Pergamon

Tetrahedron Letters 43 (2002) 2377–2380

TETRAHEDRON LETTERS

Lycopanerols H, two high molecular weight ether lipids from *Botryococcus braunii* comprising an α-tocopherol unit

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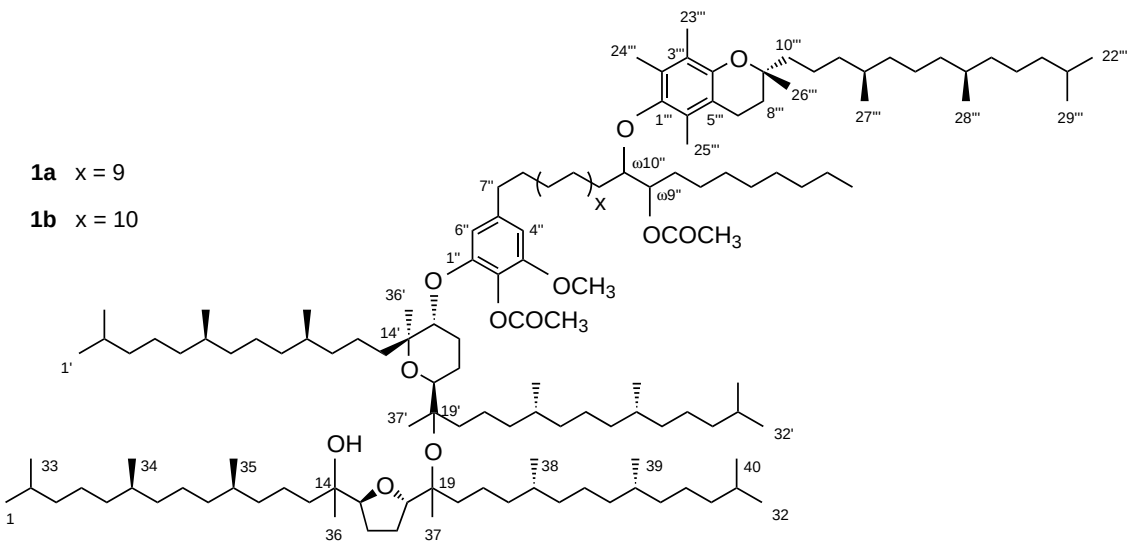
Received 22 November 2001; revised 7 February 2002; accepted 8 February 2002

Abstract—Two high molecular weight ether lipids, C_{151} and C_{153} lycopanerols H, have been isolated from the lipid extract of a strain of the green microalga *Botryococcus braunii*. These new compounds arise from the linkage via ether bridges of tetraterpenoid, *n*-alkylphenol and α-tocopherol units. Their structures have been elucidated by Mass and NMR spectral analysis.

The green microalga *Botryococcus braunii* has provided a large number of unusual secondary metabolites including several families of ether lipids of new types.[1] In the present study we describe the isolation and structure determination of two polyethers, lycopanerols H, which were isolated from an extract of a strain of this alga, grown in the laboratory. Lycopanerols constitute a family of natural products comprising from one to three lycopane units and, in some cases, an *n*-alkenyl or an *n*-alkylphenol moiety connected to each other by ether bridge(s).[2] They are synthesized by the L strains of *B. braunii*, which are characterized by the production of lycopa-14(*E*),18(*E*)-diene, an acyclic tetraterpenoid hydrocarbon.[3]



Keywords: algae; *Botryococcus braunii*; ether lipids; tetraterpenoids; alkylpyrogallol; benzopyrans.
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0040-4039/02/$ - see front matter
PII: S0040-4039(02)00291-5

The strain, originating from a freshwater lake in Yamoussoukro, Ivory Coast, was cultured under air-lift conditions (1% CO_2), at 25°C, under continuous light of 470 μE m^{-2} s^{-1} and on a chemically defined medium.[4] The freeze-dried biomass (44 g) was extracted at room temperature twice in 1 h with 500 ml heptane, and the combined extracts were concentrated under reduced pressure (6.9 g). A rubbery material was removed from the extract dissolved in $CHCl_3$, by addition of an equivalent volume of methanol,[5] the resulting extract (4.4 g) was subjected to silica gel CC by elution with heptane containing increasing amounts of diethyl ether and the fraction eluted with heptane–diethyl ether 17:3 v/v was collected (1.05 g). Further purification of this fraction, which composition appeared very complex by HPLC analysis, was achieved via (i) acetylation by treatment in pyridine–Ac_2O, for 18 h at 50°C, (ii) separation by preparative silica gel TLC of the resulting mixture (eluent heptane–diethyl ether 4:1) and then (iii) preparative silver nitrate-silica gel TLC (eluent heptane–diethyl ether 3:1). Compound **1** (80 mg) was isolated as an oily material in a yield of 0.18% of dry weight; $[\alpha]_D^{25} = -6$ (*c* 7.2, $CHCl_3$).

The LR FAB (magic bullet/NaI) mass spectrum of **1** displayed two $[M+Na]^+$ ions at *m*/*z* 2307.1 and 2335.1, in a ratio of ca. 4:1, compatible with the molecular formulae $C_{151}H_{278}O_{12}$ and $C_{153}H_{282}O_{12}$ for **1a** and **1b**, respectively, and suggesting that the mixture was made of two homologous compounds differing in molecular mass by two methylenes. The HR MALDI-TOF mass data agreed with the molecular formula $C_{151}H_{278}O_{12}$ proposed for the predominant compound **1a**, $[M+Na]^+$ at *m*/*z* 2307.1028, $C_{151}H_{278}O_{12}$+Na required 2307.1036.

The IR spectrum showed absorption bands for hydroxyl group (3580 cm^{-1}), C=O bonds of ester functions (1770 and 1740 cm^{-1}), aromatic ring (1600 cm^{-1}) and C–O bonds (1240, 1200, 1150, 1125 and 1075 cm^{-1}). The UV spectrum showed absorption band maxima at 290, 282 and 242 nm, suggesting the presence of aromatic chromophores.[6] A detailed inspection of the ^1H, ^{13}C NMR, DEPT, COSY, HMQC and HMBC data and comparisons with NMR data from other lycopanerols[2] indicated that **1** contained, among some other things, signals for a tetrahydrofuran ring (THF), a tetrahydropyran (THP) and an alkylpyrogallol unit (Table 1). From the HMBC spectrum it could be established that these three units were linked, in this order, by ether bridges, as shown in partial structure A (Fig. 1). Moreover, the absence of connectivities between H-15 (δ_H at 3.71) and H-18 (δ_H at 3.91) in the ROESY spectrum suggested that these two THF protons would exhibit an *anti* stereochemistry. As previously emphasized,[2a] the values of the coupling constants did not help in the determination of the stereochemistry of the THF ring in lycopanerols. However, the presumption of an *anti* stereochemistry came from the results of the biomimetic transformation of the natural 14(*R*),15(*R*):18(*S*),19(*S*)-diepoxy-lycopane, the likely precursor of lycopanerols occurring in *B. braunii*, only into a *trans*-THF-containing lycopane.[7]

Table 1. Selected ^{13}C (100 MHz) and ^1H (400 MHz) NMR data of lycopanerols **1** ($CDCl_3$ solution)

Position	δ ^{13}C	δ ^1H (mult., *J*, Hz)	HMBC[a]	Position	δ ^{13}C	δ ^1H (mult., *J*, Hz)	HMBC[a]
13	37.9	1.40 (m)	15, 36	ω8″	30.6	1.85 (m)	ω9″, ω10″
14	73.0		13, 15, 16, 36	ω9″	74.5	5.14 (m)	
15	85.8	3.71 (dd, 5.8, 9.5)	16, 17, 18, 36	ω10″	80.6	3.93 (m)	
16	26.5	1.77 (m); 1.81 (m)	15, 17	ω11″	25.7	1.35 (m)	ω10″
17	27.4	1.90 (m)	15, 16, 18	ω3″	31.9	1.20–1.30	ω1″, ω2″
18	83.5	3.91 (t, 7.7)	16, 17, 37	ω2″	22.8	1.20–1.30	ω1″
19	79.6		17, 20, 37, 18′	ω1″	14.2	0.86	ω2″
20	38.7	1.33 (m); 1.56 (m)	18, 37	1‴	145.8		23‴, 25‴, ω10″
36	24.6	1.19 (s)	15	2‴	128.4		23‴, 24‴
37	19.6	1.14 (s)	18	3‴	122.8		23‴, 24‴
13′	42.0	1.63 (m)	36′	4‴	147.4		7‴, 24‴
14′	79.4		15′, 36′	5‴	117.6		7‴, 8‴, 25‴
15′	82.4	4.12 (br d, 7.2)	16′, 36′	6‴	126.4		7‴, 25‴
16′	25.6	1.95 (m); 1.62 (m)	18′	7‴	20.8	2.54 (t, 7.2)	8‴, 26‴
17′	27.7	1.70 (m)		8‴	31.7	1.78 (m); 1.80 (m)	7‴, 26‴
18′	77.9	3.58 (dd, 5.1, 6.3)	17′, 37′	9‴	74.7		8‴, 26‴
19′	81.3		37′	10‴	39.8	1.55 (m)	26‴
20′	41.9	1.42 (m); 1.50 (m)	18′, 37′	23‴	13.8	2.15 (s)	
36′	20.7	1.24 (s)	15′	24‴	11.5	2.07 (s)	
37′	21.5	1.13 (s)	18′	25‴	12.9	2.10 (s)	
1″	150.1		15′, 6″	26‴	24.0	1.23 (s)	
2″	127.5		4″, 6″, $OCOC\underline{H}_3$ (2″)	OCH_3	56.1	3.79	
3″	152.1		4″, $OC\underline{H}_3$	$O\underline{C}OCH_3$ (2″)	168.8		$OCOCH_3$ (2″)
4″	104.6	6.37 (s)	6″, 7″, OCH_3	$OCO\underline{C}H_3$ (2″)	20.6	2.27 (s)	
5″	141.4		4″, 6″, 7″	$O\underline{C}OCH_3$ (ω9″)	170.9		ω9″, $OCOCH_3$ (ω9″)
6″	106.8	6.37 (s)	4″, 7″	$OCO\underline{C}H_3$ (ω9″)	21.1	2.08 (s)	
7″	36.7	2.54 (t, 7.2)	4″, 6″				

[a] Proton correlating with carbon resonance.

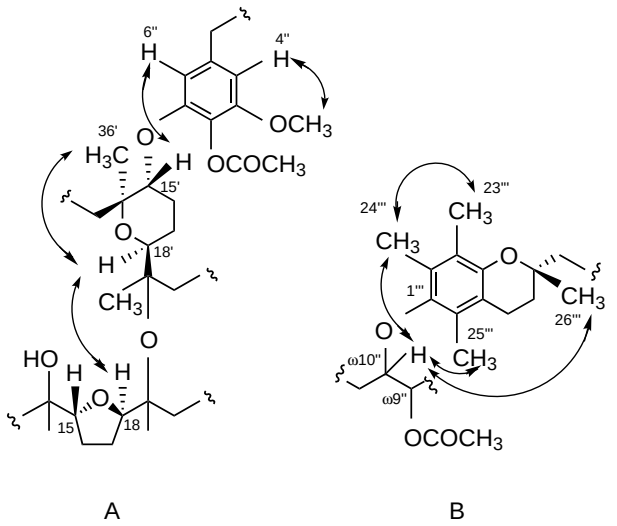

Figure 1. Partial structures A and B and selected ROE correlations.

Furthermore, the ROESY experiment (Fig. 1) revealed that Me-36′ (δ_H at 1.24), H-18′ (δ_H at 3.58) and H-15′ (δ_H at 4.12) were axially oriented in the THP ring (Fig. 1). HMBC and ROESY data established the linkage of the THP ring at C-15′ to an alkylpyrogallol derivative by a phenoxy bond. This general structural pattern, previously identified in lycopanerol E,[2b] was also supported by some fragment ions in the LR FAB mass spectrum at m/z 1716.6, 1125.9 and 430.4, which indicated the successive loss of two lycopane moieties (cleavage of the C-19–O and C-15′–O bonds) and of an alkylpyrogallol unit (cleavage of the C-ω10″–O bond).

The ^{1}H and ^{13}C NMR data (Table 1) showed also the presence of a chroman nucleus exhibiting an entirely substituted aromatic ring, with six singlets at δ_C 147.4 (C-4‴), 145.8 (C-1‴), 128.4 (C-2‴), 126.4 (C-6‴), 122.8 (C-3‴) and 117.6 (C-5‴). The HMBC spectrum established that the positions 2‴, 3‴ and 6‴ were substituted by methyl groups, suggesting the presence of an α-tocopherol moiety in **1**. The long range connectivity observed between H-ω10″ and C-1‴ indicated that the chroman ring was bound by a phenoxy bond to a methine carbon (Table 1). Moreover, the COSY and the HMBC data showed that this latter carbon was α to a methine carbon bearing an acetoxy group, as shown in partial structure B (Fig. 1).

The terpenoid side chain of tocopherol could not be identified by NMR given the co-occurrence of four other saturated terpenoid side chains from the THF and THP units and exhibiting similar proton and carbon resonances. However, the analysis of **1** by flash pyrolysis–GC–MS supported the presence of an α-tocopherol unit (pyrolysis at the Curie point temperature of 455°C). Indeed, the composition of the flash pyrolysate revealed beside the presence of C_{18}, C_{19}, C_{21}, C_{22} and C_{40} terpenoids arising from the cleavage of some C–C and C–O bonds, the formation in appreciable amount of α-tocopherol[8] and of its acetate derivative. This last compound was likely generated through the cleavage of the C-ω10″–O bond and transfer of the acyl group from the acetoxy at C-ω9″, during the pyrolysis. The occurrence of an ether bound tocopherol was also supported by the presence in the HR LSI mass spectrum of **1** of a fragment ion at m/z 430.3811, calcd for $C_{29}H_{50}O_2$ 430.3791.

Unfortunately, no alkylpyrogallol derivative was identified from the ion chromatogram of the flash pyrolysate of **1**. This could be related to a transfer problem of high molecular weight compounds from the pyrolysis unit to the GC column, as already noticed for some high molecular weight lipids.[9] Nevertheless, the size of the alkyl chain of the pyrogallol moiety was deduced to be C_{31} in **1a**, from the FAB mass data, with an ion at m/z 1125.9 assigned to $[C_{71}H_{122}O_8+Na]^+$, and resulting from the cleavage of the C-15′–O bond. Furthermore, the high intensity in the ^{1}H NMR spectrum of a polymethylene signal at δ_H 1.29 and the presence in the ^{13}C NMR spectrum of numerous intense signals at δ_C around 29 ppm were in favor of a straight alkyl chain.[10]

The NMR data, including the COSY spectrum, also showed the presence of two oxymethines embedded in the long methylene chain (H-ω9″ δ_H at 5.14, C-ω9″ δ_C at 74.5; H-ω10″ δ_H at 3.93, C-ω10″ δ_C at 80.6). Their location at these positions were based on: (i) the EI mass data obtained from the trimethylsilylated analogues[11] of **1**, with $OSiMe_3$ groups at C-14, C-2″ and C-ω9″, showing an ion at m/z 215 $[C_9H_{18}OSiMe_3]^+$, and (ii) the likely biogenetic pathway involving an oxidative condensation of α-tocopherol to a ω9–ω10 unsaturation commonly occurring at this position in alkenyl phenols from *B. braunii*, especially in lycopanerol E,[2b] the presumed precursor of **1**.

To date, ether-bound tocopherols have only been identified in some sedimentary organic matter,[12] but the nature of the second ether-linked units remains unknown. A very small number of compounds derived from the condensation of tocopherols with some natural products have been reported,[13] but lycopanerols H are the first example of high molecular weight ether lipids comprising an ether-linked tocopherol. It may be assumed that the anti-oxidative properties of α-tocopherol are maintained for the ether-linked tocopherol unit in **1**, as it is the case for a synthetic tocopherol methyl ether.[14] Lycopanerols H could play a role in the prevention of oxidative damage to lipids in the L strains of *B. braunii*.

Acknowledgements

We are grateful to Mrs. N. Morin (Ecole Normale Supérieure de Paris) for the FAB/MS analyses.

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11. The trimethylsilyl derivatives were prepared via saponification of **1** in MeOH/toluene/H_2O 5:1:1 v/v/v, 1N KOH, 1 h under reflux, followed by reaction of the product in pyridine solution with hexamethyldisilazane, in presence of trimethylsilyl chloride, 1 h at room temperature.
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