Lycopanerols I−L, Four New Tetraterpenoid Ethers from *Botryococcus braunii*

Pierre Metzger,*,† Marie-Noëlle Rager,‡ Nicole Sellier,§ and Claude Largeau†

Laboratoire de Chimie Bioorganique et Organique Physique, Service RMN, and Laboratoire de Spectrométrie de Masse, Ecole Nationale Supérieure de Chimie de Paris, 11 Rue Pierre et Marie Curie, 75231 Paris Cedex 05, France

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Four new tetraterpenoid ethers, lycopanerols I−L (**5**−**8**), were isolated from a culture of a strain of the green microalga *Botryococcus braunii* (L race). The structures were determined by means of spectral analyses and chemical degradation. The relative stereochemistry of these ethers was established by ROESY NMR. A biogenetic relationship is proposed between lycopanerols and lycopadiene (**1**), the acyclic diunsaturated tetraterpene hydrocarbon synthesized by the alga.

The green colonial microalga *Botryococcus braunii* is renowned as a rich source of lipids, not only those commonly found in algal oils such as fatty acids and triacylglycerols but more particularly unusual compounds including hydrocarbons, epoxides, alkenyl phenols, and a wide variety of ether lipids.[1] Most of these lipids are stored in the thick outer walls that surround the algal cells, such a localization allowing a rather quick extraction from the dry biomass with apolar solvents. Biochemical analyses of wild samples and laboratory-grown strains of *B. braunii* have lead to the recognition in this alga of three morphologically and ultrastructurally similar chemical races. The classification of *B. braunii* strains into the so-called A, B, and L races is based on the production of distinct types of hydrocarbons: *n*-C_{23} to *n*-C_{33} alkadienes and trienes comprising an odd number of carbon atoms (race A), C_{30} to C_{37} triterpenes named botryococcenes (race B), and an acyclic diunsaturated tetraterpene, *trs*,*trs*-lycopadiene, **1** (race L).[1] Lycopanerols, the subject of the present study, are a family of tetraterpenoid ethers closely related to lycopadiene **1**, which have been isolated from strains of the L race of *B. braunii* isolated from water samples collected in some freshwater lakes of Thailand, Ivory Coast, and India.[2]

Lycopanerols are structurally unique and markedly differ from the other classes of natural polyethers, like the THF-acetogenins currently found in Annonaceae,[3] the squalene-derived polyethers isolated from marine sources[4] and from some terrestrial plants,[5] or the isopranyl glycerol ether lipids constitutive of the membranes in Archaea.[6] Lycopanerols A, **2**, were first discovered and their structure was elucidated;[7] they comprise a *trans*-tetrahydrofuran (THF)-containing lycopane connected by an ether bridge to another lycopane comprising a tetrahydropyran ring (THP) in turn bound to a very long *n*-alkenyl chain by another ether bridge. Recently, we reported on the isolation and structural elucidation of new lycopanerols (B−H),[8] composed of one to three lycopane moieties, comprising, or not, a THP and/or a THF ring. Some of these lycopanerols comprise also an *n*-alkyl or an *n*-alkenyl chain and an *n*-alkenyl pyrogallol or a α-tocopherol unit, linked to each other by ether or phenoxy bonds, giving rise to high molecular weight ether lipids, exhibiting up to 150 carbon atoms.[8b]

Further analysis of a lipid extract from a culture of a strain of *B. braunii* originating from a lake of Ivory Coast has yielded four additional new lycopanerols. Herein we report the structures of these new compounds.

Results and Discussion

The algal biomass obtained from a three week old culture grown on a synthetic mineral medium was freeze-dried, and lipids were extracted with heptane. The concentrated crude extract was partitioned between a mixture of compounds soluble in $CHCl_3$−MeOH and an insoluble material. The soluble part was chromatographed over silica gel CC, and the obtained fractions were subsequently purified by silica gel TLC and normal-phase HPLC. These purifications resulted in the isolation of lycopadiene **1**, its mono- and diepoxide derivatives, **3** and **4**,[9] eight previously described lycopanerols, A−H,[8] and four new lycopanerols, I−L (**5**−**8**). Compounds **6**−**8** were purified and analyzed as acetate derivatives.

FABMS of lycopanerols I, **5** (Table 1), showed a set of fragment ions $[M - H_2O + H]^+$, compatible with a mixture of two series of homologous compounds increasing in molecular mass by two methylenes, successively, on one hand, $C_{66}H_{124}O_3$, $C_{68}H_{128}O_3$, $C_{70}H_{132}O_3$, $C_{72}H_{136}O_3$, and on the other hand $C_{66}H_{122}O_3$, $C_{68}H_{126}O_3$, $C_{70}H_{130}O_3$, $C_{72}H_{134}O_3$, $C_{74}H_{138}O_3$, $C_{76}H_{142}O_3$, each series exhibiting five and six degrees of unsaturation, respectively. This was further confirmed by HR MALDI-TOF-MS (Table 1). The IR spectrum showed a hydroxyl absorption at 3580 cm^{-1} and a band at 1590 cm^{-1} for an aromatic ring. The NMR spectra (Table 2) displayed resonances for a 1,3,5-trisubstituted benzene ring, as deduced from the presence in the ^1H NMR spectrum of three singlets for three protons (H-2′, H-4′, and H-6′) at δ_H 6.35, 6.32, and 6.40, respectively, and in the ^{13}C NMR spectrum of three peaks for three aromatic methine carbons at δ_C 99.7, 106.7, and 108.6, and three peaks for three aromatic quaternary carbons at δ_C 161.1, 160.7, and 145.5 (C-1′, C-3′, and C-5′, respectively). These spectra also showed signals for a methoxy group (δ_H 3.76, δ_C 55.3) and for a midchain unsaturation (δ_H 5.34, δ_C 130.0), in a *Z* configuration, as deduced from the chemical shift of the allylic carbons at δ_C 27.3.[10] The spectra also displayed signals for a trisubstituted double bond at positions 18,19, with a triplet at δ_H 5.06 and carbon signals at δ_C 123.6 (methine carbon) and 136.5 (quaternary carbon), of *E* configuration as determined from the chemical shift value of the allylic carbon C-20 (δ_C 40.1).[2a] The location of the two olefinic unsaturations at positions 18,-19 and ω9′,ω10′ could be inferred from the identification of trimethyl-6,10,14-pentadeca-2-one and *n*-nonanal in the products resulting from the reductive cleavage of the derived polyozonide. The substitution of the benzene ring

* To whom correspondence should be addressed. Fax: +33-143257975. Tel: +33-144276717. E-mail: pierre-metzger@enscp.jussieu.fr.
† Laboratoire de Chimie Bioorganique et Organique Physique, UMR CNRS 7573.
‡ Service RMN, UMR CNRS 7576.
§ Laboratoire de Spectrométrie de Masse, UMR CNRS 7573.

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Table 1. Mass Data of Lycopanerols I (**5**)

molecular formula	length of the normal chain	FAB		HR MALDI-TOF
		$[M - H_2O + H]^+$ (rel int)	resorcinol fragment[a] *m/z*	$[M - H_2O + H]^+$ (calcd); Δ mmu
		Ethers Comprising a *n*-Alkylresorcinol Moiety		
$C_{66}H_{124}O_3$	C_{19}	948 (6)	391	947.9357 (947.9518); −16.1
$C_{68}H_{128}O_3$	C_{21}	976 (34)	419	975.9772 (975.9831); −5.9
$C_{70}H_{132}O_3$	C_{23}	1004 (10)	447	1003.9942 (1004.0143); −20.1
$C_{72}H_{136}O_3$	C_{25}	1032 (2)	475	not determined
		Ethers Comprising a *n*-Alkenylresorcinol Moiety		
$C_{66}H_{122}O_3$	C_{19}	946 (5)	not detected	not determined
$C_{68}H_{126}O_3$	C_{21}	974 (25)	417	973.9643 (973.9679); −3.6
$C_{70}H_{130}O_3$	C_{23}	1002 (10)	445	1001.9941 (1001.9992); −5.1
$C_{72}H_{134}O_3$	C_{25}	1030 (2)	473	not determined
$C_{74}H_{138}O_3$	C_{27}	1058 (2)	501	not determined
$C_{76}H_{142}O_3$	C_{29}	1086 (4)	529	not determined

[a] $[M - C_{40}H_{79}O + 2H]^+$ ions.

Table 2. ^1H and ^{13}C NMR Data of Lycopanerols I (**5**)[a,b]

position	δC	δH (mult., *J*, Hz)	HMBC[c]
1,32	22.8	0.87 d (6.7)	
2,31	28.1	1.52 m	
3,30	39.5	1.13 m	
4,29	24.9	1.15−1.35 m	
5,28	37.4	1.05 m, 1.25 m	
6,27	32.9	1.37 m	
7,26	37.5	1.05 m, 1.25 m	
8,25	24.6	1.15−1.35 m	
9,24	37.5	1.05 m, 1.25 m	
10,23	32.9, 32.3	1.37 m	
11,22	37.8, 37.9	1.41 m, 1.59 m	
12	20.8	1.40 m	
13	37.8	1.40 m, 1.60 m	12, 15, 36
14	75.2		13, 15, 16, 36
15	84.3	4.05 dd (3, 8.6)	16, 17, 36
16	30.8	1.72 m	15, 18
17	24.9	2.02 m, 2.12 m	15, 18
18	123.6	5.06 t (6.7)	16, 17, 37
19	136.5		37
20	40.1	1.91 t (8.2)	18, 37
21	25.5	1.34 m	20, 37
33,40	22.7	0.87 d (6.7)	
34,39	19.8	0.84 d (6.6)	
35,38	19.8	0.84 d (6.6)	
36	23.9	1.20 s	15
37	16.0	1.45 s	18, 20
1′	161.1		15, 2′, 6′
2′	99.7	6.35 s	4′, 6′
3′	160.7		2′, 4′, OCH_3
4′	106.7	6.32 s	2′, 6′, 7′
5′	145.5		7′
6′	108.6	6.40 s	2′, 4′, 7′
7′	36.4	2.51 t (7.5)	4′, 6′, 8′
8′	29.9	1.58 m	7′
9′ to ω12′	29.8−29.4	1.25	
ω8′,ω11′	27.3	2.02 m	ω9′, ω10′
ω9′,ω10′	130.0	5.34 br	ω8′, ω11′
ω4′ to ω7′	29.8−29.4	1.25	
ω3′	32.0	1.25	
ω2′	22.8	1.25	
ω1′	14.2	0.87 t (8.6)	
OCH_3	55.3	3.76 s	

[a] Spectra recorded in $CDCl_3$. [b] *J* values in Hz are shown in parentheses. [c] Proton correlating with carbon resonance.

at C-5′ by a normal hydrocarbon chain was supported by prominent peaks in the ^1H (δ_H 1.25) and ^{13}C (δ_C 29.4−29.8) NMR spectra. Moreover, the low mass region of the FAB-mass spectrum showed two series of peaks likely related to alkyl and alkenyl resorcinol ions (Table 1). These ions were indicative of the existence of normal hydrocarbon chains comprising an odd number of carbon atoms: 19−25 for the saturated chains in **5a** and 19−29 for the unsaturated ones in **5b**. The ^1H, HMQC, and HMBC NMR data established the presence of two vicinal oxygen-bearing carbons, a quaternary (δ_C 75.2) and a tertiary (δ_H 4.05, δ_C 84.3) which are part of a −C(Me)OH−CH(OR)−CH_2−CH_2−CH═C(Me)− pattern, as deduced from $^1H-^1H$ COSY data. Finally, a ^3J HMBC correlation observed between H-15 and the aromatic carbon C-1′ established that lycopanerols I are monounsaturated lycopanes, bearing a hydroxyl at C-14, and linked at C-15 to an odd carbon numbered *n*-C_{19}−C_{25} alkyl (**5a**) or *n*-C_{19}−C_{29} alkenyl (**5b**) resorcinol monomethyl ether, via a phenoxy bond, as shown in Figure 1.

The molecular formula of the acetate derivative of lycopanerol J (**6a**), $C_{122}H_{238}O_8$, was established by HR MALDI-TOF-MS (*m/z* $[M + Na]^+$ 1854.7982). The IR spectrum demonstrated the presence of hydroxyl group (3570 cm^{-1}) and ester functions (1735 cm^{-1}). The comparison of ^1H, ^{13}C, COSY, HMQC, and HMBC (Table 3) NMR data of **6a** with those of lycopanerols F[8a] indicated the presence of two *trans*-THF-containing lycopanes, ether-linked at C-19 and C-19′, respectively. The *trans* conformation of the THF rings in lycopanerols was previously deduced from NMR data of model compounds[2] and from the biomimetic conversion of the natural *anti*-diepoxy-14(*R*),15(*R*),18(*S*),19(*S*)-lycopane (**4**), precursor of some lycopanerols, into a *trans*-THF-containing lycopane.[9b] The ^1H and ^{13}C NMR spectra of **6a** differed from those of lycopanerol F by the presence of signals for an epoxide function, δ_H 2.69 (1 H) and δ_C 63.4 (tertiary) and 61.0 (quaternary), and an acetoxy group borne by a tertiary carbon atom (δ_H 4.90, δ_C 77.4). The existence of a $-CH_2-CH(OAc)-C(OR)(Me)-CH_2-$ pattern, β to the epoxide, was revealed by some long-range proton−carbon correlations (Table 3). The HMBC spectrum did not show connectivities allowing to link this pattern and the second THF-containing lycopane. This was deduced from the general molecular formula, which indicated that they might be connected by an ether bridge, at C-14′ and C-14″, on the basis of the NMR data. This was finally confirmed by the ESI-MS/MS: a MS^3 fragmentation of the quasi-molecular ion $[M + Na]^+$ gave an ion at *m/z* 1220, most likely resulting from the cleavage of the C(14″)−O ether bond (Figure 1S; Supporting Information). Therefore, the structure of the acetate derivative of lycopanerol J was determined as **6a**, and due to the absence of acetyl signals in the NMR spectra of fraction IV which contained this ether, the natural form of lycopanerol J was assigned to be diol **6** (Figure 1).

The ESI mass spectrum of lycopanerols K isolated as triacetates (**7a**) showed six quasi-molecular ions $[M + Na]^+$, at *m/z* 2259, 2287, 2315, 2343, 2371, and 2399, suggesting that the mixture was made of six homologous compounds increasing in molecular mass by two methylenes, successively. The IR spectrum exhibited absorptions for a hydroxyl group (3570 cm^{-1}), ester functions (1770 and 1735

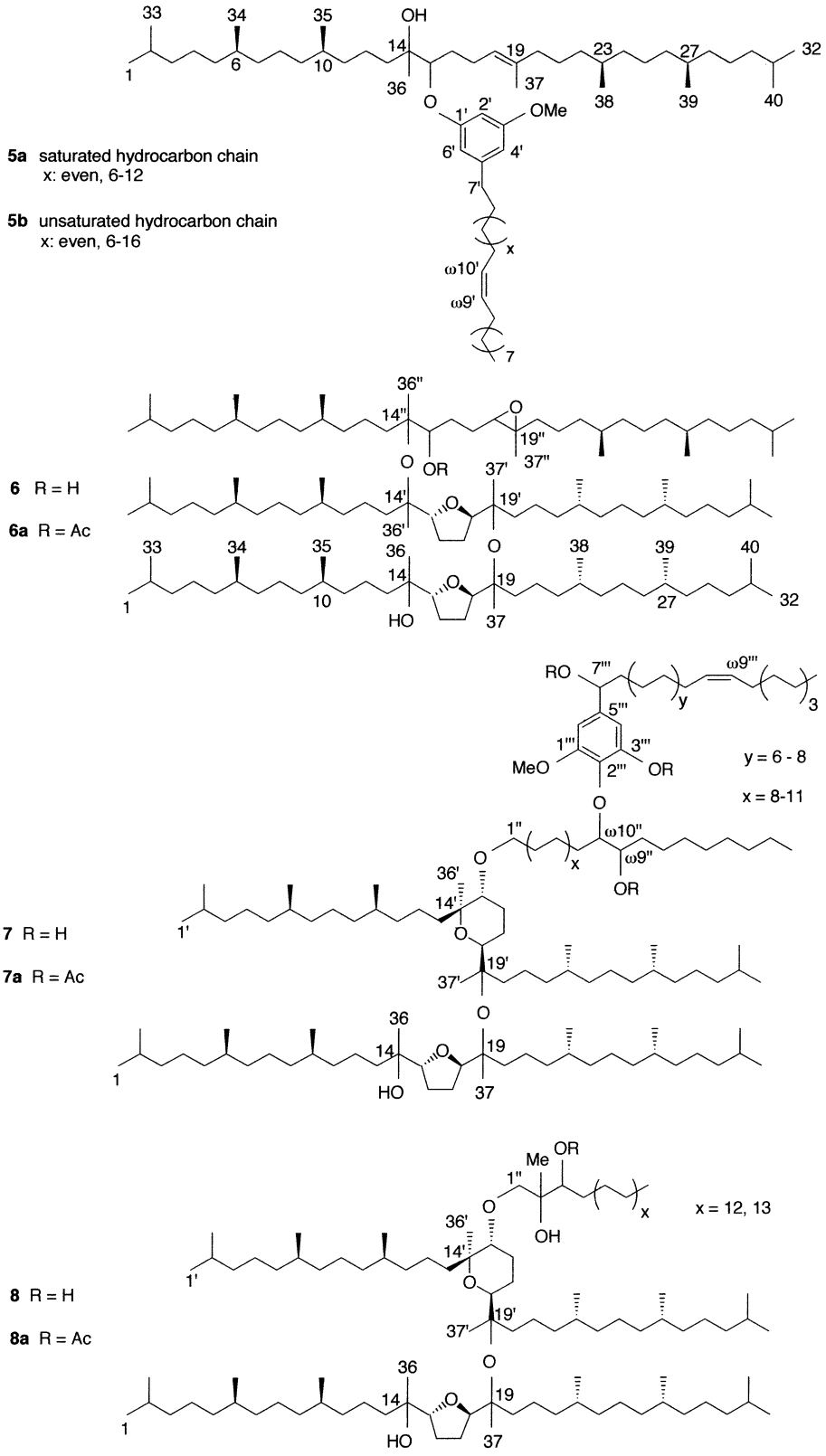

Figure 1. Lycopanerols I−L (**5**−**8**).

cm^{-1}), and an aromatic ring (1600 cm^{-1}). The comparison of the ^{1}H and ^{13}C NMR data (Table 4) with those of lycopanerols A and B[8a] clearly showed that these new compounds are derivatives of the latter. Proof of the attachment by ether bridges of a *trans*-THF-containing lycopane to a THP-containing lycopane, in turn linked to a methylenic carbon of an aliphatic chain, was shown by the observation in the HMBC spectrum of a ^{4}J connectivity between H-18′ and C-19 and ^{3}J connectivities between the two diastereotopic protons H-1″ and C-15′, respectively. In the THP ring, the equatorial positions of substituents at positions 15′ and 18′ were deduced from the coupling constant values of protons H-15′ (dd, J = 3.5, 10.4 Hz) and H-18′ (dd, J = 1.5, 9.0 Hz). The orientations of these substitutents were indirectly corroborated by a ROESY experiment which showed correlations between Me-36′ and

Table 3. Selected ^{1}H and ^{13}C NMR Data of Lycopanerol J (**6a**)[a–c]

position	δC	δH mult. (*J*, Hz)	HMBC[d]
13	38.1	1.40 m	36
14	73.0		15, 36
15	85.8	3.70 dd (5.8, 9.3)	16, 18, 36
16	26.5	1.80 m	15, 17
17	27.3	1.80 m, 1.90 m	16, 18
18	83.4	3.89 t (7.1)	17, 37
19	79.4		37, 18′
20	38.9	1.35 m, 1.56 m	18
36	24.6	1.18 s	15
37	19.6	1.12 s	18
13′	42.3	1.39 m, 1.49 m	36′
14′	80.7		15′, 36′
15′	76.7[e]	3.54 br d (7.0)	16′, 36′
16′	27.3	1.60 m	17′
17′	26.2	1.60 m	16′
18′	76.7[e]	3.54 br d (7.0)	17′, 37′
19′	80.5		18′, 37′
20′	42.3[e]	1.39 m, 1.49 m	37′
36′	21.2	1.08 s	
37′	21.2	1.10 s	
14″	78.9		36″
15″	77.4	4.90 dd (1.7, 9.9)	36″, C*H*$_3$CO
16″	31.2	1.54 m, 1.70 m	15″, 17″
17″	25.3		15″, 18″
18″	63.4	2.69 t (6.3)	37″
19″	61.0		18″, 37″
20″	39.2	n.d.	18″, 37″
36″	19.7	1.12 s	15″
37″	16.6	1.22 s	
*C*H$_3$CO	21.3	2.05 s	
CH$_3$*C*O	170.7		15″, C*H*$_3$CO

[a] Spectra recorded in $CDCl_3$. [b] *J* values in Hz are shown in parentheses. [c] Resonances for other protons and carbons of the lycopane moieties are similar to those of the corresponding atoms in **5**. [d] Proton correlating with carbon resonance. [e] Broad peak. n.d.: not determined.

H-18′. Moreover, the ^{1}H and ^{13}C NMR spectra exhibited signals for a 1,2,3,5-tetrasubstituted benzene ring with two proton signals at δ_H 6.65 (H-4‴) and 6.73 (H-6‴) and two aromatic methine carbons at δ_C 113.4 (C-4‴) and 108.7 (C-6‴). HMBC data indicated that the benzene ring bears a methoxy group at C-1‴ and an acetoxy group at C-3‴ and that it was connected at C-5‴ to methine carbon C-7‴ (δ_H 5.66, δ_C 75.6) bearing an acetoxy group and at C-2‴ to a methine carbon (δ_H 4.35, the signal of the carbon is split into two peaks of similar intensity at δ_C 81.2 and 81.1) via a phenoxy bond. Furthermore, on the basis of COSY and HMBC data a third acetoxy group was fixed α to this oxymethine carbon. The presence of two peaks for the methine carbon (δ_C 81.1 and 81.2) just like for carbons at positions 1‴, 2‴, 7‴, 8‴, and those of carboxyl functions, suggested the existence of isomerisms likely related to the configurations of the acetoxy groups at C-7‴ and C-ω9″. Recording the ^{1}H NMR spectrum at 313 K instead of 293 K had no effect on the splitting of the signals of the methyl protons of the acetoxy groups at C-ω9″ and C-7‴, suggesting that there was no steric hindrance for the rotation of these groups. The chain lengths of the non-terpenoid moieties were estimated from the ESI mass data. MS^2 fragmentations of the preponderant molecular adduct ion, *m*/*z* 2343, gave two ions at *m*/*z* 1669 and 1727 (Experimental Section and Figure 2), which were inferred to result from the cleavages of the C(19)−O ether bond and C(ω10″)−O phenoxy bond, respectively. This last ion strongly suggested that the THP ring was ether linked to a C_{32} chain (Figure 2, $x = 10$) bearing an acetoxy group and, furthermore, that the pyrogallol moiety comprised a C_{27} alkenyl chain (Figure 2, $y = 7$). The unsaturation was

Table 4. Selected ^{1}H and ^{13}C NMR Data of Lycopanerols K (**7a**)[a–c]

position	δC	δH mult. (*J* in Hz)	HMBC[d]
13	38.1	1.40 m	36
14	72.9		15, 36
15	85.7	3.71 dd (6.2, 9.2)	18, 36
16	26.5	1.70−1.80 m	
17	27.2	1.85 m	
18	83.6	3.87 t (6.7)	37
19	79.4		37, 18′
20	38.7	1.36 m, 1.56 m	37
36	24.6	1.18 s	
37	19.5	1.13 s	
13′	42.4	1.48 m	36′
14′	80.0		15′, 36′
15′	85.0	3.10 dd (3.5, 10.4)	36′, 1″
16′	25.8	1.86 H-eq m, 1.48 H-ax m	
17′	28.2	1.60 m, 1.65 m	
18′	77.8	3.50 dd (1.5, 9.0)	37′
19′	80.8		37′
20′	41.9	1.40 m, 1.56 m	37′
36′	20.7	1.11 s	
37′	21.2	1.04 s	
1″	70.3	3.23 dt (9.0, 6.8) 3.50 dt (9.0, 6.3)	
2″	30.4	1.52 m	1″
3″	26.4	n.d	1″
ω12″	25.6	1.20−1.50 m	ω10″
ω11″	30.1	1.50−1.60 m	ω9″, ω10″
ω10″	81.2, 81.1	4.35 m	ω9″
ω9″	74.7	5.03 m	ω10″, C*H*$_3$CO (ω9″)
ω8″	30.4	1.50−1.60 m	ω9″, ω10″
ω7″	25.2	1.20−1.50 m	ω9″
1‴	152.9, 153.0		6‴, OCH$_3$
2‴	138.4, 138.5		4‴, 6‴, ω10″
3‴	144.1		4‴, C*H*$_3$CO (3‴)
4‴	113.4	6.65 br s	6‴, 7‴
5‴	135.8		4‴, 6‴, 7‴
6‴	108.7	6.73 br s	4‴, 7‴, OC*H*$_3$
7‴	75.6[e]	5.66 t (6.1)	4‴, 6‴, C*H*$_3$CO (7‴)
8‴	36.2[e]	1.70−1.85 m	7‴
9‴	25.7	1.20−1.50 m	7‴, 8‴
ω8‴,ω11‴	27.3	2.02 m	ω9‴,ω10‴
ω9‴,ω10‴	130.0	5.35 t (4.6)	ω8‴,ω11‴
*C*H$_3$CO (ω9″)	21.2[e]	1.97 s, 1.98 s	
CH$_3$*C*O (ω9″)	170.7[e]		ω9″, C*H*$_3$CO (ω9″)
*C*H$_3$CO (3‴)	20.8	2.28 s	
CH$_3$*C*O (3‴)	168.6		C*H*$_3$CO (2‴)
*C*H$_3$CO (7‴)	21.4[e]	2.05 s, 2.06 s	
CH$_3$*C*O (7‴)	170.4[e]		7‴, C*H*$_3$CO (7‴)
OCH$_3$	55.9	3.83 s	

[a] Spectra recorded in $CDCl_3$. [b] *J* values in Hz are shown in parentheses. [c] Resonances for other protons and carbons of the lycopane moieties are similar to those of the corresponding atoms in **5**. [d] Proton correlating with carbon resonance. [e] These signals are split into two peaks, with a difference between the two resonances less than 0.1 ppm.

determined to be located at position ω9‴ from the identification of *n*-nonanal in the products resulting from the reductive cleavage of the derived ozonides. The stereochemistry of this carbon−carbon double bond was established to be *Z* on the basis of the chemical shift of its allylic carbons (δ_C 27.3).[10] In the EIMS spectrum of the TMS derivatives **7b** (Experimental Section and Figure 2S, Supporting Information), an ion at *m*/*z* 215 $[C_9H_{18}OSiMe_3]^+$ was indicative for a hydroxyl group at C-ω9″ in **7** and, consequently, the phenoxy bond at C-ω10″. Therefore, we conclude that these triacetates have the structure shown in **7a** (Figure 1). They comprise a *trans*-THF-containing

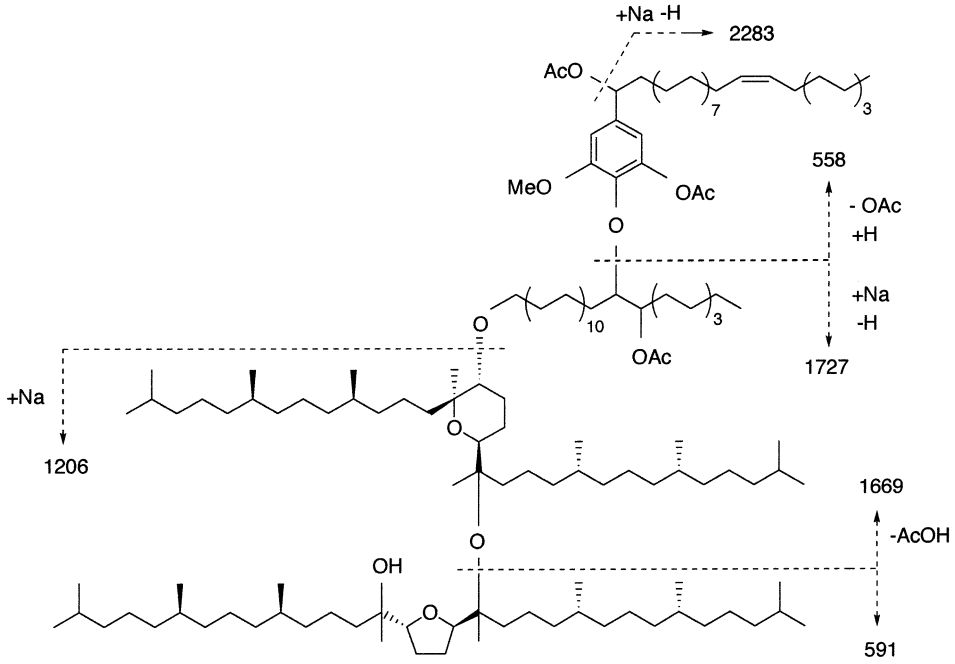

Figure 2. Fragmentation (ESI-MS) of lycopanerol **7a**, $C_{152}H_{286}O_{13}$ ([M + Na]$^+$ *m/z* 2343).

lycopane connected at C-19 via an ether bridge with C-19′ of a THP-containing lycopane ether linked at C-15′ to C-1″of an *n*-alkyl chain in turn linked at C-ω10″ to an *n*-alkenyl pyrogallol derivative via a phenoxy bond. The absence of acetyl resonances in the ^{1}H and ^{13}C NMR spectra of fraction V showed that lycopanerols K comprise three alcohol functions and a free hydroxy phenol.

When lycopanerols K (**7a**) were exposed to the air at room temperature, it was apparent that they undergo spontaneous oxidation. Indeed, the ESI mass spectrum of **7a** stored under these conditions for two weeks exhibited in addition to the pseudo-molecular ions [M + Na]$^+$ some new peaks shifted to higher masses by 16 mu, suggesting the integration of one oxygen atom. Furthermore, MS-MS data located the position of this oxidation. For example, the daughter ion spectrum of *m/z* 2359 [M + 16 + Na]$^+$ gave, in addition to a major peak at *m/z* 2299 resulting from the loss of AcOH, two minor peaks: one at *m/z* 1727, already present in the MS^2 fragmentation of the quasi-molecular ion *m/z* 2343 [M + Na]$^+$, and the other at *m/z* 1743. This latter ion strongly suggested the insertion of an oxygen atom into the C(ω10″)−O(pyrogallol) bond. Upon exposure to the air, lycopanerols K would lead to the formation of peroxides.

The FAB-MS of lycopanerols L (**8a**) displayed two quasi-molecular ions [M + Na]$^+$ at *m/z* 1717 and 1745, with peaks in a 3:1 ratio. The HR MALDI-TOF-MS showed two quasi-molecular ions at *m/z* 1716.6581 [$C_{112}H_{220}O_8$ + Na]$^+$ and 1744.6901 [$C_{114}H_{224}O_8$ + Na]$^+$. The IR spectrum showed absorption bands for hydroxyl group (3565 cm^{-1}) and ester functions (1735 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (Table 5) contained signals for a *trans*-THF-containing lycopane ether linked to a THP-containing lycopane similar to those of lycopanerols A, B, and K. From the coupling constant values of H-15′ and H-18′ and from ROESY correlations (Figure 3S; Supporting Information), it appeared that the relative stereochemistries of the substituents in these two rings were similar to those existing in three lycopanerols, A, B, and K. The comparison of ^{1}H and ^{13}C NMR data of **8a** with those of lycopanerols A indicated that the THP ring also contained a side chain, ether linked from C-15′ to the methylene C-1″. This was confirmed by the observation in the HMBC spectrum of a ^3J connectivity between H-15′ and C-1″. In addition, lycopanerols L exhibited signals for a methyl group (δ_H 1.13 s, δ_C 20.9) and a methine (δ_H 4.92 dd, δ_C 76.5) bearing an acetoxy group and

Table 5. Selected ^{1}H and ^{13}C NMR Data of Lycopanerols L (**8a**)[a–c]

position	δC	δH, mult. (*J* in Hz)	HMBC[d]
13	38.1	1.40 m	36
14	73.0		13, 15, 16, 36
15	85.8	3.70 dd (5.9, 9.4)	18, 36
16	26.5	1.79 m, 1.74 m	15, 17
17	27.3	1.87 m	18
18	83.6	3.88 t (7.1)	17, 37
19	79.4		20, 37, 18′
20	38.7	1.35 m, 1.55 m	37
36	24.6	1.18 s	15
37	19.6	1.13 s	18
13′	42.5	1.47 m	36′
14′	79.6		13′, 15′, 36′
15′	85.1	3.14 br d (7.7)	36′
16′	25.8	H_{eq}: 1.85 m, H_{ax}: 1.46 m	17′
17′	26.3	1.70 m	16′
18′	77.8	3.52 br d (8.7)	37′
19′	81.1		37′
20′	41.9[e]	1.40 m, 1.55 m	37′
36′	20.6	1.12 s	15′
37′	21.3	1.03 s	18′
1″	74.2	H_a: 3.46 d (8.7) H_b: 3.09 d (8.7)	15′, C*H*$_3$C(OH)
2″	73.3		1″, 3″, C*H*$_3$C(OH)
3″	76.5	4.92 dd (2.3, 10.4)	1″, C*H*$_3$C(OH), C*H*$_3$CO
4″	26.3	1.50 m, 1.67 m	3″, 5″
5″	30.0	1.20−1.30	3″
ω1″	14.2	0.87	
ω2″	22.8	1.20−1.30	
ω3″	32.0	1.20−1.30	
ω4″-6″	29.4−29.8	1.20−1.30	
*C*H$_3$C(OH)	20.9	1.13 s	1″ (H_a), 3″
*C*H$_3$CO	21.2	2.07 s	
CH$_3$*C*O	170.6		3″, C*H*$_3$CO

[a] Spectra recorded in $CDCl_3$. [b] *J* values in Hz are shown in parentheses. [c] Resonances for other protons and carbons of the lycopane moieties are similar to those of the corresponding atoms in **5**. [b] Proton correlating with carbon resonance. [e] Broad peak.

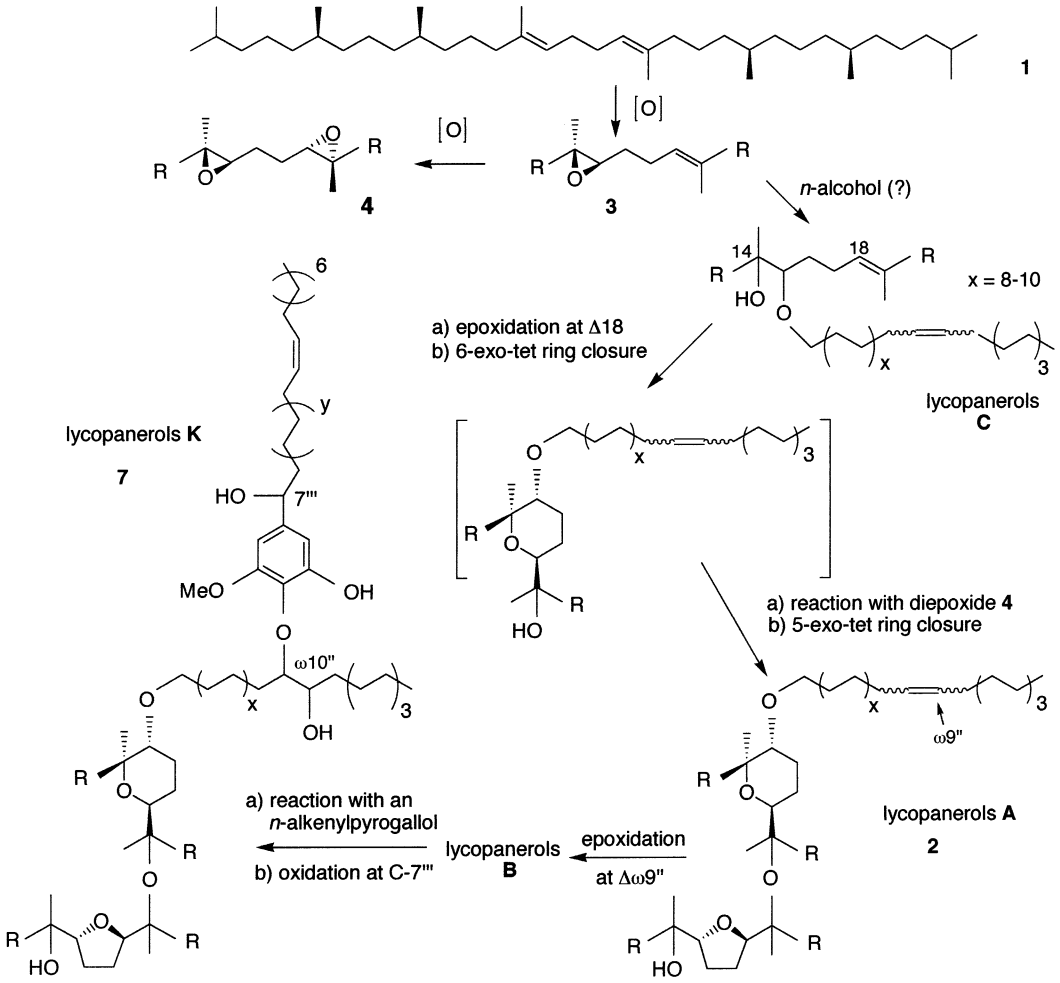

Figure 3. Proposed biogenesis of lycopanerols K (**7**; R = $C_{16}H_{33}$).

bound to a quaternary carbon atom (δ_C 73.3). Significant correlations observed in the HMBC spectrum, Table 5 and Figure 3S (Supporting Information), established that these groups belong to a $-O-CH_2-C(CH_3)OH-CH(OAc)-CH_2-$ pattern, ether linked from its left side to the THP ring, at C-15′. The alkyl moiety of the side chain (in its right side) was inferred to be unbranched owing to the high intensity in the ^1H NMR spectrum of a polymethylene signal at δ_H 1.29 and the presence in the ^{13}C NMR spectrum of numerous intense peaks at δ_C around 29 ppm. Moreover, from the mass data it could be deduced that, with the exception of the acetoxy group, this side chain comprises 30 and 32 carbon atoms in the predominant and minor lycopanerols L, respectively. Acetyl signals being absent in the ^1H and ^{13}C NMR spectra of fraction IV, the natural lycopanerols L were determined to be triols **8** (Figure 1).

Lycopanerol tetraterpenes are believed to be biosynthesized from their lycopadiene precursor, **1**, through sequential condensation of monoepoxide(s) and (or) diepoxide(s).[8a] Thus, the biogenesis of lycopanerols K, **7**, would proceed likely through the addition of a very long chain fatty alcohol to monoepoxide **3** (Figure 3) to yield lycopanerols C. Then, the pyrane ring would be generated by a 6-exo-tet ring closure of the derived 18,19-epoxide, via protonation of the epoxide and attack by the 14-hydroxy group. In accordance with Baldwin's rules,[11] the formation of the pyrane ring would be favored over a 7-endo-tet ring closure. Then, this THF intermediate would be coupled with diepoxide **4** to give lycopanerols A (**2**). Subsequently, lycopanerols K would be produced by the addition of an *n*-alkyl- or *n*-alkenylpyrogallol to the derived $\omega 9''$-epoxides, lycopanerols B, and finally oxidation would occur at the benzylic carbon atom C-7‴. Lycopanerols E would be similarly produced through the ring closure of an epoxide derived from lycopanerols I (**5**) and then coupling with diepoxide **4**. Furthermore, the structure of lycopanerol J (**6**) strongly suggests that it is the direct precursor of lycopanerol F. The biosynthetic origin of the methyl-branched chain in lycopanerols L (**8**) seems to be, however, more difficult to approach.

Experimental Section

General Experimental Procedures. Silica gel 60 (Merck, 70−230 mesh) was used for open column chromatography. TLC purifications were performed on glass plates coated with silica gel 60 (Merck, $PF_{254+266}$) and visualized by UV light. IR spectra were obtained on a Perkin-Elmer 1420. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Bruker Avance 400 DPX; chemical shifts are given on a δ scale, in ppm, and referenced to the residual solvent signals with resonances at δ_H 7.26 and δ_C 77.1 ($CDCl_3$). EIMS spectra (direct inlet at 70 eV) were recorded with a Nermag R10-10-C. GC-EI-MS were recorded at 70 eV on a HP 5989 spectrometer. FAB mass spectra were obtained with a JEOL MS 700, with inclusion of the products in a nitrobenzyl alcohol matrix and addition of NaI. ESI mass spectra were recorded with an API 3000 (Applied Biosystems). MALDI-TOF-MS were obtained with an Applied Biosystems Voyager DE-STR, at the "Service Central d'Analyse du CNRS", Vernaison, France, with inclusion of the products in a dithranol (1,8,9-trihydroxyanthracene) matrix and addition of NaI. Normal-phase HPLC analyses and quantitative separations were performed with two spherical 3 μm silica columns (0.46 × 15 cm) connected in

series, with differential refractometer detection. Acetylations were carried out according to standard procedures.[12]

Organism and Culture. The strain originated from a lake in Yamoussoukro, Ivory Coast, and was fully described in a previous paper.[2b] The strain is conserved in the laboratory by periodic replications (every 4 months) on a modified CHU 13 medium.[13] The alga was grown at 25 °C, under air-lift conditions (air enriched by 1% CO_2) and continuous illumination (470 μE m^{-2} s^{-1}) as previously described.[13] The cultures were harvested when they entered the stationary phase of growth, by filtration on 10 μm Nylon cloth, and then freeze-dried.

Extraction and Isolation. The dry biomass of the Yamoussoukro strain (22.1 g) was extracted at room temperature with heptane and the extract treated to eliminate a rubbery material as previously described.[8a] The resulting oil was separated into five fractions via silica gel CC by elution with heptane (fraction I), heptane−Et_2O, 19:1 v/v (fraction II), heptane−Et_2O, 23:2 v/v (fraction III), heptane−Et_2O, 17:3 v/v (fraction IV), and then Et_2O (fraction V), as previously described.[8a] Fraction II (1.6 g) separated by silica gel TLC, elution by heptane−Et_2O, 22:3 v/v, afforded the monoepoxide of lycopadiene (R_f 0.57), lycopanerols A, C (R_f's 0.48 and 0.25, respectively) and I (**5**; 16 mg; R_f 0.32). Fraction III (1.2 g) purified by silica gel TLC by elution with heptane−Et_2O, 21:4 v/v, afforded lycopanerols B (R_f 0.65) and F (R_f 0.5), *anti*-diepoxy lycopane (R_f 0.48), and a wide band of R_f 0.4−0.25 corresponding to a mixture of compounds. This mixture was reacted with Ac_2O−pyridine, extracted as usual, and purified by silica gel TLC, with heptane−Et_2O, 17:3 v/v, as eluent, to give the acetate derivatives of lycopanerols E (R_f 0.36). Fraction IV (1.05 g) was also reacted with Ac_2O−pyridine, extracted, and purified by silica gel TLC with heptane−Et_2O, 41:9 v/v, as eluent to give the acetate derivatives of lycopanerols D and G (R_f's 0.58 and 0.76, respectively) and two bands (R_f's 0.53−0.34 and 0.32−0.05) corresponding to mixtures of compounds. The first of these two bands was purified by silica gel-$AgNO_3$ TLC, with heptane−Et_2O, 3:1 v/v, as eluent, affording a major band of R_f 0.62 containing J, as **6a** (45 mg). The second band (R_f's 0.32−0.05) purified by silica gel TLC (eluent heptane−Et_2O−MeOH, 69:30:1 v/v/v) afforded lycopanerols L, as **8a** (25 mg; R_f 0.53). After acetylation, fraction V (0.25 g) was separated via silica gel CC by elution with heptane containing increasing amounts of Et_2O, and the fraction eluted with heptane−Et_2O, 17:3 v/v, was collected (0.12 g) and further purified by silica gel-$AgNO_3$ TLC (eluent heptane−Et_2O, 13:7 v/v). The band exhibiting R_f 0.54 (23 mg) was finally purified by normal-phase HPLC (heptane−THF, 25:1, 1.5 mL/min). Repeated injections gave lycopanerols K, as **7a** (12 mg; t_R 12 min).

Lycopanerol I (5): clear oil; LR FAB(magic bullet)-MS and HR MALDI-TOF-MS, see Table 1; IR (CCl_4) ν_{max} 3580, 3000, 2950, 2920, 2840, 1590, 1460, 1430, 1370, 1360, 1190, 1145, 1060, 720 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2.

Lycopanerol J (6a): clear oil; HR MALDI-TOF-MS obsd 1854.7982 ($C_{122}H_{238}O_8Na$ requires 1854.8190, Δ −11.8 mmu); ESI-MS/MS *m/z* (rel int) 1855 $[M + Na]^+$ (100); daughter ion spectrum of *m/z* 1855 gives *m/z* 1795 $[M + Na - AcOH]^+$, *m/z* 1220 $[M + Na - AcOH - C_{40}H_{78}O - H]^+$; daughter ion spectrum of *m/z* 1795 gives *m/z* 1220 $[M + Na - AcOH - C_{40}H_{78}O - H]^+$, *m/z* 1204 $[M + Na - AcOH - C_{40}H_{78}O_2 - H]^+$; IR ($CCl_4$) ν_{max} 3570, 2950, 2920, 2860, 1735, 1460, 1370, 1360, 1230, 1150, 1070 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3.

Lycopanerols K (7a): clear oil; ESI-MS (NaI) *m/z* 2315.44 $[C_{150}H_{282}O_{13} + Na]^+$, *m/z* 2343.57 $[C_{152}H_{286}O_{13} + Na]^+$, *m/z* 2371.56 $[C_{154}H_{290}O_{13} + Na]^+$, *m/z* 2399.53 $[C_{156}H_{294}O_{13} + Na]^+$; LR MALDI-TOF-MS *m/z* 2259.1 $[C_{146}H_{274}O_{13} + Na]^+$, *m/z* 2287.1 $[C_{148}H_{278}O_{13} + Na]^+$, *m/z* 2315.1 $[C_{150}H_{282}O_{13} + Na]^+$, *m/z* 2343.2 $[C_{152}H_{286}O_{13} + Na]^+$, *m/z* 2371.2 $[C_{154}H_{290}O_{13} + Na]^+$, *m/z* 2399.3 $[C_{156}H_{294}O_{13} + Na]^+$; HR MALDI-TOF-MS obsd *m/z* 2315.1583 ($C_{150}H_{282}O_{13}Na$ requires 2315.1301, Δ 28.2 mmu), *m/z* 2343.1530 ($C_{152}H_{286}O_{13}Na$ requires 2343.1616, Δ −8.6 mmu), *m/z* 2371.2094 ($C_{154}H_{290}O_{13}Na$ requires 2371.1929, Δ 16.5 mmu); ESI-MS/MS *m/z* (rel int) 2343 $[M + Na]^+$ (60); daughter ion spectrum of *m/z* 2343 gives *m/z* 2283 $[M + Na - AcOH]^+$; daughter ion spectrum of *m/z* 2283 gives *m/z* 2223 $[M + Na - 2AcOH]^+$, *m/z* 2163 $[M + Na - 3AcOH]^+$, *m/z* 1727 $[M + Na - AcOH - C_{36}H_{61}O_4]^+$, *m/z* 1669 $[M - AcOH - C_{40}H_{79}O_2]^+$; IR ($CCl_4$) ν_{max} 3570, 2950, 2920, 2840, 1770, 1735, 1600, 1500, 1460, 1365, 1230, 1190, 1150, 1120, 1090, 1070, 1020, 720 cm^{-1}; ^1H and ^{13}C NMR data, see Table 4.

Peroxides of lycopanerols K: ESI-MS (NaI) *m/z* (rel int) 2331.4 $[C_{150}H_{282}O_{14} + Na]^+$ (30), 2359.5 $[C_{152}H_{286}O_{14} + Na]^+$ (100), 2387.5 $[C_{154}H_{290}O_{14} + Na]^+$ (45), 2415.6 $[C_{156}H_{294}O_{14} + Na]^+$ (13); daughter ion spectrum of *m/z* 2359 gives *m/z* 2299 $[C_{152}H_{286}O_{14} + Na - AcOH]^+$, *m/z* 1743 $[C_{152}H_{286}O_{14} + Na - AcOH - C_{36}H_{61}O_4]^+$, *m/z* 1727 $[C_{152}H_{286}O_{14} + Na - AcOH - C_{36}H_{61}O_5]^+$.

Lycopanerols L (8a): clear oil; HR MALDI-TOF-MS obsd *m/z* 1716.6581 ($C_{112}H_{220}O_8Na$ requires 1716.6700, Δ −10.9 mmu), *m/z* 1744.6901 ($C_{114}H_{224}O_8Na$ requires 1744.7013, Δ −11.2 mmu); IR (CCl_4) ν_{max} 3565, 2950, 2920, 2840, 1735, 1460, 1370, 1230, 1150, 1070, 720 cm^{-1}; ^1H and ^{13}C NMR data, see Table 5.

Ozonolysis. Lycopanerols **5** and **7a** (each ca. 1 mg), in CH_2Cl_2 solution (1 mL), were treated by air enriched in ozone, at −15 °C. The resulting ozonides were reduced by addition of 5 mg of triphenylphosphine, and the reaction mixtures were concentrated under a flux of N_2 and analyzed by GC−MS (CP Sil 5CB capillary column (25 m × 0.25 mm), temperature program: 5 min at 120 °C and then progressing up to 300 °C at 5 °C min^{-1}).

Trimethylsilyl Ether Derivatives 7b. The acetate derivatives **7a** were saponified by reflux for 10 min in MeOH−H_2O−toluene, 7:1:2. After extraction with Et_2O and washing of the organic phase with water, the recovered lycopanerols **7** were trimethylsilylated according to a standard procedure[12] and analyzed by EI(70 eV)-MS, direct inlet.

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Supporting Information Available: Chemical structures of lycopanerols B, D, E, F, G, and H; ESI-MS, ESI-MS/MS, LR MALDI-TOF-MS, ^1H, ^{13}C, and DEPT, COSY, HMBC, HMQC, and ROESY spectra of **7a**; ESI-MS fragmentation of **6a** (Figure 1S); EI-MS fragmentation of **7b** (Figure 2S); HMBC and ROESY correlations of **8a** (Figure 3S). This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) For a review see: Metzger, P.; Largeau, C. Chemicals of *Botryococcus braunii*. In *Chemicals from Microalgae*; Cohen, Z., Ed.; Taylor & Francis: London, 1999; pp 205−260.
(2) (a) Metzger, P.; Casadevall, E. *Tetrahedron Lett.* **1987**, *28*, 3931−3934. (b) Metzger, P.; Allard, B.; Casadevall, E.; Berkaloff, C.; Couté, A. *J. Phycol.* **1990**, *26*, 258−266. (c) Metzger, P.; Pouet, Y.; Summons, R. *Phytochemistry* **1997**, *44*, 1071−1075.
(3) Cavé, A.; Figadère, B.; Laurens, A.; Cortes, D. *Prog. Chem. Org. Nat. Prod.* **1997**, *70*, 81−288.
(4) Fernández, J. J.; Souto, M. L.; Norte, M. *Nat. Prod. Rep.* **2000**, *17*, 235−246.
(5) (a) Harding, W. W.; Lewis, P. A.; Jacobs, H.; McLean, S.; Reynolds, W. F.; Tay, L.-L.; Yang, J.-P. *Tetrahedron Lett.* **1995**, *36*, 9137−9140. (b) Miller, S. L.; Tinto, W. F.; McLean, S.; Reynolds, W. F.; Yu, M.; Carter, C. A. G. *Tetrahedron* **1995**, *44*, 11959−11966.
(6) De Rosa, M., Gambacorta, A., Gliozzi, A. *Microbiol. Rev.* **1986**, *50*, 70−80.
(7) Metzger, P.; Aumelas, A. *Tetrahedron Lett.* **1997**, *38*, 2977−2980.
(8) (a) Rager, M.-N.; Metzger, P. *Phytochemistry* **2000**, *54*, 427−437. (b) Metzger, P.; Rager, M.-N. *Tetrahedron Lett.* **2002**, *43*, 2377−2380.
(9) (a) Delahais, V.; Metzger, P. *Phytochemistry* **1997**, *44*, 671−678. (b) Metzger, P. *Tetrahedron* **1999**, *55*, 167−176.
(10) Pfeffer, P. F.; Luddy, F. E.; Unruh, J.; Shoorley, J. N. *J. Am. Oil Chem. Soc.* **1977**, *54*, 380−386.
(11) Baldwin, J. E. *J. Chem. Soc., Chem. Commun.* **1976**, 734−736.
(12) Kates, M. In *Techniques of Lipidology*; Burton, R. H., Knippenberg, P. H., Eds.; Elsevier: Amsterdam, 1986.
(13) Metzger, P.; Berkaloff, C.; Casadevall, E.; Couté, A. *Phytochemistry* **1985**, *24*, 2305−2312.

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