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Lycopanerols A, di-Tetraterpenoid tetraether derivatives from the Green Microalga *Botryococcus braunii*, L strain

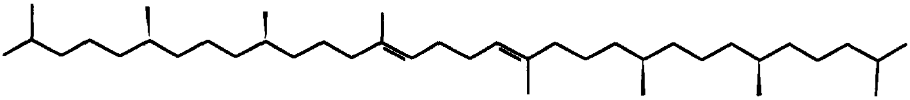
Pierre Metzger*, [a] **and André Aumelas**[b]

[a] Laboratoire de Chimie Bioorganique et Organique Physique - associé au CNRS - Ecole Nationale Supérieure de Chimie de Paris, 11, Rue P. et M. Curie - 75231 Paris Cedex 05- France

[b]Centre de Biochimie Structurale, Faculté de Pharmacie, 15, Avenue Charles Flahault - 34060 Montpellier Cedex 1 - France.

Abstract : A series of polyethers of a new type, lycopanerols A, consisting of tetraterpenoid and unbranched aliphatic moieties, was isolated from a L strain of the green microalga *Botryococcus braunii*. Their structures were established on the basis of chemical degradation and of Mass and NMR data. © 1997 Elsevier Science Ltd.

The L strains of the green colonial microalga *Botryococcus braunii* are characterized by the production of lycopadiene **1**, an acyclic tetraterpenoid hydrocarbon[1]. This compound is synthesized in variable amounts by the alga (0.07-8% of dry wt) depending on the origin of the strain[2] ; it could be at the origin of lycopane found in some lacustrine sediments [3a,b]. From the oil extracted from the dry algal biomass with heptane, we isolated also a monoepoxide derived from lycopadiene and its corresponding diol[4]. We have further investigated the extracts and isolated several series of polyethers we named lycopanerols. In this paper we report the isolation and the structural elucidation of compounds of one of these series, lycopanerols A, **2**.



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B. braunii (Yamoussoukro strain, Ivory-Coast) was cultured under air-lift conditions (1% CO_2), at 25°C, under continuous light of 470 $\mu E.m^{-2}.s^{-1}$ and on a chemically defined medium[5]. The extraction of the dry biomass with heptane furnished a resin-like extract from which a rubbery material was removed by dissolution in $CHCl_3$ and addition of an equivalent volume of MeOH. The remaining filtrate, free of "rubber", was subjected to silica gel column chromatography and the fraction eluted with heptane/ether (19:1) was collected. Further purification of this fraction by TLC over silica gel furnished a clear oil (12.6% of dry wt); $[\alpha]_D^{20} = -6.9°$ (c. 4.58, *n*-heptane). The HRFAB(NBA-LiCl) mass spectrum displayed three $[M+Li]^+$ adduct ions at m/z 1596.6512, 1624.6764 and 1652.7133, in a ratio of *c.a* 12:35:53, corresponding to the molecular formulae $C_{108}H_{212}O_5$ ($[M+Li]^+$ calcd. 1596.6495), $C_{110}H_{216}O_5$ ($[M+Li]^+$ calcd. 1624.6808) and $C_{112}H_{220}O_5$ ($[M+Li]^+$ calcd. 1652.7121) respectively; these data suggested that the mixture was made of a series of homologous compounds increasing in molecular mass by two methylenes successively.

The IR spectrum showed absorption bands for hydroxyl group (3580 cm^{-1}) and C-O bonds (1095 and 1070 cm^{-1}). The presence of a tertiary alcool function was deduced from trimethylsilylation and from the

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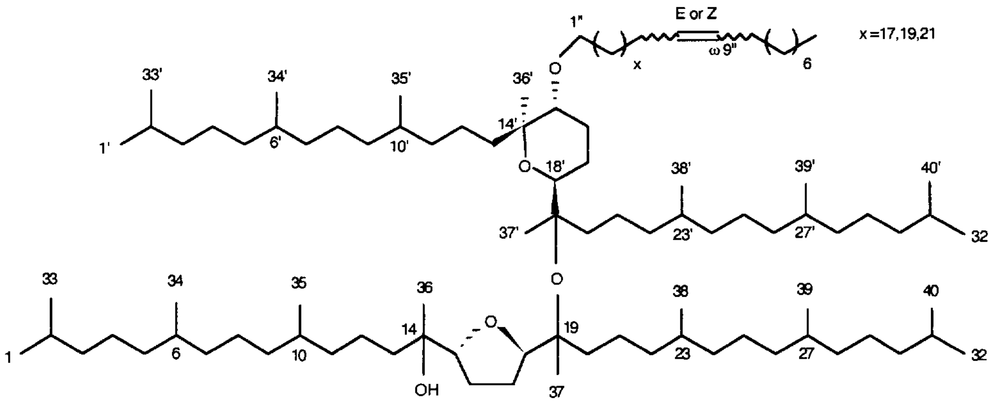
Fax: : 33-1-43-25-79-75 ; E. mail : pgmetzg@ext.jussieu.fr* absence of reactivity of **2 with acetic anhydride in pyridine. Joined to the absence of C=O band in the IR spectrum and of signal for acetal function in the ^{13}C NMR spectrum, these results indicated that the four other oxygens were part of ether bridges. Moreover the presence of a $-CH_2-CH=CH-CH_2-$ unsaturation was evident from the NMR data which indicated also the presence of both *cis* (δ_H 5.34, *t* ; δ_C 129.88) and *trans* (δ_H 5.36, *t* ; δ_C 130.37) configurations, in a 3:1 ratio. The existence of these two geometries was confirmed by the ^{13}C chemical shifts of the allylic carbons : δ*cis* 27.25 and δ*trans* 32.70.



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The nature of the carbon skeletons was determined by submitting lycopanerols A to a total reduction *via* (i) hydrogenation over rhodium on charcoal in heptane, followed by (ii) ether cleavage with HI over red phosphorous (under reflux for 18hr) and finally by (iii) reduction of the resulting iodide derivatives with Zn/HCl (under reflux for 4hr). GC-MS analysis of the resulting hydrocarbons showed the formation of lycopane and *n* $-C_{28}$, *n* $-C_{30}$ and *n* $-C_{32}$ alkanes. In order to locate the unsaturation, we submitted the mixture of lycopanerols A to ether cleavage by dibromotriphenylphosphorane[6]. Thus, besides a complex mixture of brominated tetraterpenoids, we isolated a series of *cis* and *trans* *n* $-C_{28}$, C_{30} and C_{32} bromo-1-alkenes[7], which upon ozonolysis (in CH_2Cl_2, at -15°C) and reductive cleavage of the ozonides with triphenylphosphine, furnished n-C_{19}, C_{21} and C_{23} ω-bromoaldehydes[8]; this established that *cis* and *trans* unsaturations were located at ω9" positions in the normal chains.

The LRFAB(NBA-LiCl) mass spectrum of **2** showed peaks at m/z 1188 and 591 indicative of ions $[C_{80}H_{157}O_4+Li]^+$ and $[C_{40}H_{79}O_2]^+$ respectively, thus establishing the linkage of two tetraterpenoids likely *via* ether bridge. Moreover the EI-mass spectrum of the trimethylsilyl derivatives showed an ion at m/z 341 $[C_{18}H_{36}OSiMe_3]^+$, indicating that the hydroxyl was at C-14 on a tetraterpene moiety. The complete elucidation of the structure was achieved by 1D and 2D NMR. In a view to suppress the overlapping of some ^1H signals with those of allylic protons, spectra were recorded on the dihydroderivatives **3** prepared by catalytic (PtO_2) reduction of **2**. The assignment of the proton and carbon resonances of **3** (Table 1 and note 9) was based on the analysis of TOCSY, DQF-COSY, ROESY , $^1H-^{13}C$ HSQC and HMBC data. The ^{13}C NMR spectrum displayed nine signals which chemical shifts were in agreement with nine oxygen-bearing carbons : four quaternary (C-14, C-19, C-14' and C-19'), four methine (C-15, C-18, C-15', and C-18') and one methylene (C-1"). Recording the ^1H NMR spectrum at 315 K instead of 300 K optimized the resolution; there were now six resolved signals, in the CH-O region when two overlapped at 300 K (H-1"a and H-18'). The diastereotopic protons H-1"a and H-1"b gave two doublets of triplets at δ 3.50 and 3.23. H-15' was assigned on the basis of its NOE with these two protons. The combination of DQF-COSY, TOCSY and HMBC data revealed partial structures comprising a tetrahydrofuran (THF) and a tetrahydropyran (THP) as shown in Fig.1. Moreover, the long range connectivity observed between C-19 and H-18' in the HMBC experiment demonstrated the linkage of the THF and THP units *via* the C-19-O-C-19' ether bridge.

Table 1. Selected chemical shifts (ppm) and HMBC connectivities of lycopanerols A ($CDCl_3$, 315K, 600 MHz).

Position	δ_C	δ_H(mult, J Hz)	HMBC [a]	Position	δ_C	δ_H(mult, J Hz)	HMBC [a]
13	38.05	1.40(m)	36	13'	42.43	1.47(m)	36'
14	72.95		16b, 36	14'	79.75		13', 36'
15	85.75	3.70(dd, 9.7, 5.8)	16b, 18, 36	15'	84.88	3.10(dd, 10.2, 2.6)	1"a,b, 17'ax,36'
16	26.47	a:1.78(m); b:1.74(m)	17a, b	16'	25.72	eq:1.84(m); ax:1.46(m)	
17	27.22	a:1.88(m); b:1.85(m)	16a, b	17'	28.15	eq:1.65(m); ax:1.60(m)	
18	83.76	3.86(dd, 8.4, 6.6)	17a, 37	18'	77.69	3.54(dd, 9.7, 1.7)	37'
19	79.34		18', 20a, 37	19'	80.81		17'ax, 18', 37'
20	38.72	a:1.54(m); b:1.35(m)	18, 37	20'	41.94	a:1.56(m); b:1.40(m)	37'
36	24.53	1.16(s)	13, 15	36'	20.65	1.10(s)	
37	19.54	1.12(s)	18	37'	21.24	1.03(s)	18'
1"	70.17	a:3.50(dt, 8.8, 6.2) b:3.23(dt, 8.8, 6.6)	2"a, b				
2"	30.39	a:1.51(m); b:1.48(m)	1"a, b				
3"	26.38	1.30(m)	2"a, b				

[a] Protons correlating with carbon resonance.

The equatorial positions of substituents at C-15' and C-18' in the THP ring were deduced from the coupling constant values of H-15' (*dd* , *J* =2.6, 10.2 Hz) and H-18' (*dd* , *J* =1.7, 9.7 Hz) signals. Moreover, the H-18'/Me-36' dipolar interaction clearly confirmed the axial orientation of Me-36'.

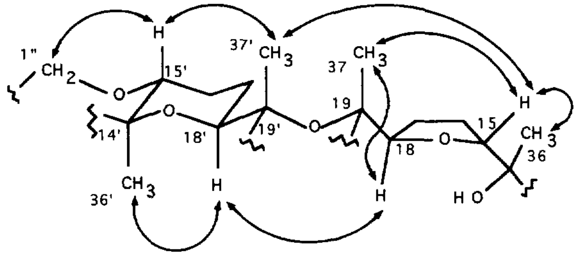
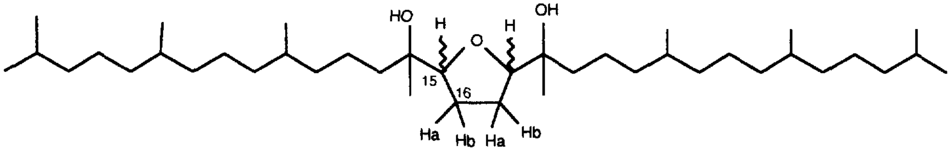


Figure 1. Partial structure of 3 and selected dipolar interactions.

The configuration of the THF ring could not be deduced from the coupling constants observed for H-15 and H-18. Indeed, the comparison of these values (Table 1) with those obtained from model compounds, *cis*-and *trans*- THF 4[10], prepared through epoxidation of 1 (with 2 eq. of *m*-CPBA in CH_2Cl_2, at room temperature) and then cyclized using perchloric acid (in THF-H_2O 9:1, at room temperature), did not furnish a clear indication. Indeed, the coupling constants observed for H-15 were 5.2 Hz in *cis*- THF **4** and 6.4 Hz in *trans*- THF **4**. A *trans* stereochemistry was suggested by the low Δδ values observed for the THF protons of **3**, δH16a-δH16b = 0.04ppm and δH17a-δH17b = 0.03ppm, by comparison to the one observed in *cis*- THF **4**: δH16a - δH16b = 0.11ppm, while H-16a and H-16b overlapped in *trans*- THF **4**. Confirmation of the



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existence of a *trans*- THF in **3** came from the ROESY experiment (Fig. 1). Indeed, the observation of the H-18'/H-18 and Me-37'/H-15 dipolar interactions, both with the absence of the H-18'/H-15 connectivity would be only consistent with such a geometry. Moreover, the Me-37/H-15, Me-37'/H-15 and Me-36/H-15 (strong) NOEs suggest that these three methyl groups and the H-15 proton are located on the same side of the THF ring. Thus, whole structure of lycopanerols A, isolated in abundance from the L strain of *B. braunii,* is *trans*- THF containing lycopane connecting at C-19 *via* an ether bridge with C-19' of a second lycopane containing a THP ring, which in turn is connected at C-15' *via* another ether bridge to a *n*-C_{28}, C_{30} or C_{32} alkenyl chain comprising a *cis* or a *trans* unsaturation at ω9" position.

Natural ethers exhibiting THF and THP rings such as the bioactive acetogenins from *Annonaceae*[11a,b] or squalene derivatives from red seaweeds of the genus *Laurencia*[12] are continuously increasing in number. Lycopanerols A are the first reported THF, THP tetraterpenoids which in addition comprise unbranched aliphatic moieties. If numerous ether lipids exhibiting ether bridges between aliphatics and(or) phenolics have been reported to occur in noticeable amounts in the A strains of *B. braunii*[13], no THF or THP derivative were however isolated from this alga up to now.

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8. GC-EIMS: $BrCH_2(CH_2)_{17}CHO$, $[M-H_2O]^+$ m/z 344, 342; $BrCH_2(CH_2)_{19}CHO$, $[M-H_2O]^+$ m/z 372, 370; $BrCH_2(CH_2)_{21}CHO$, $[M-H_2O]^+$ m/z 400, 398.
9. Compounds **3** also displayed the other following carbon resonances of (i) tetraterpenoid moieties, CH_3: 22.71 (C-1, 1', 32, 32'), 22.63 (C-33, 33', 40, 40'), 19.96, 19.91, 19.76 (C-34, 34', 39, 39'), 19.70 ; CH_2: 39.44 (C-3, 3', 30, 30'), 37.94, 37.61, 37.52, 37.46, 37.41, 37.36, 24.83 (C-4, 4', 29, 29'), 24.53 (C-8, 8', 25, 25'), 21.43, 20.98, 20.82; CH: 33.05, 32.90, 32.84, 32.71, 28.20 (C-2, 2', 31, 31'), and (ii) normal chain, CH_2: 31.94 (C-ω-2"), 29.71, 29.55, 29.36, 22.71 (C-ω-1"), CH_3: 14.08 .
10. HRFAB (NBA-LiCl)MS: *trans*-THF **4**, $[M+Li]^+$ m/z 615.6263 (calcd. 615.6267 for $C_{40}H_{80}O_3Li$); *cis*-THF **4**, $[M+Li]^+$ m/z 615.6265 (calcd: 615.6267 for $C_{40}H_{80}O_3Li$). These two isomers were distinguished by their IR spectrum: *trans*-**4** exhibited a free OH absorption at 3590 cm^{-1}, while its *cis* isomer showed a free OH bond (3620 cm^{-1}) and an intramolecular one (3595 cm^{-1}).
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