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Polyhalogenated homosesquiterpenic fatty acids from *Plocamium cartilagineum*

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Abstract

We describe the composition of novel polyhalogenated homosesquiterpenic acids from the red alga *Plocamium* collected during summer on Maltese Islands and Corsica. The compounds, predominantly derivatives with unique groups (bromine, chlorine, and diene), were identified by means of ^{1}H and ^{13}C NMR, MS, IR and UV spectra.

Keywords: Polyhalogenated homosesquiterpenic acids; Red alga; *Plocamium*; NMR, MS, IR and UV spectra

1. Introduction

Plocamium cartilagineum (L.) Dixon (Gigartinales) has a wide range of distribution (Cormaci et al., 1997; Frick et al., 1996). This species is cosmopolitan in all of the worlds' temperate oceans. *P. cartilagineum* grows in the mid to low intertidal zone and down to 20 m subtidally. It can live in sheltered to moderately exposed and exposed open coast habitats. This species is generally epilithic, growing on rocks, sand covered rocks or shell fragments. *P. cartilagineum* has also been found as an epiphyte on articulated coralline algae (Bischoff-Bäsmann and Wiencke, 1996).

Red algae of the genus *Plocamium* have been shown to be a rich source of halogenated monoterpenes and other compounds (Faulkner, 2000; Abreu et al., 1997). In the course of our investigation of the chemical composition of algae of fresh water (Rezanka et al., 1983; Rezanka and Podojil, 1984) and marine origin (Rezanka et al., 1988; Dembitsky et al., 1993), we describe in this paper the elucidation of the structure of four new polyhalogenated homosesquiterpenic acids from *P. cartilagineum* collected on Maltese Islands and Corsica.

2. Results and discussion

A fresh sample of *P. cartilagineum* was separately and successively extracted with a mixture of CH_2Cl_2 and MeOH. Fractionation of the extracts by chromatography on Sephadex LH-20 afforded three main fractions. The third fractions were purified by means of a preparative RP–HPLC to yield four acids (Table 1 and Fig. 1). Compound (**1**) was determined to have the molecular formula $C_{16}H_{19}O_3BrCl_2$ by HREIMS with major fragment peaks at m/z 372, 374 and 376 $[M–HCl]^+$, m/z 293 and 295 $[M–HCl–Br]^+$ and m/z 257 $[M–2xHCl–Br]^+$. The IR spectrum was consistent with the presence of an α, β-unsaturated ketone (1685 cm^{-1}) and a carboxylic group (1710 cm^{-1}). Compound **1** showed an UV maximum at 243 nm, typical for a conjugated en-one system. The ^{1}H NMR spectrum showed vinylidene protons at δ_H 6.16 and 5.86, which were found to be attached to C-14 (δ_C 126.8) from the HMQC experiment, and showed long-range correlations with C-4 (^{3}J) and C-3 (^{2}J) (δ_C 200.8 and 143.4, respectively) in the HMBC spectrum.

A weak carbon signal at δ_C 173.9 (C-1), which showed a ^{2}J interaction with δ_H 3.24 (H_2-2), was assigned to a carboxylic acid. The H_2-2 signal also exhibited HMBC interactions with C-3, C-4 and C-14. Finally, the positions of the two methyl groups were determined by the HMBC experiment (see Table 2). For example, Me-15

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Table 1
Content of novel polyhalogenated homosesquiterpenic acids from the red alga *Plocamium cartilagineum* collected on Maltese Islands and Corsica (in mg on 100 g of dried alga)

Acid	Maltese Islands	Corsica
A (**1**)	5.4	–
B (**2**)	2.3	–
C (**3**)	–	3.8
D (**4**)	–	1.9

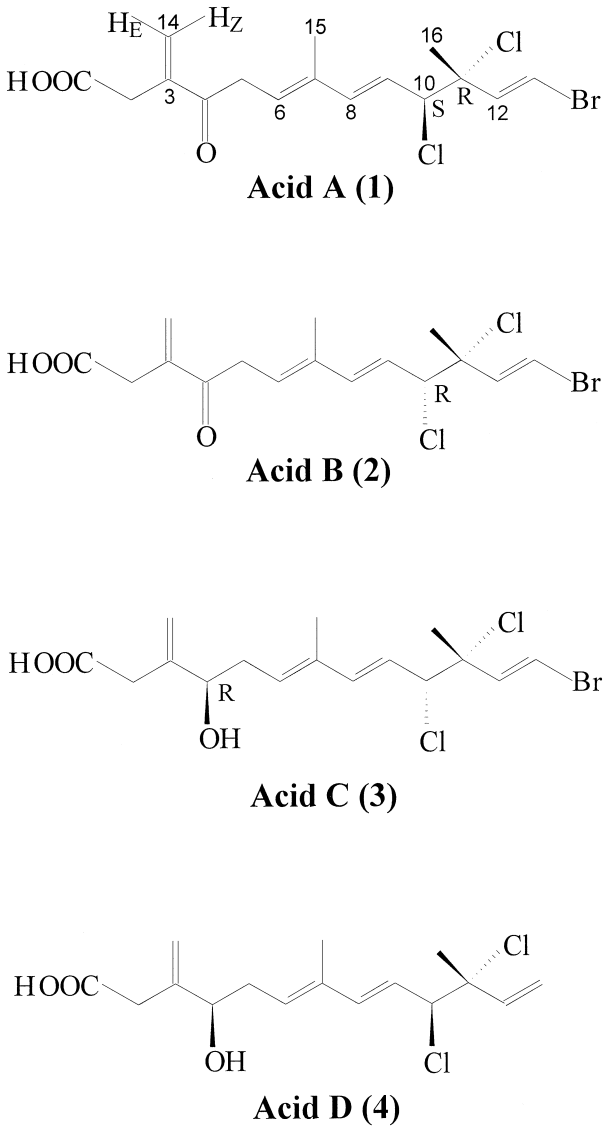

Fig. 1. The structure of acids A–D (**1**–**4**) from *Plocamium cartilagineum*.

had correlations with C-6, C-7 and C-8 and Me-16 with C-10, C-11 and C-12. The ^{1}H NMR spectrum of **1** (Table 2) displayed also two methyl signals at δ 1.78 and 1.56. DQF–COSY measurements allowed the following spin systems to be discerned: an AB quartet $-$CH=CHX at δ 6.45 (H-12) and δ 6.58 (H-13) and a three-proton AMX system $(R)_3C$–CH=CH–CHX– at δ 6.36 (H-8), δ 6.42 (H-9) and δ 4.55 (H-10).

The *E* stereochemistry of trisubstituted Δ^6 double bond is based on the relative upfield shift of the vinylic methyl group (C-15) (δ_C 15.7) and NOESY γ-correlations between this methyl group and the methylene group at C-5 (Blunt et al., 1985).

The stereochemistry of the double bonds Δ^8 and Δ^{12} was assigned as *E* on the basis of the interproton coupling constants J_{8H-9H} = 16.1 Hz and $J_{12H-13H}$ = 13.4 Hz. The coupling constants of olefinic protons in (3*E*,7*E*)-2,6-dimethyloctadiene polyhalogenated monoterpenes lie in the range of 13.5 to 15.9 Hz (Jongaramruong and Blackman, 2000). In the ^{13}C NMR spectrum of **1** (Table 3), the chemical shift of C-13 δ 110.8 indicated a vinyl bromide functionality. The remaining three sp^2 olefinic carbons absorb at δ 132.4 (C-12), δ 124.6 (C-8), and δ 136.5 (C-9). The halogen substitution at C-5 and C-6 was established by comparison of the ^{1}H NMR, ^{13}C NMR, and MS of 1 with those of known compounds (Grible, 1996). The proton and carbon chemical shifts of the C-16 methyl group δ 1.56 and δ 26.2 suggest a (10*S**,11*R**) stereochemistry, in accordance with the empirical rules of Mynderse and Faulkner (1975) and Crews and Kho-Wiseman (1974).

Thus, NMR spectroscopy indicated that the structure of compound **1** is that of (6*E*,8*E*,12*E*)-3-methylene-4-oxo-7,11-dimethyl-(10*S**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid.

Acid B (**2**) was isolated from the same fraction as **1** and a sesquiterpene skeleton with the formula $C_{16}H_{19}O_3BrCl_2$ was indicated by HREIMS; it had the same molecular weight as **1** but the some spectral characteristics were different. In support of this, the main MS fragment peaks were at *m*/*z* 293 and 295. The ^{13}C NMR spectrum (Table 3) indicated that Cl on C-10 had an opposite configuration, i.e. *R**. This assumption was proved by the ^{1}H NMR chemical shift of H-16 protons, in keeping with literature data (Mynderse and Faulkner, 1975; Crews and Kho-Wiseman, 1974). The stereochemistry at the C-10 and C-11 centers was assigned on the basis of NMR spectra (see above) and positive optical rotation ($[\alpha]_D^{21} = +37.6^\circ$). Acid B (**2**) is (6*E*,8*E*,12*E*)-3-methylene-4-oxo-7,11-dimethyl-(10*R**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid.

Acid C (**3**), the most polar of the four compounds, had the formula $C_{16}H_{21}O_3BrCl_2$, as determined by HREIMS. Comparison of its ^{1}H and ^{13}C NMR spectral data with those of compounds **1** and **2** suggested that the structure of **3** was similar, except for the presence of one substituent. The change of ketone group on C-4 to a hydroxyl group. Giving rise to another chiral carbon was indicated by the optical rotation. The fact that the end of the molecule has the same chemical shifts in ^{1}H and ^{13}C NMR as acid B (**2**), proved that the two were

Table 2
^{1}H NMR data of homosesquiterpenic acids (**1**–**4**) from *Plocamium cartilagineum*

Hydrogen no.	**1**	**2**	**3**	**4**
2	3.24 *d* (1H, *J* = 16.3)	3.21 *dd* (1H, *J* = 16.8)	3.26 *dd* (1H, *J* = 16.6)	3.28 brs (2H)
	3.29 *dd* (1H, *J* = 16.3)	3.29 *dd* (1H, *J* = 16.8)	3.31 *dd* (1H, *J* = 16.6)	
4	–	–	4.02 *dd*(1H, *J* = 8.1;6.1)	3.95 *dd*(1H, *J* = 7.9;5.2)
5	2.85 (1H, *J* = 16.1)	2.94 *dd* (1H, *J* = 15.6)	2.28 *m* (1H)	2.24 *m* (1H)
	2.64 (1H, *J* = 16.1)	2.81 *dd* (1H, *J* = 15.6)	2.31 *m* (1H)	2.33 *m* (1H)
6	5.75 *d* (1HJ = 0.7)	5.72 *dd* (1H, *J* = 0.9)	5.12 *sept* (1H, *J* = 6.5;1.1)	5.15 *sept* (1H, *J* = 6.9;1.4)
8	6.36 *dd* (1H, *J* = 16.1;0.7)	6.31 *dd* (1H, *J* = 15.9;0.9)	6.31 *dd* (1H, *J* = 15.9;0.9)	6.34 *dd* (1H, *J* = 15.3)
9	6.42 *dd* (1H, *J* = 8.6;16.1)	6.47 *dd* (1H, *J* = 8.4;15.9)	6.49 *dd* (1H, *J* = 8.4;15.9)	6.53 *dd* (1H, *J* = 15.3;8.5)
10	4.55 *dd* (1H, *J* = 0.7;8.6)	4.57 *dd* (1H, *J* = 0.9;8.4)	4.54 *dd* (1H, *J* = 0.9;8.4)	4.45 *dd* (1H, *J* = 8.5;1.0)
12	6.45 *dd* (1H, *J* = 13.4)	6.41 *dd* (1H, *J* = 13.8)	6.41 *dd* (1H, *J* = 13.8)	6.02 *dd* (1H, *J* = 10.6;16.5)
13	6.58 *dd* (1H, *J* = 13.4)	6.60 *dd* (1H, *J* = 13.8)	6.60 *dd* (1H, *J* = 13.8)	5.21 *dd* (1H, *J* = 10.6;1.0)
				5.38 *dd* (1H, *J* = 16.5;1.0)
14E	5.86 *brs* (1H)	5.91 *brs* (1H)	5.88 *brs* (1H)	5.91 *brs* (1H)
14Z	6.16 *brs* (1H)	6.18 *brs* (1H)	6.17 *brs* (1H)	6.15 *brs* (1H)
15	1.78 *s* (3H)	1.84 *s* (3H)	1.85 *s* (3H)	1.78 *s* (3H)
16	1.56 *s* (3H)	1.58 *s* (3H)	1.59 *s* (3H)	1.56 *s* (3H)

Table 3
^{13}C NMR data of homosesquiterpenic acids (1–**4**) from *Plocamium cartilagineum*

Carbon no.	**1**	**2**	**3**	**4**
1	173.9	174.1	175.8	174.4
2	37.4	37.1	37.2	37.1
3	143.4	141.9	143.2	142.9
4	200.8	199.5	74.6	77.0
5	44.8	44.1	36.2	34.7
6	123.9	122.4	124.7	120.8
7	136.1	134.9	135.7	134.0
8	124.6	125.5	124.9	125.8
9	136.5	135.8	136.2	137.1
10	68.2	69.7	69.3	70.6
11	72.1	73.0	72.9	71.8
12	132.4	133.1	132.7	139.1
13	110.8	111.2	110.1	115.8
14	126.8	126.9	127.0	126.5
15	15.7	15.8	15.8	15.9
16	26.2	29.6	29.7	24.6

identical. The strongly shielded nature of H-4 (δ 4.02) and C-4 (δ 74.6) indicated the presence of a hydroxyl group, and its *R** configuration was determined by means of Mosher's esters. Treatment of each hydroxy acid with (*R*)-(−)-MTPACl or (*S*)-(+)-MTPACl converted them into (*S*)- and (*R*)-2-methoxy-2-trifluoromethyl phenylacetic acid (MTPA) esters (**3s**, and **4s** and **3r** and **4r**, respectively). The values of $\Delta\delta = [\delta$(S-MTPA ester) − δ(R-MTPA ester)] in the ^{1}H NMR spectra suggested that the absolute configurations at C-4 of compounds **3** and **4** were (R) (Ohtani et al., 1991a,b). The data are shown in Table 4. Compound **3** could also be denoted (6*E*,8*E*,12*E*)-3-methylene-(4*R*)-hydroxy-7,11-dimethyl-(10*R**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid.

Table 4
^{1}H NMR (500 MHz) spectral data for Mosher's esters ($CDCl_3$)

Hydrogen no	Compound no. (Δin Hz)[a]	
	3	**4**
2	+27	+31
5a	−7	−8
5b	−21	−23
6	−34	−39
14*E*	+11	+10
14*Z*	+23	+19

[a] Resonances of H directly attached to esterified carbon are not analyzed and/or values < |3 Hz| have not been included.

Acid D (**4**), with the molecular formula $C_{16}H_{22}O_3Cl_2$, had a homosesquiterpene structure, as in **1**. However, the chain lacked one bromine atom. A 3-chloro-1-butenyl end of the chain was indicated by mass fragmentation (base peak at *m*/*z* 89) and ^{1}H NMR assignments of a tertiary methyl group and clean vinylic ABX system at δ 5.21 (H-13a), δ 5.38 (H-13b) and δ 6.02 (H-12). Therefore, the bromine at the end of chain (on C-13) is obviously missing. The further part of the molecule is the same as that of acid C (**3**). All the above data indicate that acid D (**4**) is (6*E*,8*E*,12*E*)-3-methylene-(4*R*)-hydroxy-7,11-dimethyl-(10*S**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid.

In terms of biosynthesis, halogenated homosesquiterpenic acids may be biosynthesized by elongation of the know a halogenated monoterpenes described in *Plocamium* many times (Jongaramruong and Blackman, 2000; Crews and Kho-Wiseman, 1974; König at al., 1990).

Although this alga has a worldwide distribution, which is restricted to temperate seas, it is of interest to

note that this Mediterranean Sea collection afforded new metabolites and provides yet another example of chemical variability of marine natural products.

3. Experimental

The algal material *Plocamium cartilagineum* (L.) Dixon (Gigartinales) was collected at two places at Maltese Islands and Corsica.

UV spectra were measured by a Cary 118 (Varian) apparatus in EtOH within the range of 200–350 nm. A Perkin-Elmer Model 1310 (Perkin-Elmer, Norwalk, CT, USA) infrared spectrophotometer was used for scanning infrared spectroscopy of methyl esters as neat film. NMR spectra were recorded on a Brüker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H), 125.7 MHz (^{13}C). High- and also low-resolution mass spectra were recorded using a VG 7070E-HF spectrometer (Micromass, Manchester, UK). The ionizing condition was 70 eV.

The air dried samples (250 and 300 g) from both collections were separately extracted with mixture of CH_2Cl_2–MeOH (1:1, v/v). The extracts were concentrated under N_2. The total lipid extract, viscous dark oil, was subjected to a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:5) and further separated by semi-preparative RP–HPLC, a C_{18} reversed phase column (5 μm, 7.8×250 mm, Supelco, USA) was employed. A linear gradient from 20% H_2O and 80% acetonitrile to 1% water and 99% acetonitrile over 25 min, flow rate 2 ml/min was used to separate all of the compounds in crude extract. The acids **1**–**4** were detected by UV absorption at 208 nm.

(*S*)-MTPA esters. To a stirred solution of ~1.0 mg of hydroxy compound in 0.3 ml of dry pyridine was added 20 μl of (−)-MTPA chloride. After stirring the mixture under N_2 at room temperature for 1 h, the solvent was removed by blowing with N_2. The residue was redissolved in 2 ml of 30 ml EtOAc–hexane and filtered through a Sep-Pak silica column. After removing the solvent under vacuum, the residue was separated by reversed-phase HPLC (ODS column, 100% MeCN) to yield ~1.0 mg of *S* ester as a colorless gum; ^{1}H NMR spectra ($CDC1_3$), see Table 4.

(*R*)-MTPA esters. Prepared as described for ***S*** esters. From ~1.0 mg of compound and 20 μl of (+)-MTPA chloride 0.9 mg of *R* esters were obtained as a colorless gum; ^{1}H NMR spectra ($CDCl_3$), see Table 4.

Acid A (**1**) is (6*E*,8*E*,12*E*)-3-methylene-4-oxo-7,11-dimethyl-(10*S**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid (5.4 mg; 0.0054%), $[\alpha]_D$ −43.1° (*c* 0.17, MeOH); UV λ_{max}(EtOH) (log ε) 243 nm (2.37); IR (film) ν_{max} 2950, 2910, 1710 (COOH), 1685 cm^{-1} (α, β-unsaturated ketone); LREIMS *m*/*z* (% of base peak) 372 (15), 374 (16) and 376 (5) [M–HCl]$^+$, *m*/*z* 293 (11) and 295 (5) [M–HCl–Br]$^+$, *m*/*z* 257 (15) [M–2xHCl–Br]$^+$ HREIMS (*m*/*z*) 408.3247, (calc. for $C_{16}H_{19}O_3{}^{79}Br^{35}Cl_2$ 408.3251). NMR data, see Tables 2 and 3.

Acid B (**2**) is (6*E*,8*E*,12*E*)-3-methylene-4-oxo-7,11-dimethyl-(10*R**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid (2.3 mg; 0.0023%), $[\alpha]_D$ +37.6° (*c* 0.14, MeOH); UV λ_{max} (EtOH) (log ε) 243 nm (2.37); IR (film) ν_{max} 2950, 2910, 1710 (COOH), 1685 cm^{-1} (α, β-unsaturated ketone); LREIMS *m*/*z* (% of base peak) 372 (14), 374 (15) and 376 (4) [M-HCl]$^+$, *m*/*z* 293 (9) and 295 (4) [M–HCl–Br]$^+$, *m*/*z* 257 (13) [M–2xHCl–Br]$^+$ HREIMS (*m*/*z*) 408.3247, (calc. for $C_{16}H_{19}O_3{}^{79}Br^{35}Cl_2$ 408.3251). NMR data, see Tables 2 and 3.

Acid C (**3**) is (6*E*,8*E*,12*E*)-3-methylene-(4*R*)-hydroxy-7,11-dimethyl-(10*R**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid (3.8 mg; 0.0038%), $[\alpha]_D$ +30.1° (*c* 0.14, MeOH); UV λ_{max} (EtOH) (log ε) 243 nm (2.37); IR (film) ν_{max} 3450 (OH), 2950, 2910, 1715 (COOH); LREIMS *m*/*z* (% of base peak) 374 (17), 376 (19) and 378 (7) [M–HCl]$^+$, *m*/*z* 295 (10) and 297 (4) [M–HCl–Br]$^+$, *m*/*z* 259 (11) [M–2xHCl–Br]$^+$ HREIMS (*m*/*z*) 410.3406, (calc. for $C_{16}H_{21}O_3{}^{79}Br^{35}Cl_2$ 410.3409). NMR data, see Tables 2 and 3.

Acid D (**4**) is (6*E*,8*E*,12*E*)-3-methylene-(4*R*)-hydroxy-7,11-dimethyl-(10*S**,11*R**)-dichloro-13-bromo-trideca-6,8,12-trienoic acid oil (1.9 mg; 0.0019%), $[\alpha]_D$ 15.4° (*c* 0.18, MeOH); UV λ_{max}(EtOH) (log ε) 243 nm (2.37); IR (film) λ_{max} 3450 (OH), 2950, 2910, 1715 (COOH); LREIMS *m*/*z* (% of base peak) 296 (14) and 298 (6) [M–HCl]$^+$, *m*/*z* 259 (17) [M–2xHCl]$^+$ HREIMS (*m*/*z*) 332.3483, (calc. for $C_{16}H_{22}O_3{}^{35}Cl_2$ 332.3489). NMR data, see Tables 2 and 3.

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