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Braunicetals: Acetals from condensation of macrocyclic aldehydes and terpene diols in *Botryococcus braunii*

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ABSTRACT

Two series of braunicetals were isolated from the green microalga *Botryococcus braunii*. Based on spectroscopic and chemical evidence, their structures were determined to be acetals formed by the condensation of C_{32} and C_{34} macrocyclic aldehydes with C_{33} and C_{34} methylated squalene diols (series I), or a C_{40} lycopaene diol (series II).

1. Introduction

Most naturally occurring macrocyclic compounds containing only carbon atoms in the rings are isoprenoids like, for example, the 14-membered macrocycles of the cembrene family (Wessjohann et al., 2005). By comparison, non-isoprenoid macrocycles containing only carbon atoms in the rings are relatively rare. Besides the 15- and 17-membered macrocycles muscone and civetone (Ruzicka, 1966), few compounds have been reported. More recently, macrocycloalkanes were identified in some higher plants (Red'kina et al., 1990; Baik et al., 1996; Mimica-Dukic et al., 1998). Furthermore, some macrocyclic alkanes with 15–34 carbon atoms, discovered in some sediments and crude oils, were considered as the signature of the alga *Botryococcus braunii* and derived from cell wall materials (Audino et al., 2002a, b). Our recent discovery of compounds exhibiting non-isoprenoidal macrocycles in the living *B. braunii*, prompted us to report their structure determination.

B. braunii is a green colonial microalga, recently reclassified in the Trebouxiophyceae on the basis of 18S rRNA evidence (Senousy et al., 2004). Common in freshwater, brackish lakes and reservoirs at varying latitudes (Tyson, 1995 and references therein), this species is characterized by a high production of hydrocarbons and unusual lipids stored for the most part in the outer walls (Largeau et al., 1980; Metzger et al., 1985a, 1990). These walls are closely associated with each other, thus ensuring colony cohesion, and are composed of a structural element termed algaenan, an aliphatic chemically resistant biopolymer (Largeau et al., 1986; Tegelaar et al., 1989), embedded within lipids including hydrocarbons. Based on the type of the synthesized hydrocarbons, strains of *B. braunii* have been sub-classified into three chemical races that are morphologically similar. The race A biosynthesizes *n*-alkadienes and trienes with an odd-carbon number between C_{25} and C_{33} (Metzger et al., 1985a, 1991). The race B produces predominantly C_{30}–C_{37} acyclic and cyclic triterpenes called botryococcenes (Maxwell et al., 1968; Metzger et al., 1985a, b; David et al., 1988) and also low amounts of C_{31}–C_{34} methylated squalenes (Huang and Poulter, 1989; Summons et al., 2002; Achitouv et al., 2004). The race L produces lycopadiene, an acyclic tetraterpene (Metzger et al., 1990). In addition to hydrocarbons and some classical lipids, these algae also produce a number of non-classical lipids, including high molecular weight polymers: aliphatic polyaldehydes and their derived polyacetals resulting from the condensation of some aldehyde functions with terpene diols (Metzger and Largeau, 2002). As part of recent investigations, two series of acetals comprising macrocyclic

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units, we name braunicetals, were discovered. Herein, we report the chemical investigations on lipids of five strains of *B. braunii*, which have resulted in the identification of these compounds.

2. Results and discussion

2.1. Braunicetals I

The heptane extract of the Overjuyo strain (*B. braunii* race B) was subjected to alumina column chromatography (CC) using heptane, toluene and chloroform/methanol as eluting solvents. The toluene fraction was purified by silica gel TLC to yield braunicetals I as an inseparable mixture of compounds (**1**–**6**; Fig. 1). The LR-APCI mass spectrum of the mixture showed four quasi-molecular ions $[M+H]^+$ at *m/z* 925, 939, 953 and 967, with peaks in a 5:11:9:25 relative ratio, suggesting that the mixture could be made of four homologous compounds increasing in molecular mass by one methylene group, successively. The molecular formulae: $C_{65}H_{112}O_2$, $C_{66}H_{114}O_2$, $C_{67}H_{116}O_2$ and $C_{68}H_{118}O_2$ were deduced from the HR-ESIMS analysis. The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of a long hydrocarbon chain, and a terpenoid pattern. In the olefinic part of the ^1H NMR spectrum, a triplet for four protons at δ 5.35 suggested the presence of two disubstituted –CH=CH– unsaturations of *Z* stereochemistry on the basis of the

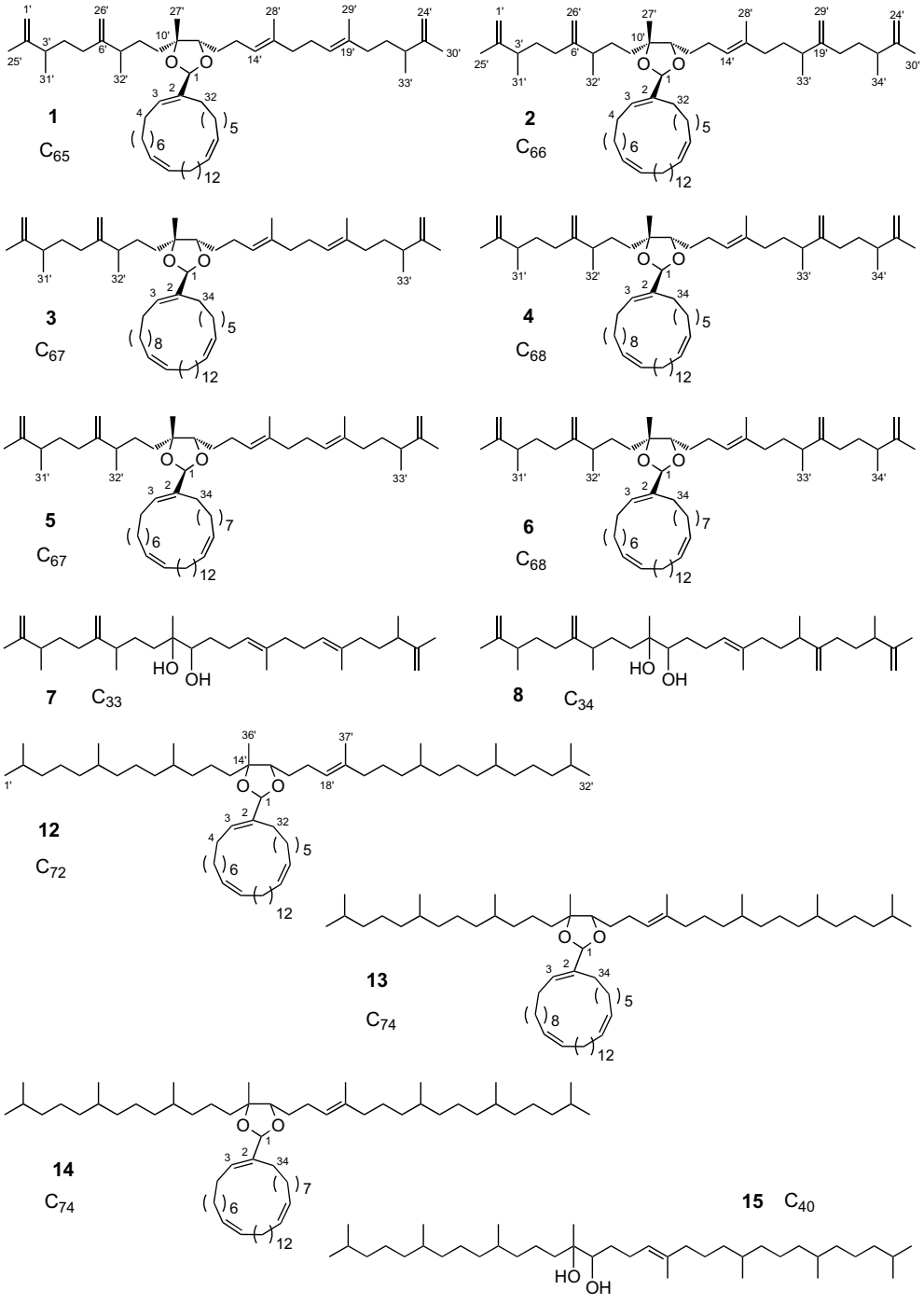

Fig. 1. Structures of compounds **1**–**8** and **12**–**15**.

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data for braunicetal **6**[a] in $CDCl_3$

Position	δH, multiplicity (J in Hz)	δC, multiplicity	HMBC[b]
Macrocyclic moiety			
1	5.19, *s*	105.6, *t*	3, 32
2		137.8, *s*; 137.7, *s*	1, 3, 4, 32
3	5.52, *t* (7.1)	131.9, *d*	1, 4, 32
4	2.04, *m*	ca 29.1	3
5–9, 14–23, 28–33	1.30, *br*	29.0–30.3	
10, 13, 24, 27	2.02, *m*	27.2, *t*; 27.1, *t*	
11, 12, 25, 26	5.35, *t* (5.4)	130.1, *d*	
34	2.04, *m*	26.7, *t*	1, 3
Terpene moiety			
1′, 24′	4.68, *m*	109.7, *t*; 109.6, *t*	3′, 25′
2′, 23′		150.1, *s*; 150.1, *s*	1′, 3′, 25′
3′, 22′	2.15, *m*	41.2, *d*; 41.1, *d*	1′, 4′, 25′, 31′
4′, 21′	1.52, *m*; 1.40, *m*	33.5, *t*; 33.4, *t*	3′, 5′
5′, 20′	1.90, *m*	31.6, *t*; 31.5, *t*	4′, 7′, 26′
6′, 19′		154.8, *s*; 154.6, *s*	5′, 7′, 26′, 32′
7′	2.06, *m*	40.7, *d*	5′, 8′, 26′, 32′
8′	1.45, *m*; 1.35, *m*	ca 29.0, *t*	7′, 32′
9′	1.52, *m*; 1.40, *m*	34.0, *t*	8′
10′		82.2, *s*	9′, 11′, 27′
11′	3.56, *dd* (2.5, 9.4)	84.7, *d*	1, 12′, 27′
12′	1.54, *m*; 1.33, *m*	ca 29.0	11′
13′	2.04, *m*	26.1, *t*	11′
14′	5.11, *m*	123.5, *d*	13′, 16′, 28′
15′		136.2, *s*	13′, 16′, 28′
16′	1.90, *m*	37.6, *t*	17′, 28′
17′	1.50, *m*; 1.35, *m*	34.1, *t*	16′, 18′
18′	2.04, *m*	39.8, *d*	
25′, 30′	1.65, *s*	19.0, *q*	1′
26′, 29′	4.72, *m*	107.7, *t*; 107.4, *t*	
27′	1.23, *s*	21.7, *q*	11′
28′	1.59, *s*	16.2, *q*	14′, 16′
31′, 34′	1.02, *d* (6.9)	19.9, *q*	3′, 4′
32′, 33′	1.02, *d* (6.9); 1.00, *d* (7.2)	20.6, *q*; 20.3, *q*	7′

[a] Given the predominance of C_{34} tetramethylsqualene diol and C_{34} macrocyclic aldehydes among the hydrolysis products, braunicetal **6** is arbitrary chosen for the carbon numbering.
[b] Protons correlating with carbon resonances; for the terpene moiety, only the correlations concerning positions 1′–18′, 25′–28′, 31′ and 33′ are given.

chemical shift of their allylic carbons (δ_C 27.2, 27.1) (Pfeffer et al., 1977); another triplet at δ_H 5.52 for one proton (H-3) indicated the presence of a trisubstituted unsaturation. In the HMBC experiment, correlations from H-3 to quaternary carbon C-2 (δ 137.7), H-3 to methine carbon C-1 (δ 105.6), H-1 (*s*, δ 5.19) to C-11′ (δ 84.7), and H-11′ (δ 3.56) to C-10′ (δ 82.2) suggested the presence of an acetal function. This was confirmed by acid hydrolysis of the mixture of **1–6** with aqueous HCl in THF/chloroform solution. Purification of the reaction products by silica gel TLC gave a triterpene diol fraction and a mixture of aldehydes. Analysis of the diols as trimethylsilyl ethers by GC/MS and coinjection on GC with standards showed the presence of two methylated squalene diols, $C_{33}H_{58}O_2$, **7**, and $C_{34}H_{60}O_2$, **8** (Fig. 1). Recently identified in the hydrolysates of high molecular weight polyacetals from two strains of the B race of *B. braunii* (Metzger et al., 2007), these vicinal diols exhibit three and four non-isoprenoid methyl groups (Me-31′ to Me-34′ in **1–6**), respectively. GC/EIMS analysis of the aldehyde fraction showed the presence of two compounds exhibiting abundant M^+ ions at *m/z* 456 **9** and 484 **10**, respectively. HR-EIMS established the formulae $C_{32}H_{56}O$ and $C_{34}H_{60}O$, respectively. NMR data established the existence of α-unsaturated aldehydes by the presence in the spectra of a singlet for aldehyde proton at δ_H 9.35 and a signal for a carbonyl carbon, deshielded by a conjugated carbon–carbon double bond at δ_C 195.4. In addition, analysis of the NMR spectra showed that two non-conjugated –CH=CH– unsaturations were present in the hydrocarbon chain. These findings combined with the absence of signals for terminal methyl groups in the ^{1}H and ^{13}C NMR spectra pointed to the existence of triunsaturated macrocyclic compounds bearing a formyl group on an olefinic carbon, (see gross structure **a** in Fig. 2). In order to locate the double bonds in the chain, an aliquot of the aldehyde fraction was submitted to ozonolysis, and the resulting ozonides were cleaved under reductive conditions. GC–MS analysis of the products showed the formation of three α,ω-dialdehydes: *n*-C_9, C_{11} and C_{14} (**b**), in a ca. 1:2:3 proportion, and two α-acid, ω-aldehydes, *n*-C_8 and C_{10} (**c**), in a ca. 1:2 proportion (Fig. 2). Based on the reductive conditions used to cleave the ozonides, it is very likely that the aldehyde functions derive from the oxidation of the five olefinic =CH– present in the ring system, and the carboxyl group from the oxidation of the olefinic quaternary carbon, =C(CHO)–, with concomitant loss of the initial formyl group (Fig. 2). All these analytical data are, however, insufficient to determine the relative position of the unsaturations.

There exists a common structural feature between these macrocyclic aldehydes and some polyaldehydes typical of *B. braunii* that form the structural element of the alga cell wall, the algaenan: both exhibit α,β-unsaturated aldehyde functions and two non-conjugated unsaturations for one CHO group (Berthéas et al., 1999; Gelin et al., 1994; Metzger and Largeau, 2002; Metzger et al., 1993, 2007). It has been shown that these polyaldehydes (structure **11** in Fig. 3) originate from the condensation-polymerization of diunsaturated α,ω-dialdehydes, predominantly a *n*-C_{32} in the investigated strain, via an aldolization-dehydration mechanism (Fig. 3; Metzger et al., 1993). By analogy, it can be assumed that the present macrocyclic aldehydes **9** and **10** originate from the intramolecular condensation of some *n*-C_{32} and C_{34} dialdehydes, respectively. In the *n*-C_{32} dialdehyde, it was shown that the two carbon-carbon double bonds are located at C-9 and C-ω9, respectively (Fig. 3; Metzger et al., 1993). The intramolecular condensation of this molecule would lead to a single cyclic compound **9** because of

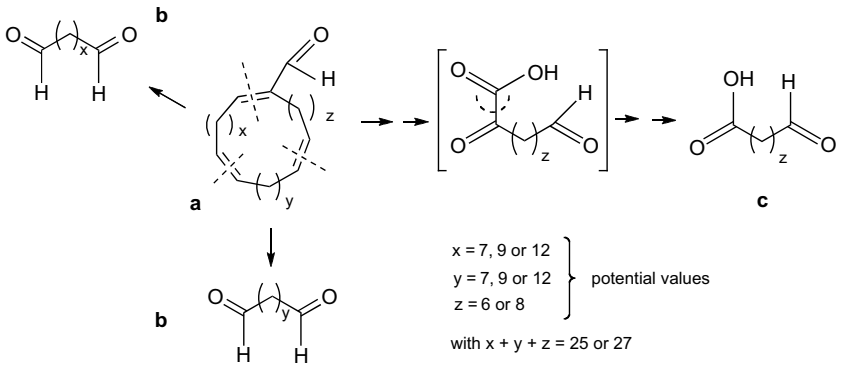

Fig. 2. Gross structure **a** for macrocyclic aldehydes and ozonolysis products.

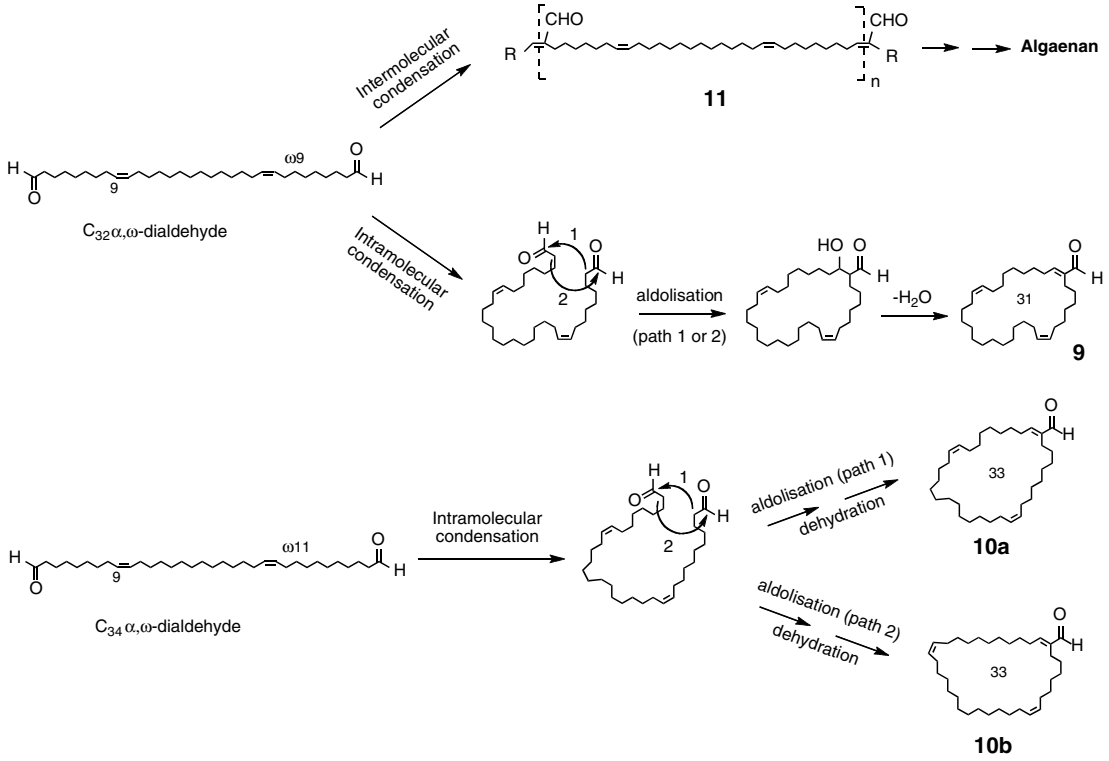

Fig. 3. Proposed biosynthetic schemes of 31- and 33-membered macrocyclic aldehydes **9** and **10**, and polyaldehydes **11** in *B. braunii*.

the molecular symmetry. Now, if we take into account the respective proportions on the one hand of the macrocyclic C_{32} and C_{34}, and on the other hand of the ozonolysis products, it is very likely that two C_{34} macrocyclic isomers are present in the mixture. Each isomer would originate from the intramolecular condensation of an *n*-C_{34} dialdehyde, diunsaturated at C-9 and C-ω11 (Fig. 3). Considering the position of these unsaturations the *n*-C_{34} dialdehyde does not display any element of symmetry. As a consequence, depending on whether a given carbonyl group acts as nucleophile or as electrophile (paths 1 and 2 in Fig. 3), two macrocyclic aldehydes **10a** or **10b** would be formed from the *n*-C_{34} dialdehyde.

Based on the above spectroscopic data and chemical degradations, braunicetals I are characterized as a series of six acetals (**1**–**6**) resulting from the condensation of 31- and 33-membered macrocyclic aldehydes **9** and **10** with C_{33} and C_{34} methylated squalene diols **7** and **8** (Fig. 1). The proposed stereochemistry of the acetal ring was supported by the ROESY data. Clear effects were observed between H-11′ at δ 3.56 and H-27′ at δ 1.23 and between H-1 at δ 5.19 and H-12′ at δ 1.54.

A similar series of braunicetals I was also isolated from another strain of *B. braunii* race B originating from Martinique; it was comprised, however, of a lower proportion of C_{34} tetramethyl squalene diol unit **8** and a corresponding higher proportion of the C_{33} trimethyl squalene diol **7**. Braunicetals I exhibit another analogy with the polymers building up the structural element of the cell walls in *B. braunii* race B: these polymers are aliphatic polyacetals derived from the condensation of polyaldehydes **11** with some methylated squalene diols (Metzger et al., 2007).

2.2. Braunicetals II

Braunicetals II (**12**–**14**; Fig. 1) were isolated from the heptane extract of a strain originating from Ivory Coast as a mixture of compounds exhibiting on LR-APCI mass spectrometry analysis two $[M+H]^+$ ions at *m/z* 1031 and 1059, with peak intensity in a 5:3 ratio. HR-ESIMS indicated molecular formulae $C_{72}H_{134}O_2$ and $C_{74}H_{138}O_2$. Inspection of the ^1H, ^{13}C, HMQC and HMBC NMR spectra indicated the presence of two different acetal-containing moieties IIa and IIb, with ^1H singlets at δ_H 5.24 and 5.14 correlating with ^{13}C signals at δ_C 105.5 and 106.0, respectively (Fig. 4). The similar magnitude of these signals suggested that these two acetal structures were equally represented. Four resonances for four oxygen-bearing

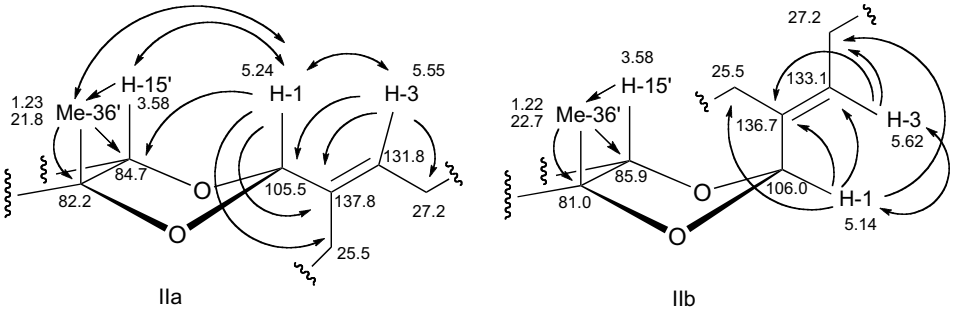

Fig. 4. Selected ^1H and ^{13}C NMR data, and HMBC (→) and ROE (↔) correlations in the acetal rings of conformers IIa and IIb in braunicetals II.

carbons were also noticed at δ_C 85.9 and 84.7 (quaternary carbons) and 82.3 and 81.1 (methine carbons). Moreover, the ^1H and ^{13}C NMR spectra also suggested the presence of a long methylenic chain (intense signal at δ_H 1.30 and numerous peaks in the region δ_C 29.9–29.0), comprising two non-conjugated unsaturations (δ_H 5.35, δ_C 130.0) of *cis* stereochemistry. The ^1H NMR spectrum exhibited also signals for eight secondary methyl groups ($0.87 < \delta_H < 0.83$), a vinylic methyl at δ_H 1.60 and two tertiary methyls at δ_H 1.23 and 1.22, suggesting on the whole the presence of a terpene moiety in the molecules. Acid hydrolysis, through the isolation and identification of a mixture of macrocyclic aldehydes **9** and **10** and a tetraterpene diol **15,** demonstrated that the isolated compounds were braunicetals of a different type from that described above. The tetraterpene is 14,15-dihydroxy-lycopa-18(*E*)-ene closely related to *trs,trs*-lycopadiene, the specific hydrocarbon of *B. braunii* race L. This diol was formerly isolated from the hydrolysis of a very high molecular weight polyacetal (Berthéas et al., 1999), a polymer rather similar to the one present in algae of race B, but in the present case it derives from the condensation of polyaldehydes with dihydroxy-lycopaene.

The existence of acetals IIa was confirmed by HMBC correlations (particularly H-1/C-15′; Fig. 4) and by a ROESY experiment establishing that the acetal proton is on the same side of the acetal ring as Me-36′ and H-15′. Unfortunately, several HMBC and ROESY experiments did not allow confirmation of the stereochemistry of acetal isomers IIb proposed in Fig. 4, probably due to unfavourable geometries of these molecules.

3. Concluding remarks

This study provides evidence for the existence of macrocyclic compounds comprising only carbon atoms in the rings, in two chemical races of *B. braunii*, B and L. Attempts to isolate similar compounds from algae of *B. braunii* race A failed, perhaps due to inappropriate purification conditions. To the best of our knowledge, braunicetals constitute the first example of very large macrocycles comprising no heteroatom, in algae. Series of C_{15}-C_{34} macrocyclic alkanes were previously identified in some sediments and crude oils containing remains of *B. braunii* (Audino et al., 2001, 2002a, 2002b; Grice et al., 2001). It was assumed that these hydrocarbons would be formed in the sediments during the diagenetic process, by an olefin metathesis reaction of the unsaturated polyaldehyde, the structural network of *B. braunii* cell walls, rather than the reduction of some unidentified biomarkers (Audino et al., 2002b). The much narrower distribution of the macrocycle units in braunicetals, restricted to C_{31} and C_{33} rings, supports this hypothesis.

Some structural considerations speak for a common biosynthetic pathway leading to polyaldehydes and macrocyclic aldehydes in *B. braunii*, i.e. by aldolisation/dehydration of diunsaturated α,ω-dialdehydes. In *B. braunii*, the reaction that converts monomers to polyaldehydes and macrocycles leads predominantly to the former compounds. If we take into account the totality of the polymers structurally based on this network, i.e. those extractable with solvents and their insoluble forms deposited in the cell walls (i.e. the algaenan), polyaldehydes account for ca. 10–25% of the dry algal biomass (Berthéas et al., 1999; Metzger et al., 2007). By comparison, braunicetals are only minor components, accounting for ca. 0.1% of the dry biomass. A similar balance between intermolecular and intramolecular reactions is observed in polymer synthesis when neat monomers are used, while, for statistical reasons, the formation of cyclic oligomers is favoured if polymerisations are carried out in solution (Hodge, 2001).

Braunicetals I and II exhibit common structural features with polyaldehydes-polyacetals synthesized by *B. braunii* races B and L, respectively. All these compounds originate from the acetalization of C_{32} (or C_{34}) aldehyde units with tri- or tetraterpene diols. Recent work on polyaldehydes-polyacetals of *B. braunii* race B has shown that increasing condensations of polyaldehydes-polyacetals occur via epoxidation of the terpenoid units (at C-14′ in **1**–**6**) followed by epoxide opening and subsequent acetalization of the resulting diols with aldehyde functions of the polyaldehyde-polyacetal backbone (Metzger et al., 2007). By a similar way macrocyles could be covalently anchored to the polymer (Fig. 5). Braunicetals could also be mechanically trapped by their macrocycles onto the linear unit of the polyaldehydes, i.e. the connecting forces are non-covalent interactions, and thus form a rotaxane moiety (Huang and Gibson, 2005) (Fig. 5). The incorporation of macrocycles into the polyaldehydes/polyacetals would explain the elastic properties of these polymers and their ability to swell in some solvents like chloroform or tetrahydrofuran (Metzger and Largeau, 2002), both properties commonly observed in synthetic polyrotaxanes (Takata, 2006).

4. Experimental

4.1. General experimental procedures

The NMR spectra were recorded on a Bruker Avance 400 DPX at 400 MHz for ^1H and 100 MHz for ^{13}C, using $CDCl_3$ as solvent. Chemical shifts were referenced relative to residual proton signal (7.26 ppm) or to the central line of ^{13}C multiplet (77.1 ppm) of $CDCl_3$. GC–(EI)MS analyses were performed with a HP 5890 chromatograph coupled to a HP 5989 mass spectrometer operating at 70 eV. The chromatograph was equipped with a CPSil-5CB fused-silica capillary column (25 m × 0.25 mm) coated with polydimethyl-siloxane (film thickness 0.25 μm). The temperature program was from 220 to 300 °C at 2 °C min^{-1}. HR-EI mass spectra were ob-

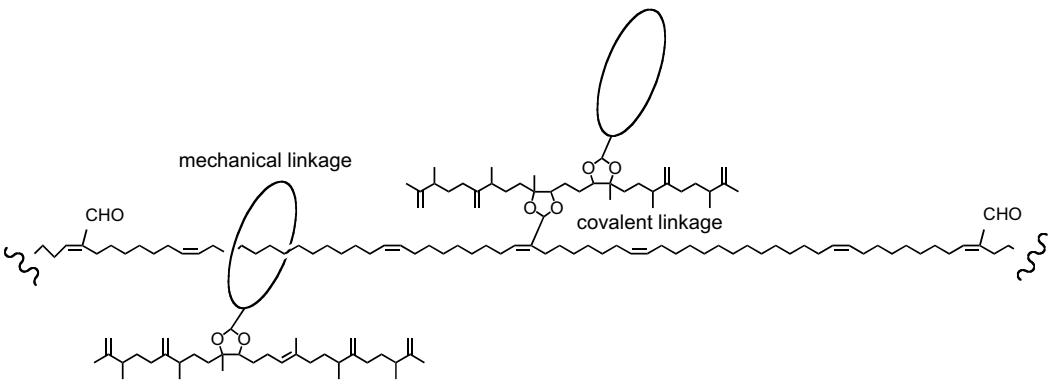

Fig. 5. Proposed models for the incorporation of braunicetals I into polyaldehydes/polyacetals of *B. braunii* via covalent or mechanical linkage.

tained on a Jeol MS 700. LR APCI-MS analyses were performed using an API 3000 apparatus; injection by direct inlet via the loop. HR ESI-TOF-MS were obtained using a Waters-TOF-MS apparatus, at the "Service Central d'Analyse du CNRS", Vernaison, France.

4.2. Algal strains and culture conditions

The strains originated from lakes located in Australia (Jillamatong; Metzger et al., 1997), Bolivia (Overjuyo; Metzger et al., 1988), France (Lingoult; Metzger et al., 1985a), Ivory Coast (Yamoussoukro; Metzger et al., 1990) and Martinique (La Manzo; Metzger et al., 1985a). The strains are conserved in the laboratory by periodic replications (every 4 months) on a modified CHU 13 medium (Largeau et al., 1980). The algae were grown at 25 °C, under batch air-lift conditions (air enriched with 1% CO_2) and continuous illumination (170 μE m^2 s^{-1}) as previously described (Metzger et al., 1985a). After 3 weeks of growth, the cultures entered the stationary phase, they were harvested by filtration on 10 μm Nylon cloth, and the biomasses were then freeze-dried.

4.3. Extraction and isolation

The dry biomass from each strain was extracted twice for 1 h with heptane (2 × 300 ml), at room temperature. The combined extracts were concentrated under reduced pressure and the resulting oil submitted to column chromatography (CC). Oils obtained from algae of race B (La Manzo and Overjuyo) were separated by alumina CC. After a first elution with heptane furnishing the hydrocarbon fraction, a second fraction eluted with toluene was collected and further separated by silica gel TLC, elution by heptane:diethyl ether 19:1 v/v. Typically, the dry biomass of the Overjuyo strain (7.2 g), furnished 4.2 g of heptane extract, 0.355 g of toluene fraction and finally 8 mg of a mixture of acetals **1**–**6** (R_f 0.61; yield 0.11% of dry wt). From strain of La Manzo a mixture of acetals **1**–**6**, exhibiting a similar R_f (0.61) was also obtained (yield: 0.08% of dry wt). The oil extracted from Yamoussoukro strain (race L) was separated by silica gel CC, by elution with heptane and then heptane:diethyl ether 19:1 v/v, as previously described (Rager and Metzger, 2000). Separation by silica gel TLC afforded a mixture of acetals **12**–**14** (R_f 0.63; yield 0.13% of dry wt). Purification of the oils extracted from strains Jillamatong and Lingoult (race A) did not furnish any macrocycle-comprising compound.

4.4. Braunicetals ***1*–*6***

Clear oil; LR-APCIMS *m/z* 925 $[M+H]^+$; 939 $[M+H]^+$; 953 $[M+H]^+$; 967 $[M+H]^+$; HR –TOF-MS *m/z* $[M+Na]^+$ 947.8497 (calc. for $C_{65}H_{112}O_2Na$, 947.8560), 961.8644 (calc. for $C_{66}H_{114}O_2Na$, 961.8717), 975.8832 (calc. for $C_{67}H_{116}O_2Na$, 975.8873), 989.9011 (calc. for $C_{68}H_{118}O_2Na$, 989.9030); ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data see Table 1.

4.5. Braunicetals ***12*–*14***

Clear oil; LR-APCIMS *m/z* 1031 $[M+H]^+$; 1059 $[M+H]^+$; HR-ESI-TOF-MS *m/z* $[M+Na]^+$ 1054.0292 (calc. for $C_{72}H_{134}O_2Na$ 1054.0282), 1082.0596 (calc. for $C_{74}H_{138}O_2Na$ 1082.0595); ^1H ($CDCl_3$, 400 MHz) NMR data: δ 5.62 (*t*, *J* = 7.0 Hz, H_{IIb}-3), 5.55 (*t*, *J* = 7.0 Hz, H_{IIa}-3), 5.34 (*m*, H-11, H-12, H-25, H-26), 5.24 (*s*, H_{IIa}-1), 5.14 (*s*, H_{IIb}-1), 5.13 (*t*, *J* = 5.3 Hz, H-18′), 3.58 (*m*, H_{IIa}-15′, H_{IIb}-15′), 2.02 (*m*, H-4, H-10, H-13, H-24, H-27, H-17′), 1.96 (*t*, *J* = 7.1 Hz, H-20′), 1.60 (*s*, H-37′), 1.52 (*m*, H-2′, H-31′), 1.37-1.25 (*br*, intense signal of CH_2 and CH protons of the terpene and ring moieties), 1.23 (*s*, H_{IIa}-36′), 1.22 (*s*, H_{IIb}-36′), 1.15 and 1.06 (*m*, other CH_2 protons of the terpene moiety), 0.87–0.83 (overlapping signals of terpene methyl protons); selected ^{13}C ($CDCl_3$, 100 MHz) NMR data: see Fig. 4.

4.6. Acid hydrolysis

Typically, braunicetals (ca. 3 mg) in $CHCl_3$ solution (1 ml) were diluted with THF (2 ml) and reacted under a nitrogen atmosphere with 0.1 ml of concentrated aqueous 12 N HCl for 4 h at room temperature and with magnetic stirring. The mixture was then diluted with diethyl ether, washed with water until neutrality, and the organic phase, dried with Na_2SO_4, was concentrated under reduced pressure. The resulting oils purified by preparative silica gel TLC, using heptane:diethyl ether (9:1, v/v) as eluent, yielded macrocyclic aldehydes **9**, **10** and the known terpene diols **7**, **8** (Metzger et al., 2007) and **15** (Berthéas et al., 1999), depending on the strains.

4.7. Macrocyclic aldehydes ***9*** *and* ***10***

Clear oil; **9** LR-EIMS *m/z* (rel. int.): 456 $[M]^+$ (55), 438 $[M\text{-}H_2O]^{+\cdot}$ (15), 427 (4), 425 (5), 149 (22), 135 (36), 121 (42), 109 (51), 95 (93), 81 (91), 67 (87), 55 (100); HR-EIMS *m/z* $[M]^+$ 456.4351 (calc. for $C_{32}H_{56}O$, 456.4307); **10** LR-EIMS *m/z* (rel. int.): 484 $[M]^+$ (52), 466 $[M\text{-}H_2O]^+$ (15), 455 (3), 453 (4), 149 (19), 135 (36), 121 (42), 109 (51), 95 (92), 81 (91), 67 (87), 55 (100); HR-EIMS: *m/z* $[M]^+$ 484.4653 (calc. for C_{34} $H_{60}O$, 484.4634). ^1H NMR (400 MHz, $CDCl_3$; proton numbering according to a C_{32} aldehyde) 9.35 (*s*, H-1), 6.44 (*t*, $J_{3,4}$ = 7.4 Hz, H-3), 5.35 (4H, *m*), 2.34 (*dt*, $J_{4,5}$ = 7.4, 7.5 Hz, H-4), 2.23 (*t*, $J_{31,32}$ = 6.4 Hz, H-32), 2.02 (8H, other allylic protons), 1.50 (*m*, H-5), 1.29 (*br*, other methylene protons); ^{13}C NMR (100 MHz, $CDCl_3$; carbon numbering according to a C_{32} aldehyde) 195.4 (C-1), 155.5 (C-3), 143.9 (C-2), 130.2-130.0 (other olefinic carbons), 29.8–28.8 [$(CH_2)_n$], 24.1 (C-32).

4.8. Ozonolysis of ***9*** *and* ***10***

A CS_2 solution (1 ml) of the mixture of aldehydes **9** and **10** (0.5 mg) was ozonized at −15 °C for 5 min, until the characteristic blue colour of ozone persisted. Excess of O_3 was removed at room temperature under a stream of N_2 and the ozonides decomposed by addition of 3 mg of triphenylphosphine. The reaction mixture was concentrated and directly analysed by GC-(EI)MS.

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