acyclic tetraterpene containing a trisubstituted double bond (δ_H 5.15 *t*, H-18; δ_C 123.1 *d*, C-18 and 136.3 *s*, C-19) of *trans* stereochemistry, according to the chemical shift of the allylic carbon 20 (δ_C 40.0 *t*), with a vinylic methyl (δ_H 1.61 *s*, δ_C 15.9 *q*) and a trisubstituted epoxide (δ_H 2.71 *t*, H-15; δ_C 61.0 *s*, C-14 and 63.4 *d*, C-15). All mass and NMR data appeared identical to those

Table 1. GC–CI(NH_3) mass spectral analysis of the polymethylated squalene epoxide fraction

Formula	Compound	$[M+H]^+$	$[M+NH_4]^+$	$[M+H-H_2O]^+$	Relative %
		(m/z) (rel. int.)			
$C_{32}H_{54}O$		455 (100)	472 (53)	437 (46)	3.4
$C_{32}H_{54}O$	**5**	455 (100)	472 (38)	437 (42)	11.6
$C_{33}H_{56}O$	**6**	469 (100)	486 (42)	451 (58)	28.5
$C_{34}H_{60}O_3$		517 (44)		499 (100)	5.2
$C_{33}H_{56}O$	**7**	469 (100)	486 (51)	451 (41)	15.9
$C_{34}H_{58}O$	**8**	483 (100)	500 (45)	465 (61)	32
Others					3.4

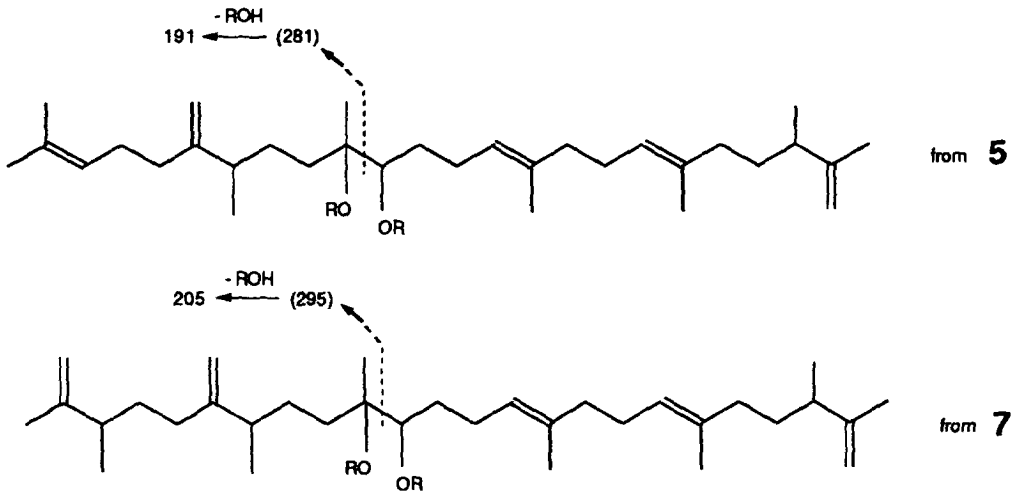


Fig. 2. Mass fragmentation pattern of the trimethylsilyl derivatives from the diols derived from compounds **5** and **7** (R = -$SiMe_3$).

Table 2. ^{1}H NMR data of compounds **5–8**

H	5*	6	7	8
1	1.61 *s*	1.61 *s*	4.69 *m*	4.69 *m*
3	5.11 *t* (6.6)	5.11 *t* (6.6)	2.16 *m*	2.16 *m*
4	2.11 *m*	2.11 *m*	1.50 *m*	1.50 *m*
			1.40 *m*	1.40 *m*
5	1.97 *t* (6.9)	1.97 *t* (6.9)	1.90 *m*	1.90 *m*
7	2.06 *m*	2.06 *m*	2.06 *m*	2.06 *m*
8	1.50 *m*	1.50 *m*	1.50 *m*	1.50 *m*
	1.35 *m*	1.35 *m*	1.35 *m*	1.35 *m*
9	1.66 *m*	1.66 *m*	1.66 *m*	1.66 *m*
	1.60 *m*	1.60 *m*	1.60 *m*	1.60 *m*
11	2.69 *t* (6.3)	2.69 *t* (6.3)	2.69 *t* (6.3)	2.69 *t* (6.3)
12	1.60 *m*	1.60 *m*	1.60 *m*	1.60 *m*
	1.52 *m*	1.52 *m*	1.52 *m*	1.52 *m*
13	2.13 *m*	2.13 *m*	2.13 *m*	2.13 *m*
14	5.15 *t* (6.5)	5.15 *t* (6.5)	5.15 *t* (6.5)	5.15 *t* (6.5)
16	1.90 *t* (7.8)	1.92 *m*	1.90 *t* (7.8)	1.92 *m*
17	2.07 *m*	1.50 *m*	2.07 *m*	1.50 *m*
		1.37 *m*		1.37 *m*
18	5.10 *t* (6.8)	2.06 *m*	5.10 *t* (6.8)	2.06 *m*
20	2.09 *m*	1.92 *m*	2.09 *m*	1.92 *m*
21	1.38 *m*	1.50 *m*	1.38 *m*	1.50 *m*
		1.40 *m*		1.40 *m*
22	2.12 *m*	2.16 *m*	2.12 *m*	2.16 *m*
24	4.67 *m*	4.69 *m*	4.67 *m*	4.69 *m*
25	1.69 *s*	1.69 *s*	1.66 *s*	1.66 *s*
26	4.73 *m*	4.73 *m*	4.72 *m*	4.72 *m*
27	1.23 *s*	1.23 *s*	1.23 *s*	1.23 *s*
28	1.61 *s*	1.61 *s*	1.61 *s*	1.61 *s*
29	1.58 *s*	4.72 *m*	1.58 *s*	4.72 *m*
30	1.66 *s*	1.66 *s*	1.66 *s*	1.66 *s*
31	1.00 *d* (6.8)	1.00 *d* (6.8)	1.02 *d* (6.8)	1.02 *d* (6.8)
32	1.02 *d* (6.8)	1.00 *d* (6.8)	1.00 *d* (6.8)	1.00 *d* (6.8)
33		1.02 *d* (6.8)	1.02 *d* (6.8)	1.00 *d* (6.8)
34				1.02 *d* (6.8)

* *J* (Hz) in parentheses.

Table 3. ^{13}C NMR data of tetramethylsqualene (**3**)* and polymethylsqualene epoxides **5–8**

C	3	5	6	7	8
1	109.3	17.7	17.7	109.6	109.6
2	149.7	131.6	131.6	149.9	149.9
3	41.0	124.3	124.3	41.1	41.1
4	33.4	26.6	26.6	33.5	33.5
5	31.6	33.6	33.6	31.7	31.7
6	154.6	153.8	153.8	154.3	154.3
7	39.5	40.2	39.7	40.1	40.1
8	37.5	30.9	30.9	30.9	30.9
9	34.0	36.8	36.8	36.8	36.8
10	135.1	61.1	61.1	61.1	61.1
11	123.9	63.3	63.3	63.3	63.3
12	28.2	24.9	24.9	24.9	24.9
13	28.2	29.0	29.0	29.0	29.0
14	123.9	123.3	123.1	123.3	123.1
15	135.1	135.9	136.2	135.9	136.2
16	34.0	39.7	34.0	39.7	34.0
17	37.5	26.6	37.5	26.6	37.5
18	39.5	124.0	40.2	124.0	40.2
19	154.6	135.3	154.8	135.3	154.8
20	31.6	37.5	31.3	37.5	31.3
21	33.4	33.4	33.4	33.4	33.4
22	41.0	40.7	41.1	40.7	41.1
23	149.7	150.1	150.0	150.1	150.0
24	109.3	109.4	109.6	109.4	109.6
25	18.9	25.7	25.7	18.9	18.9
26	107.1	107.8	107.8	107.6	107.8
27	16.0	16.7	16.7	16.7	16.7
28	16.0	16.0	16.0	16.0	16.0
29	107.1	16.0	107.6	16.0	107.6
30	18.9	19.0	18.9	19.0	18.9
31	19.7	20.2	20.3	19.8	19.8
32	20.1	19.7	20.3	20.3	20.3
33	20.1		19.8	19.7	20.2
34	19.7				19.8

* Data from ref. [20].

obtained for the monoepoxide of *trans,trans*-lycopadiene (**4**), produced by treatment of this hydrocarbon with *m*-chloroperbenzoic acid. Furthermore, acid hydrolysis of **9** yielded 14,15-dihydroxylycopa-18-ene (**10**) which underwent periodate cleavage to yield a 1:1 mixture of 6*R*, 10*R*, 14-trimethylpentadecan-2-one (**12**), $[\alpha]_D = +0.31°$, and of the γ-unsaturated C_{22} aldehyde **13**. Ozonolysis of compound **13**, followed by reductive cleavage of the ozonide, yielded the trimethylpentadecanone **12** with the same optical rotation as above. These results confirmed that the epoxide was located at C-14, C-15 and that unsaturation occurred at C-18. Moreover, the identical optical rotations found for compound **12** obtained either from periodate cleavage of compound **10** or from ozonolysis either of compound **13** or of natural phytol [21], indicated that the four methine stereocentres in compound **9** were *R*. Fractions III–V from column chromatography were investigated for the occurrence of dihydroxylycopaene (**10**). To avoid interference after TLC separation between bands of compound **10** and of oxo-carotenoids present in high amount, fractions III–V were acetylated prior to preparative TLC. In this way, the monoacetate **11** could be easily isolated. After reduction of compound **11**, the resulting diol accounted for 0.3% of the dry biomass. It exhibited mass and NMR spectra identical to those of compound **10** obtained by acid hydrolysis of **9**. Moreover, the optical rotation values, $[\alpha]_D = -2.5°$, indicated they have the same absolute stereochemistry.

The absolute configuration of the hydroxyl group at C-15 in diol **10**, obtained by acid hydrolysis of epoxide **9**, was established by application of the ^1H Mosher's method [23, 24]. The diol **10** was transformed into *S*- and *R*-methoxy(trifluoromethyl) phenyl acetates (MTPA esters) **14** and **15**, respectively. The $\Delta\delta_H$ values (δS-δR) obtained from derivatives **14** and **15** are given in Fig. 3; on the basis of these data, C-15 was assigned the *R* configuration. Consequently, the absolute configuration of the epoxide group in compound **9** could be assigned as 14*R*,15*R*. Thus, the absolute configuration of (−)*trans,trans*-epoxylycopaene (**9**) is proposed to be 6*R*,10*R*,14*R*,15*R*, 23*R*,27*R*.

In the A race of *B. braunii*, aliphatic epoxides with normal hydrocarbon chains are key intermediates in the biosynthesis of unusual ether lipids and are produced sometimes in very large amounts by the alga [25]. Work presently in progress suggests that epoxylycopaene (**9**) is a precursor for a new class of ether lipids, i.e. the ditetraterpenoid ethers. Towards the end of the present study, it was reported that the B race contained botryaxanthin A, a new class of carotenoid [26]. This pigment is an acetal very likely originating from the condensation of echinenone and dihydroxy-tetramethylsqualene derived from the hydration of epoxide **8**. Moreover, other studies from our laboratory strongly suggest that these terpenoid epoxides (**5–9**) are the direct precursors of some important building blocks of the rubbery biopolymers present in the thick outer walls of the alga.

EXPERIMENTAL

General. CC: silica gel (70–230 mesh) and alumina (activity II); TLC: silica gel 60 PF; ^1H (250 or 500 MHz) and ^{13}C (62.5 MHz) NMR: $CDCl_3$, TMS as int. standard. Acetylation and trimethylsilylations were carried out according to standard procedures [27].

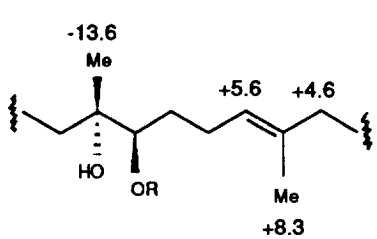
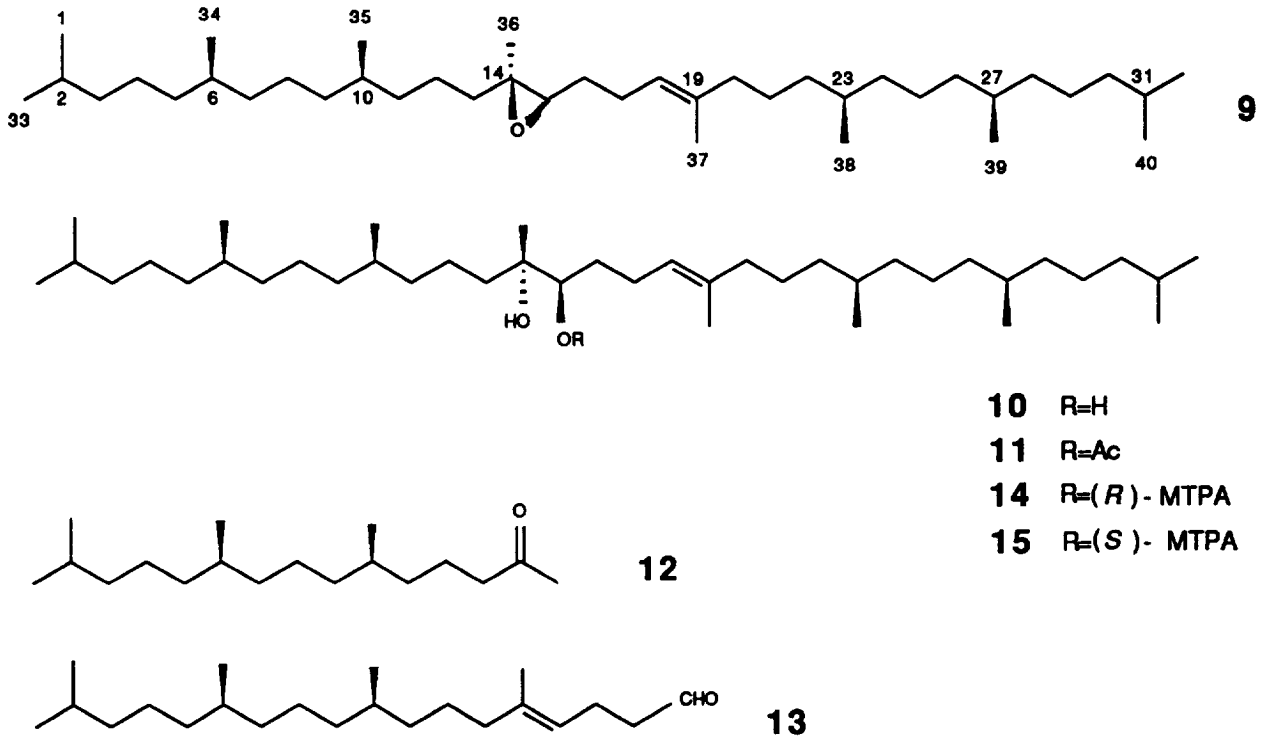


Fig. 3. $\Delta\delta_H$ values (Hz) from ^1H NMR data of compounds **14** and **15** (R = MTPA).



2,3-, 6,7- and 10,11-epoxysqualenes were synthesized by epoxidation of squalene and purified as previously reported [28].

Botryococcus braunii *strains and culture conditions.* The strains investigated originated from Martinique (strain MLM_2 [27]) (B race) and from the Ivory Coast (strain Yamoussoukro [21]) (L race). After suitable growth periods under air-lift conditions and continuous illumination [29], the biomasses were freeze-dried, extracted twice for 1 hr with heptane, and the combined extracts concentrated under red. pres.

Polymethylsqualene epoxides **5–8** *and their derivatization.* A 15.4 g sample of oil extracted from the Martinique strain was chromatographed over alumina (300 g, CC); elution with heptane (3300 ml) (95% of the extract) and toluene (1200 ml) (1.8% of the extract). Purification of the toluene fr. by silica gel TLC (heptane–Et_2O, 9:1) yielded a mixt. of methylsqualene epoxides **5–8**, exhibiting the same R_f (0.47) as a standard of 10,11-epoxysqualene. GC-CI MS (NH_3) (fused silica column CPSil-5CB, 25 m, progr. 220–300° at 2° min^{-1}) (Table 1); GC-HR-EI-MS *m/z*: $C_{32}H_{54}O$ (**5**) found 454.4167 (requires 454.4175), $C_{33}H_{56}O$ (**6**) found 468.4340 (requires 468.4331), $C_{33}H_{56}O$ (**7**) 468.4312 (requires 468.4331), $C_{34}H_{58}O$ (**8**) 482.4465 (requires 482.4488); IR υ_{max} (CCl_4): 3070, 2960, 2920, 2860, 2850, 1640, 1455, 1380, 1370, 1120 and 890 cm^{-1}. The mixt. was then fractionated by prep. TLC over silica gel–$AgNO_3$ (10%) using toluene–EtOAc (7:3). Two frs were recovered: A, R_f 0.63 (30%) and B, R_f 0.70 (70%). 2 mg of A and B were hydrolysed separately in 2 ml of THF–H_2O (9:1) with 0.1 ml of 30% $HClO_4$, for 2 hr at room temp. The reaction mixt. was extracted with CH_2Cl_2 and washed using aq. 10% Na_2CO_3 and then H_2O. After trimethylsilylation, the crude produce was analysed by GC-EI-MS (25 eV); capillary column CPSil-5CB, 25 m, progr. from 220 to 300° at 4° min^{-1}.

Epoxylycopaene (**9**). The concentrated crude extract of the Ivory Coast strain (2.95 g) was dissolved in 50 ml $CHCl_3$ and an equal vol. of MeOH added. Concentration of the filtrate obtained after elimination of a rubbery material gave an oil (0.7 g) which was sepd by silica gel (48 g) CC into 5 frs: I (180 ml heptane) (8.5% of oil), II (240 ml heptane–Et_2O, 19:1) (62.2%), III (300 ml heptane–Et_2O, 23:2) (12%), IV (460 ml heptane–Et_2O, 17:3) (12.8%) and V (180 ml Et_2O) (15%). Fr. I contained lycopadiene (**4**). Fr. II on prep. silica gel TLC (heptane–Et_2O, 22:3) gave epoxylycopaene (**9**) (R_f 0.57) as an oily compound. $[\alpha]_D = -3.5°$ (heptane, *c* 3.45). HREI-MS *m/z*: $C_{40}H_{78}O$ found 574.6080 (requires 574.6053); IR υ_{max} (CCl_4): 2950, 2920, 2860, 1660, 1460, 1380, 1375, 1365 and 1160 cm^{-1}; CI(NH_3)MS (probe) *m/z* (rel. int.): 572 $[C_{40}H_{58}O+NH_4]^+$ (63), 575 $[C_{40}H_{58}O+H]^+$ (100), 574 $[C_{40}H_{58}O+NH_4-H_2O]^+$ (22), 557 $[C_{40}H_{58}O+H-H_2O]^+$ (19); ^1H NMR (250 MHz): δ 5.15 (H-18, *t*, $J = 7.0$ Hz), 2.71 (H-15, *t*, $J = 6.3$ Hz), 2.15 (H-17, *m*), 1.95 (H-20, *t*, $J = 7.3$ Hz), 1.60 (Me-37, *s*), 1.24 (Me-36, *s*), 1.7–1.4 (overlapping CH and CH_2), 0.83–0.88 (other methyls); ^{13}C NMR: δ 136.3 (C-19), 123.1 (C-18), 63.4 (C-15), 61.0 (C-14), 40.0 (C-20), 39.4 (C-3, C-30), 39.2 (C-13), 37.5 (C-7, C-26), 37.3 (C-5, C-9, C-28), 37.1 (C-11), 36.7 (C-22), 32.8 (C-6, C-23, C-27), 32.7 (C-10), 29.0 (C-17), 28.0 (C-2, C-31), 25.4 (C-21), 24.9 (C-12), 24.8 (C-4, C-16, C-29), 24.5 (C-8, C-25), 22.7 (C-33, C-40), 22.6 (C-1, C-

32), 19.8 (C-34, C-38, C-39), 19.7 (C-35), 16.6 (C-36), 15.9 (C-37).

Epoxidation of **4**. Lycopadiene (**4**) (100 μmol) in $CHCl_3$ (10 ml) was reacted with *m*-chloroperbenzoic acid (105 μmol) for 3 hr at room temp. The reaction mixt. was washed with 5% NaOH and then with H_2O. Prep. TLC over silica gel TLC (heptane–Et_2O, 22:3) yielded a pure monoepoxide of lycopadiene exhibiting identical mass and NMR data to **9**.

Hydrolysis of **9**. 20 mg of compound **9** in 2 ml of THF–H_2O (9:1) were hydrolysed with 0.1 ml 30% $HClO_4$ for 2 hr as described for **5–8**. TLC of the crude product over silica gel TLC yielded 18 mg of dihydroxylycopaene (**10**) (heptane–Et_2O, 1:1, R_f 0.47). Oil; $[\alpha]_D = -2.5°$ (heptane, *c* 5.6). CI (NH_3) MS (probe) *m/z* (rel. int.): 610 $[C_{40}H_{80}O_2+NH_4]^+$ (74), 593 $[C_{40}H_{60}O_2+H]^+$ (13), 575 $[C_{40}H_{60}O_2+H-H_2O]^+$ (100), 340 (19), 299 (19), 292 (21), 286 (68); IR υ_{max} (CCl_4): 3630, 3400, 2950, 2920, 2850, 1460, 1375, 1365 and 1050 cm^{-1}; ^1H NMR (250 MHz): δ 5.15 (H-18, *t*, *J* = 6.6 Hz), 3.41 (H-15, *dd*, *J* = 10.0, 2 Hz), 2.20 (H-17, *m*), 2.11 (H-17, *m*), 1.95 (H-20, *t*, *J* = 7.2 Hz), 1.63 (Me-37, *s*), 1.52–1.20 (overlapping CH and CH_2) 1.15 (Me-36, *s*), 0.87–0.83 (other methyls); ^{13}C NMR: δ 136.6 (C-19), 123.6 (C-18), 78.3 (C-15), 74.7 (C-14), 40.1 (C-20), 39.4 (C-3, C-30), 37.7, 37.5, 37.3, 36.7, 36.5 (C-13), 32.8 (C-6, C-10, C-23, C-27), 31.3 (C-16), 28.0 (C-2, C-31), 25.5 (C-21), 25.2 (C-17), 24.8, (C-4, C-29), 24.5 (C-8, C-25), 23.6 (C-36), 22.7 (C-33, C-40), 22.6 (C-1, C-32), 20.8 (C-12), 19.8 (C-34, C-38, C-39), 19.7 (C-35), 16.0 (C-37).

Metaperiodate cleavage of **10**. Dihydroxylycopaene (**10**) (50 mg) and sodium metaperiodate supported on silica gel (3 g) [30] were stirred in CH_2Cl_2 (7 ml) for 48 hr at 20°. The solid was then filtered off and washed with CH_2Cl_2 (3 × 20 ml). Evapn of the solvent from the combined filtrate and washings gave 47 mg of crude products. Prep. TLC over silica gel (heptane–Et_2O, 9:1) gave compounds **12** (21 mg) and **13** (23 mg) as clear oils. Compound **12**: $[\alpha]_D = +0.31°$ (heptane, *c* 3.15); CI(NH_3)MS (probe) *m/z* (rel. int.): 286 $[C_{18}H_{36}O+NH_4]^+$ (100), 269 $[C_{18}H_{36}O+H]^+$ (5), 250 $[C_{18}H_{36}O-H_2O]^+$ (4); IR υ_{max} (CCl_4): 2950, 2920, 2860, 1715, 1460, 1375, 1365 and 1355 cm^{-1}. **13**: $[\alpha]_D = -0.97°$ (heptane, *c* 3.07); CI(NH_3) MS (probe) *m/z* (rel. int.): 340 $[C_{22}H_{42}O+NH_4]^+$ (100), 323 $[C_{22}H_{42}O+H]^+$ (18), 304 $[C_{22}H_{42}O-H_2O]^+$ (4); IR υ_{max} (CCl_4): 2950, 2920, 2860, 2700, 1725, 1660, 1460, 1380, 1375 and 1360 cm^{-1}.

Ozonolysis of **13**. A CS_2 soln (2 ml) of **13** (20 mg) was treated with O_3 at −78° until the characteristic blue colour of O_3 persisted. Then, excess O_3 was removed under a N_2 stream and the reaction mixt. treated with solid triphenylphosphine (20 mg). The resulting soln was allowed to warm to room temp. and concentrated under red. pres. Prep. silica gel TLC yielded a C_{18} ketone (14 mg) exhibiting identical optical rotation and spectral (IR, NMR, mass) features to **12**.

Monoacetate derivative of **10**. Combined frs III–V were reacted with Ac_2O in pyridine by standard procedures. The crude product was purified by CC on silica gel (elution with heptane containing increasing amount of Et_2O). The fr. eluted with heptane–Et_2O (17:3) yielded 32 mg of **11** upon prep. silica gel TLC (heptane–Et_2O, 3:2). CI(NH_3) MS (probe) *m/z* (rel. int.): 652 $[C_{42}H_{82}O_3+NH_4]^+$ (2), 635 $[C_{42}H_{82}O_3+H]^+$ (3), 617 $[C_{42}H_{82}O_3-H_2O]^+$ (100), 575 $[C_{42}H_{82}O_3+H-CH_3CO_2H]^+$ (44); IR υ_{max} (CCl_4): 3600, 2950, 2920, 2850, 1740, 1460, 1375, 1365 and 1240 cm^{-1}; ^1H NMR (250 MHz): δ 5.09 (H-18, *t*, J = 6.2 Hz), 4.86 (H-15, *t*, *J* = 6.0 Hz), 2.11 (CH_3CO, *s*), 1.97 (H-17, *m*), 1.93 (H-20, *t*, *J* = 7.3 Hz), 1.62 (H-16, *m*), 1.57 (Me-37, *s*), 1.60–1.0 (overlapping CH and CH_2), 1.24 (Me-36, *s*), 0.83–0.88 (other methyls); ^{13}C NMR: δ 171.1, 136.4 (C-19), 123.1 (C-18), 79.2 (C-15), 74.3 (C-14), 40.0 (C-20), 39.4 (C-3, C-30), 38.1 (C-13), 37.7, 37.4, 37.3 (C-22), 32.8 (C-6, C-10. C-23, C-27), 29.3, 28.0 (C-2, C-31), 25.4 (C-21), 24.8 (C-4, C-29), 24.5 (C-8, C-25), 23.9 (C-36), 22.7 (C-33, C-40), 22.6 (C-1, C-32), 21.1 (CH_3CO), 20.8 (C-12), 19.8 (C-34, C-35, C-38, C-39), 15.9 (C-37).

Reduction of **11**. Compound **11** (30 mg) in dry Et_2O (10 ml) was treated with $AlLiH_4$ (10 mg) under reflux for 1 hr. Excess hydride was destroyed by addition of a few drops of 10% HCl, the reaction mixt. was extracted with Et_2O and then washed with H_2O. Prep. silica gel TLC (heptane–Et_2O, 1:1) yielded 26 mg of compound **10**.

Preparation of MTPA esters **14** *and* **15**. A soln of compound **10** (7.0 mg) in dry CH_2Cl_2 (1 ml) was treated with *S* (+)-MTPA chloride (6 mg), 4-dimethylamino pyridine (25 mg) and triethylamine (10 μl), and the mixt. stirred at room temp. for 2 hr and evapd to dryness under vacuum. The residue was chromatographed on prep. silica gel TLC (heptane–Et_2O, 41:9) to give MTPA ester **14** (9.5 mg). Similar treatment of compound **10** with (*R*) (−)-MTPA chloride gave compound **15** (9.2 mg).

REFERENCES

1. Metzger, P., Largeau, C. and Casadevall, E., in *Progress in the Chemistry of Organic Natural Products*, Vol. 57, eds W. Herz, G. W. Kirby, W. Steglich and C. Tann. Springer, Wien, 1991, p. 1.
2. Metzger, P. and Casadevall, E., *Phytochemistry*, 1991, **30**, 1439.
3. Metzger, P. and Casadevall, E., *Phytochemistry*, 1992, **31**, 2341.
4. Metzger, P., *Phytochemistry*, 1994, **36**, 195.
5. Metzger, P. and Pouet, Y., *Phytochemistry*, 1995, **40**, 543.
6. Metzger, P., Allard, B., Casadevall, E., Berkaloff, C. and Couté, A., *Journal of Phycology*, 1990, **26**, 258.
7. Cox, R. E., Burlingame, A. L., Wilson, D. M., Eglington, G. and Maxwell, J. R., *Journal of the Chemical Society, Chemical Communications*, 1973, 284.

8. Galbraith, M. N., Hillen, L. W. and Wake, L. V., *Phytochemistry*, 1983, **22**, 1441.
9. Metzger, P., Casadevall, E., Pouet, M. J. and Pouet, Y., *Phytochemistry*, 1985, **24**, 2995.
10. Murakami, M., Nakano, H., Yamaguchi, K., Konosu, S., Nakayama, O., Matsumoto, Y. and Iwamoto, H., *Phytochemistry*, 1988, **27**, 455.
11. David, M., Metzger, P. and Casadevall, E., *Phytochemistry*, 1988, **27**, 2863.
12. Huang, Z., Poulter, C. D., Wolf, F. R., Somers, T. C. and White, J. D., *Journal of the American Chemical Society*, 1988, **110**, 3959.
13. Huang, Z. and Poulter, C. D., *Journal of Organic Chemistry*, 1988, **53**, 5390.
14. Huang, Z. and Poulter, C. D., *Phytochemistry*, 1989, **28**, 3043.
15. Huang, Z. and Poulter, C. D., *Journal of the American Chemical Society*, 1989, **111**, 2713.
16. Poulter, C. D., *Accounts of Chemical Research*, 1990, 70.
17. Wolf, F. R., Nemethy, E. K., Blanding, J. H. and Bassham, J. A., *Phytochemistry*, 1985, **24**, 733.
18. Metzger, P., David, M. and Casadevall, E., *Phytochemistry*, 1987, **26**, 129.
19. Metzger, P. and Casadevall, E., *Tetrahedron Letters*, 1983, **24**, 4013.
20. Huang, Z. and Poulter, C. D., *Phytochemistry*, 1989, **28**, 1467.
21. Metzger, P. and Casadevall, E., *Tetrahedron Letters*, 1987, **28**, 3931.
22. Metzger, P., Bishoff, R., Pouet, Y. and Casadevall, E., *Phytochemistry*, 1993, **32**, 875.
23. Dale, J. A., Dull, D. L. and Mosher, H. S., *Journal of Organic Chemistry*, 1969, **34**, 2543.
24. Othani, I., Kusumi, T., Kashman, Y. and Kakisawa, H., *Journal of the American Chemical Society*, 1991, **113**, 4092.
25. Metzger, P. and Casadevall, E., *Phytochemistry*, 1992, **31**, 2341.
26. Okada, S., Matsuda, H., Murakami, M. and Yamaguchi, K., *Tetrahedron Letters*, 1996, **37**, 1065.
27. Kates, M., *Techniques of Lipidology*, 2nd edn, eds R. H. Burton, and P. H. van Knippenberg. Elsevier, Amsterdam, 1986, p. 519.
28. Katayama, M. and Marumo, S., *Tetrahedron Letters*, 1976, **17**, 1237.
29. Metzger, P., Berkaloff, C., Casadevall, E. and Couté, A., *Phytochemistry*, 1985, **24**, 2305.
30. Gupta, D. N., Hodge, P. and Davies, J. E., *Journal of the Chemistry Society, Perkin Transactions I*, 1981, 2970.