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MERODITERPENES FROM THE BROWN ALGA *CYSTOSEIRA CRINITA* OFF THE FRENCH MEDITERRANEAN COAST

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Key Word Index—*Cystoseira crinita*; Cystoseiraceae; brown algae; meroditerpenes (tetraprenyltoluquinols); quantitative analysis; chemotaxonomy.

Abstract—Three new meroditerpenes related to zosterdiol A have been isolated from the brown alga *Cystoseira crinita*. Their structures have been established mainly by means of 2D NMR experiments: COSY, HCCORR and HMBC correlations. They have also been quantitatively analysed and their geographical variation has been studied from a chemotaxonomic point of view.

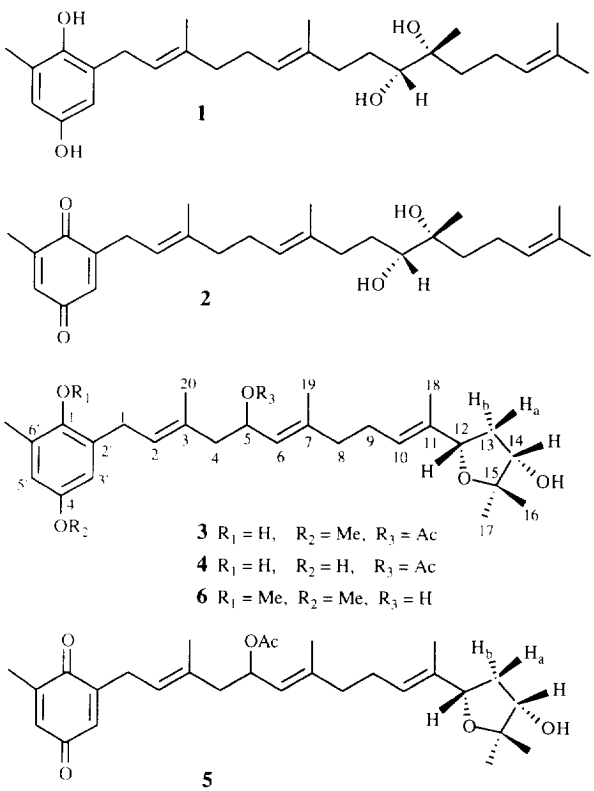
INTRODUCTION

In the course of our continuing phytochemical investigation of the family of the marine algae Cystoseiraceae [1–5], we have now investigated the lipid extract from *Cystoseira crinita* (Desfontaines) Duby, collected off the French Riviera coast. Sicilian authors [6] have isolated the linear meroditerpene **1** and its quinone derivative **2** from this species, but these compounds have not been found in the French variety. We now report the isolation and structure elucidation of three new tetraprenyltoluquinols (**3**–**5**) related to zosterdiol A (**6**), a meroditerpene isolated from *C. zosteroides* (Turner) C. Agardh [7] which is a deep-water species collected off the Sicilian coast. The seasonal and geographical variations in these compounds were also examined.

RESULTS AND DISCUSSION

The ether extract of dried *C. crinita*, collected near Toulon, France, was fractionated by liquid chromatography using silica gel. The fractions that eluted with hexane–ether (2:3) and (3:7) were further purified by HPLC on normal phase silica to give compounds **3** and **5** from the first fraction and **4** from the second.

Compound **3**, $C_{30}H_{44}O_6$ (HRMS), was an optically active oil which showed hydroxyl (3400 cm^{-1}), aromatic ring (1600 cm^{-1}) and acetate (1730 cm^{-1}) absorptions in its IR spectrum. The UV spectrum showed two maxima at 210 and 290 nm corresponding to an hydroquinonoid moiety. This function was confirmed by the ions at *m/z* 151 (100%), 189 and 191 in the mass spectrum which are characteristic fragments of the terpenoid derivatives of methyl-hydroquinone [8]. The ion at *m/z* 189 is particularly significant because it is obtained by the intra-



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molecular condensation of a phenolic OH group *ortho* to the first isoprene unit of the terpenoid chain.

The ^{1}H NMR spectrum in C_6D_6 (Table 1) showed the following substructures: Ar–CH_2–CH = C(Me)– (**A_3**), in which the benzylic methylene appeared as the AB part of an ABX system centred at δ3.48 (1H, *dd*, J = 15.6, 6.9 Hz) and 3.32 (1H, *dd*, J = 15.6, 6.8 Hz), the olefinic proton (X part of the ABX) gave rise to a doublet of doublets at δ5.52 (1H, *dd*, J = 6.9, 6.8 Hz) and the methyl group appeared at δ1.77. The second substructure (**B_3**), C–CH_2–CH(OAc)–CH = C(Me)–, was characterized by two *dd*, one at δ2.48 (J = 13.6, 8.1 Hz) and the other at δ2.20 (J = 13.6, 5.7 Hz) corresponding to the methylene protons, a *ddd* looking like a *td* at δ6.02 (J = 9, 8.1, 5.7 Hz) which was attributable to the proton geminal to the acetate group; a broad singlet at δ1.76 due to the methyl group and a *dq* at δ5.33 (J = 9, 1.2 Hz) for the olefinic proton. The third substructure, C–CH_2–CH_2–CH=C(Me)–(**C_3**), showed two triplets at δ2.07 (J = 7.3 Hz) and 5.64 (J = 7 Hz) for the non-allylic methylene and the olefinic proton, respectively; the allylic methylene appeared as a multiplet at δ2.18 and the methyl group as a singlet at δ1.70. The fourth substructure (**D_3**) was [=CMe]–CH(O–C–)–CH_2–CH(OH)–, characterized by a *br t* at δ4.34 (J = 7.3 Hz) corresponding to the allylic proton geminal to an oxygen function; a *dd* δ3.82 (J = 6.2, 5.7 Hz) corresponding to the proton geminal to the secondary hydroxyl group; and two multiplets at δ2.20 and 1.80, respectively, for the methylene protons. Structures **A_3**, **B_3**, **C_3** and **D_3** were confirmed by means of a COSY experiment.

In addition, there were peaks corresponding to two aromatic hydrogens with a *meta* coupling (J = 3 Hz) centred at δ6.76 and 6.71, respectively. Five methyl groups were also seen: one was directly bonded to an aromatic carbon (δ2.27); one was a methoxyl that was also linked to an aromatic carbon (δ3.55); two were geminal to a carbon bearing an oxygen function (Me–C–O) (δ1.36 and 1.22) and another corresponded to the acetate group.

Table 1. NMR data for compounds **3** and **4**

	3		**4**	
Position	^{13}C NMR* 100 MHz (C_6D_6)	^{1}H NMR 400 MHz (C_6D_6)	^{13}C NMR* 100 MHz (C_6D_6)	^{1}H NMR 400 MHz (C_6D_6)
1′	147.1	—	146.3	—
2′	128.6	—	128.3	—
3′	113.7	6.76 *d* (3)	114.6	6.66 *d* (3)
4′	154.0	—	150.5	—
5′	114.3	6.71 *d* (3)	115.9	6.65 *d* (3)
6′	126.1	—	125.9	—
Me 6′	16.6	2.27 *s*	16.6	2.20 *s*
OMe 4′	55.3	3.55 *s*	—	—
1	30.6	3.48 *dd* (15.6, 6.9) 3.32 *dd* (15.6, 6.8)	29.9	3.44 *dd* (15.2, 7) 3.34 *dd* (15.2, 6.8)
2	126.4	5.52 *dd* (6.8, 6.9)	126.6	5.52 *dd* (6.8, 7)
3	133.1	—	132.8	—
4	45.9	2.48 *dd* (13.6, 8.1) 2.20 *dd* (13.6, 5.7)	45.9	2.49 *dd* (13.5, 8.0) 2.20 *dd* (13.5, 5.6)
5	69.5	6.02 *ddd* (9, 8.1, 5.7)	69.8	6.05 *ddd* (8.9, 8.0, 5.6)
6	124.3	5.33 *dq* (9, 1.2)	124.2	5.33 *dq* (8.9, 1.2)
7	140.2	—	140.3	—
8	39.3	2.07 *t* (7.3)	39.2	2.06 *t* (7.4)
9	26.0	2.18 *m*	26.0	2.18 *m*
10	124.4	5.64 *t* (7)	124.8	5.66 *t* (7)
11	136.7	—	136.4	—
12	79.9	4.34 *brt* (7.3)	80.0	4.34 *brt* (7.4)
13	40.0	2.20 *m* 1.80 *m*	39.8	2.28 *m* 1.73 *m*
14	78.4	3.82 *dd* (6.2, 5.7)	78.4	3.79 *dd* (6, 5.8)
15	82.6	—	82.7	—
16	26.2	1.22 *s*	26.1	1.19 *s*
17	23.0	1.36 *s*	22.9	1.38 *s*
18	12.3	1.70 *s*	12.3	1.69 *s*
19	16.9	1.76 *s*	16.9	1.75 *s*
20	16.5	1.77 *s*	16.4	1.76 *s*
Ac C=O	170.3	—	170.8	—
Ac Me	20.9	1.84 *s*	20.9	1.83 *s*

* ^{13}C assignments are based on a HCCORR experiment.

The ^{13}C spectrum of this compound (Table 1) showed peaks corresponding to 30 carbon atoms (in agreement with the MS data). Their multiplicities were determined by DEPT sequence as eight methyl groups (one being a methoxyl and another an acetoxymethyl), five methylenes, three sp^3 methynes (– CH–O), five sp^2 methynes (–CH=C) and nine quaternary carbons: one of them was sp^3 oxygen-bonded, four others were aromatic, three were olefinic and one was the carbonyl of the acetate function at δ170.3.

All these spectroscopic data are in agreement with a meroditerpene structure with a C-1′, C-2′, C-3′ and C-5′ tetrasubstituted aromatic ring bearing hydroxyl, methyl and methoxyl groups as well as a diterpenoid side chain. The position of the phenolic hydroxyl at C-1′ being confirmed by the fragment at *m/z* 189 in the mass spectrum. By comparison with the NMR data of the side chain of zosterdiol A [7], we have suggested that the substructure $\mathbf{D}_3$ could be completed by a tetrahydrofuran ring as shown in formula **3**. This hypothesis was unambiguously confirmed by 2D heteronuclear NMR experiments (one bond and long-range coupling) which, in addition, have permitted us to link the substructures in the order: $\mathbf{A}_3$ to $\mathbf{D}_3$ via $\mathbf{B}_3$ and then $\mathbf{C}_3$. The correlations observed in the ^1H–^{13}C long range by inverse detection (HMBC) for **3** are summarized in Table 2. These data permitted the unambiguous assignment of all carbon signals as well as the substitution pattern of the aromatic ring.

Table 2. Long-range 2D ^1H–^{13}C correlations for compound **3**

C	δ(in $CDCl_3$)	Type	Long-range connected proton (HMBC in $CDCl_3$)
1′	146.5	C	Me 6′, 3′, 5′
2′	127.8	C	
3′	113.0	CH	5′
4′	153.2	C	OMe 4′, 3′, 5′
5′	113.9	CH	Me 6′, 3′
6′	125.9	C	Me 6′
Me 6′	16.3	CH_3	5′
OMe 4′	55.6	CH_3	
1	30.5	CH_2	3′
2	125.5	CH	20
3	133.5	C	4, 5, 20
4	45.4	CH_2	2, 20
5	69.5	CH	
6	123.3	CH	19
7	140.4	C	8, 9, 19
8	38.9	CH_2	6, 19
9	25.7	CH_2	
10	124.9	CH	18
11	135.9	C	12, 18
12	79.8	CH	10, 14, 18
13	39.4	CH_2	
14	78.3	CH	16, 17
15	82.7	C–O	16, 17
16	25.8	CH_3	14, 17
17	22.5	CH_3	16
18	12.1	CH_3	12
19	16.8	CH_3	8
20	16.5	CH_3	4
Ac C=O	170.7	C=O	Ac Me
Ac Me	21.1	CH_3	

The position of the C-18, C-19 and C-20 methyl signals above δ20 and in the ^{13}C NMR spectrum of **3** (Table 1) indicates the all *E* configuration of the double bonds of the isoprenoid side chain [9, 10]. The relative stereochemistry at the chiral centres at C-12 and C-14 was determined by a homonuclear ^1H–^1H NOESY experiment which showed H-12 and H-14 to be on the same face of the tetrahydrofuran ring, as for zosterdiol A [7].

The structure of metabolite **4** ($C_{29}H_{42}O_6$ by HRMS), an optically active oil with ν_{max} 3400 cm^{-1} (OH), 1600 cm^{-1} (aromatic ring) and 1730 cm^{-1} (acetate), and λ_{max} 212 and 290 nm, was readily established by comparison of its spectral properties with those of **3**. The ^{13}C NMR spectrum of **4** in C_6D_6 (Table 1) differed from that of **3** only by the absence of the methoxyl signal at δ55.3 and its ^1H NMR spectrum by the absence of the singlet at δ3.55 (Table 1). These data showed unambiguously that **4** was the 4′-demethoxylated derivative of **3**.

Compound **5**, $C_{29}H_{40}O_6$ (HRMS), was an optically active oil. UV absorption at 252 nm (ε 4380) indicated a *p*-benzoquinone moiety [11], which was confirmed by IR bands at 1650, 1640 and 1610 cm^{-1}, and by MS fragments at *m/z* 175 and 137. In the ^1H NMR spectrum (Table 3) H-5′ appeared at δ6.08 as a *dq* (J = 2.6, 1.7 Hz) due to long-range couplings with H-3′ and Me-6′, while H-3′ (δ6.29) was a *dt* (J = 2.6, 1.8 Hz) coupled with H-5′ and H-1. The latter was, in turn, vicinally coupled with a vinyl proton at δ5.10 leading to a *br d* (J = 7.3) at δ2.94. The ^{13}C NMR spectrum of this compound (Table 3) showed peaks corresponding to 29 carbon atoms (in agreement with the MS data). Their multiplicities were determined by DEPT sequence as seven methyl groups (one being an acetoxymethyl), five methylenes, three sp^3 methynes (– CH–O), five sp^2 methynes (–CH=C) and nine quaternary carbons: one sp^3 oxygen-bonded, two aromatics, three olefinics and three carbonyls corresponding to the benzoquinone moiety (δ187.2) and the acetate function (δ170.0), respectively. Comparison with the NMR data of the side chain of compound **3** together with the information given by 2D homo- and heteronuclear NMR experiments led us to propose that **5** was the quinone derivative from **3**. The signals of the side chain affected by the presence of the benzoquinone moiety were C-1, C-2 and C-3 (^{13}C NMR, Table 3), and H-1, H-2 and H-20 (^1H NMR, Table 3), respectively. The correlations observed in the ^1H–^{13}C one bond (HCCORR) and long range by inverse detection (HMBC) for **5** are summarized in Table 4. These data permitted the unambiguous assignment of all carbon signals as well as the substitution pattern of the quinonic ring.

Geographical variations in C. crinita

We have now started a study of the geographical variation in the meroditerpenoid composition from *C. crinita* (Desfontaines) Duby. For this purpose, the alga

Table 3. NMR data for compounds **5** and **6**

	5		**6***	
Position	^{13}C NMR† 50 MHz (C_6D_6)	^{1}H NMR 200 MHz (C_6D_6)	^{13}C NMR 62.9 MHz ($CDCl_3$)	^{1}H NMR 250 MHz ($CDCl_3$)
1′	187.2	—	150.3	—
2′	147.5	—	134.8	—
3′	132.1	6.29 *dt* (2.6, 1.8)	112.7	6.54 *br s*
4′	187.2	—	155.5	—
5′	133.0	6.08 *dq* (2.6, 1.7)	113.7	6.54 *br s*
6′	145.5	—	131.6	—
Me 6′	15.5	1.58 *d* (1.2)	16.3	2.23 *s*
OMe 1′		—	60.2	3.66 *s*
OMe 4′		—	55.2	3.72 *s*
1	27.7	2.94 *br d* (7.3)	28.6	3.35 *d* (7)
2	122.0	5.10 *tq* (7.3, 1.1)	126.3	5.37 *t* (7)
3	135.4	—	132.6	—
4	45.6	2.41 *dd* (13.4, 7.2) 2.19 *dd* (13.4, 6.2)	47.8	2.17 *m*
5	69.1	5.91 *ddd* (8.9, 7.2, 6.2)	66.2	4.45 *ddd* (8.5, 8, 5.2)
6	124.2	5.23 *dq* (8.9, 1.2)	128.5	5.14 *d* (7.5)
7	140.2	—	137.1	—
8	39.2	2.04 *m*	38.9	2.03 *m*
9	25.9	2.17 *m*	25.3	218 *m*/2.05 *m*
10	124.0	5.59 *t* (7.6)	124.4	5.50 *t* (7)
11	136.8	—	136.4	—
12	79.7	4.22 *t* (7.3)	79.7	4.30 *t* (7)
13	39.9	2.10 *m*/1.75 *m*	39.1	2.40/1.80 *m*
14	78.2	3.66 *dd* (6.2, 6.3)	78.3	3.88 *br t* (3.5)
15	82.6	—	83.0	—
16	26.0	1.08 *s*	26.0	1.18 *s*
17	22.8	1.27 *s*	22.5	1.29 *s*
18	12.2	1.61 *d* (1.7)	12.3	1.59 *s*
19	16.8	1.70 *d* (1.1)	16.0	1.61 *s*
20	16.2	1.54 *d* (1.6)	16.4	1.76 *s*
Ac C=O	170.0	—	—	—
Ac Me	20.9	1.83 *s*	—	—

*^{13}C and ^{1}H NMR data of ref. [7] added for comparison with those of compounds **3** and **4**.
†^{13}C assignments are based on a HCCORR experiment.

was collected off the French Riviera coast from Le Brusc (Six-Fours) to Boulouris (Saint Raphaël) at five separate locations: Le Brusc, Le Mourillon (Toulon), Les Gorges du Loup (Porquerolles Island), Les Issambres (Roquebrune sur Argens) and Boulouris. The different collections were treated and extracted in an identical fashion. Preliminary examination of the ether extract from each collection by TLC revealed that all samples possessed the usual mixtures of lipids, pigments, fatty acids and sterols. In addition to these compounds, extracts of *C. crinita* contained several meroditerpenoids (R_f 0.24–0.53 in ethyl acetate–isooctane, 3:2). The ether extracts were analysed by semi-preparative HPLC (ethyl acetate–isooctane, 3:2) for the presence of compounds **3**–**5**. Moreover, a precise determination of **3** and **4** was achieved by analytical normal-phase HPLC (ethyl acetate–isooctane, 1:1) for the alga collected in August 1992 at Le Brusc. The concentrations of metabolites calculated as mg per g of alga dry weight were 0.42 and 0.14 for **3** and **4**, respectively.

This study showed that the geographical variation in the meroditerpenoid composition from the French Riviera variety was not significant because the major metabolites were **3** and **4** for all locations studied, except for Le Mourillon and Les Issambres where they are replaced by **5**. But, as this last compound is the quinone derivative from **4** and as **3** is its methoxy derivative, we can consider that the French Riviera variety of *C. crinita* is characterized by chemical structure **4** and its derivatives (**3** and **5**). So, *C. zosteroides*, with zosterdiol A (**6**) as the major meroditerpenoid constituent from its lipid extract is chemically related to this *Cystoseira* species. By contrast, the Sicilian variety, which is characterized by structure **1** seems to constitute a second 'chemical variety' of *C. crinita*. A study of species collected off the coast of other Mediterranean countries (i.e. Italy, Spain, North Africa),

Table 4. Long-range 2D ^1H–^{13}C correlations for compound **5**

C	δ(in C_6D_6)	Type	Long-range connected proton (HMBC in C_6D_6)
1′	187.2	C=O	Me 6′
2′	147.5	C	1
3′	132.1	CH	1
4′	187.2	C=O	
5′	133.0	CH	Me 6′
6′	145.5	C	Me 6′
Me 6′	15.5	CH_3	
1	27.7	CH_2	
2	122.0	CH	1, 20
3	135.4	C	1, 20
4	45.6	CH_2	2, 20
5	69.1	CH	
6	124.2	CH	19
7	140.2	C	19
8	39.2	CH_2	19
9	25.9	CH_2	8
10	124.0	CH	12, 18
11	136.8	C	18
12	79.7	CH	14, 18
13	39.9	CH_2	
14	78.2	CH	16, 17
15	82.6	C–O	16, 17
16	26.0	CH_3	14, 17
17	22.8	CH_3	16
18	12.2	CH_3	12
19	16.8	CH_3	6
20	16.2	CH_3	2, 4
Ac C=O	170.0	C=O	Ac Me
Ac Me	20.9	CH_3	

must now be undertaken to verify the possible presence of any other chemical varieties of *C. crinita*.

EXPERIMENTAL

General. MS: direct inlet, 70 eV; ^1H NMR: 200 and 400 MHz; ^{13}C NMR: 50 and 100 MHz. Chemical shifts are quoted in ppm (δ) relative to TMS and coupling constants are in Hertz. Final purification of all metabolites was achieved by HPLC on silica gel (Lichrosorb Si-60, 5 μm), with RI monitoring.

Plant material. Cystoseira crinita (Desfontaines) Duby was collected near Toulon, France in August 1991 and 1992 for isolation of compounds **3**–**5**. A voucher specimen of this species is deposited in the Herbarium of Dr Pellegrini, Laboratoire de Biologie Marine Fondamentale et Appliquée, University of Marseille II, France.

Extraction and purification. The shade-dried material from the Le Brusc collection (3000 g) was ground and extracted with Et_2O at room temp. After filtration and evaporation of solvent, 13 g of a crude extract were obtained and subjected to CC on silica gel eluted with a solvent gradient from hexane to Et_2O. The two new compounds, **3** and **4**, were eluted with hexane–Et_2O (2:3) and (3:7), respectively. They were subsequently purified by semi-preparative normal phase HPLC (EtOAc–isooctane, 2:3 to give 300 mg **3** and 50 mg **4**.

In the same way, the shade-dried material from Le Mourillon collection (1000 g) gave 3.5 g of a crude extract which was subjected to CC on silica gel. The fraction eluted with hexane–Et_2O (2:3) was purified by HPLC (EtOAc–isooctane, 2:3) to give 62 mg **5**.

Compound **3**. Oil; $[\alpha]_D^{25}$ 0.6° (CH_2Cl_2; *c* 2.0); IR ν_{max}^{film} cm^{-1}: 3400, 3050, 2900, 1730, 1655, 1600, 1470, 1380, 1240, 1150, 1060, 1020, 857; UV λ_{max}^{MeOH} nm (ε): 210 (15220), 290 (2800); HRMS: $[M]^+$ 500.3142 (calc. for $C_{30}H_{44}O_6$, 500.3138); EIMS (70 eV) *m/z* (rel. int.): 500 (3), 440 (5), 271 (8), 229 (5), 191 (55), 189 (70), 175 (15), 151 (100), 121 (17), 71 (31), 55 (34), 43 (66); ^{13}C NMR (50 MHz, $CDCl_3$): δ12.1 (C-18), 16.3 (Me 6′), 16.5 (C-20), 16.8 (C-19), 21.1 (AcMe), 22.5 (C-17), 25.7 (C-9), 25.8 (C-16), 30.5 (C-1), 38.9 (C-8), 39.4 (C-13), 45.4 (C-4), 55.6 (OMe4′), 69.5 (C-5), 78.3 (C-14), 79.8 (C-12), 82.7 (C-15), 113.0 (C-3′), 113.9 (C-5′), 123.3 (C-6), 124.9 (C-10), 125.5 (C-2), 125.9 (C-6′), 127.8 (C-2′), 133.5 (C-3), 135.9 (C-11), 140.4 (C-7), 146.5 (C-1′), 153.2 (C-4′), 170.7 (AcC=O); ^1H NMR (200 MHz, $CDCl_3$): δ1.20 (3H, *s*, H-16), 1.27 (3H, *s*, H-17), 1.61 (3H, *s*, H-18), 1.64 (1H, *m*, H_a-13), 1.70 (3H, *s*, H-19), 1.81 (3H, *s*, H-20), 1.93 (3H, *s*, AcMe), 2.02 (2H, *m*, H-8), 2.05 (2H, *m*, H-9), 2.17 (3H, *s*, H_a-4), 2.20 (3H, *s*, Me-6′), 2.35 (1H, *m*, H_b-13), 2.39 (1H, *m*, H_b-4), 3.22 (1H, *dd*, *J* = 15.3 and 6.9 Hz, H_a-1), 3.36 (1H, *dd*, *J* = 15.3 and 7 Hz, H_b-1), 3.73 (3H, *s*, OMe4′), 3.95 (1H, *dd*, *J* = 5.8 and 6 Hz, H-14), 4.29 (1H, *br t*, *J* = 7.3 Hz, H-12), 5.10 (1H, *dq*, *J* = 8.8 and 1.5 Hz, H-6), 5.32 (1H, *dd*, *J* = 6.9 and 7 Hz, H-2), 5.46 (1H, *br t*, *J* = 7.7 Hz, H-10), 5.66 (1H, *ddd*, *J* = 8.8, 8 and 5.9 Hz, H-5), 6.50 (1H, *d*, *J* = 2.9 Hz, H-3′), 6.55 (1H, *d*, *J* = 2.9 Hz, H-5′); ^1H and ^{13}C NMR in C_6D_6: Table 1.

Compound **4**. Oil; $[\alpha]_D^{25}$ 0.7° (CH_2Cl_2; *c* 1.6); IR ν_{max}^{film} cm^{-1}: 3400, 3050, 2900, 1730, 1655, 1600, 1470, 1380, 1240, 1150, 1020, 857; UV λ_{max}^{MeOH} nm (ε): 212 (13650), 290 (3440); HRMS: $[M]^+$ 486.2984 (calc. for $C_{29}H_{42}O_6$, 486.2981); EIMS (70 eV) *m/z* (rel. int.): 486 (3), 426 (5), 408 (3), 257 (10), 189 (8), 177 (80), 175 (30), 137 (100), 121 (28), 81 (20), 55 (42), 43 (65); ^{13}C NMR (100 MHz, $CDCl_3$): δ12.0 (C-18), 16.2 (C-20), 16.3 (Me 6′), 16.7 (C-19), 21.3 (AcMe), 22.5 (C-17), 25.6 (C-9), 25.7 (C-16), 29.6 (C-1), 38.8 (C-8), 39.3 (C-13), 40.3 (C-4), 69.8 (C-5), 78.4 (C-14), 79.9 (C-12), 82.9 (C-15), 113.9 (C-3′), 115.4 (C-5′), 123.2 (C-6), 124.9 (C-10), 125.4 (C-2), 125.7 (C-6′), 127.6 (C-2′), 133.5 (C-3), 135.8 (C-11), 140.4 (C-7), 145.9 (C-1′), 149.2 (C-4′), 171.0 (AcC=O); ^1H NMR (400 MHz, $CDCl_3$): δ1.19 (3H, *s*, H-16), 1.27 (3H, *s*, H-17), 1.58 (3H, *s*, H-18), 1.68 (3H, *s*, H-19), 1.77 (3H, *s*, H-20), 1.80 (1H, *m*, H_a-13), 1.92 (3H, *s*, AcMe), 2.07 (2H, *t*, *J* = 7 Hz, H-8), 2.11 (1H, *m*, H_a-4), 2.16 (3H, *s*, Me-6′), 2.20 (2H, *m*, H-9), 2.25 (1H, *m*, H_b-13), 2.36 (1H, *m*, H_b-4), 3.20 (1H, *dd*, *J* = 15.3 and 7 Hz, H_a-1), 3.27 (1H, *dd*, *J* = 15.3 and 7 Hz, H_a-2), 3.94 (1H, *br t*, *J* = 6 Hz, H-14), 4.28 (1H, *br t*, *J* = 7.1 Hz, H-12), 5.07 (1H, *dq*, *J* = 8.3 and 1.3 Hz, H-6), 5.29 (1H, *dd*, *J* = 7 and 7.2 Hz, H-2), 5.45 (1H, *br t*, *J* = 7.1 Hz, H-10), 5.67 (1H, *ddd*, *J* = 8.3, 8.0 and 5.8 Hz, H-5), 6.39 (1H, *d*, *J* = 3 Hz, H-5′), 6.46 (1H, *d*, *J* = 3 Hz, H-3′); ^1H and ^{13}C NMR in C_6D_6: Table 1.

Compound **5**. Oil; $[\alpha]_D^{25}$ 0.8° (CH_2Cl_2; *c* 1.8); IR ν_{max}^{film} cm^{-1}: 3400, 3050, 2900, 1730, 1650, 1640, 1610, 1470, 1380, 1240, 1150, 1020, 900; UV λ_{max}^{MeOH} nm (ε): 205 (17350), 252 (4380); HRMS: $[M]^+$ 484.2830 (calc. for $C_{29}H_{40}O_6$, 484.2825); EIMS (70 eV) *m/z* (rel. int.): 484 (3), 424 (5), 256 (8), 177 (30), 175 (50), 137 (48), 71 (49), 55 (44), 43 (100); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1 (C-18), 16.0 (Me 6'), 16.4 (C-20), 16.8 (C-19), 21.3 (AcMe), 22.5 (C-17), 25.8 (C-16), 25.8 (C-9), 27.6 (C-1), 38.9 (C-8), 39.4 (C-13), 45.3 (C-4), 69.4 (C-5), 78.3 (C-14), 79.8 (C-12), 82.7 (C-15), 121.5 (C-2), 123.3 (C-10), 124.8 (C-6), 132.2 (C-3'), 133.2 (C-5'), 135.6 (C-3), 135.9 (C-11), 140.0 (C-7), 145.9 (C-6'), 147.9 (C-2'), 170.1 (AcC=O), 187.8 (C-1' and C-4'); ^1H NMR (400 MHz, $CDCl_3$): δ 1.21 (3H, *s*, H-16), 1.28 (3H, *s*, H-17), 1.62 (3H, *br s*, H-18), 1.65 (1H, *m*, H_a-13), 1.67 (3H, *br s*, H-20), 1.71 (3H, *br s*, H-19), 2.02 (3H, *s*, AcMe), 2.03 (2H, *m*, H-8), 2.06 (3H, *d*, *J* = 1.2 Hz, Me 6'), 2.11 (1H, *m*, H_a-4), 2.18 (2H, *m*, H-9), 2.20 (1H, *m*, H_b-13), 2.28 (1H, *m*, H_b-4), 3.12 (2H, *d*, *J* = 7.2 Hz, H-1), 3.96 (1H, *dd*, *J* = 6.2 and 6.3 Hz, H-14), 4.30 (1H, *t*, *J* = 7.3 Hz, H-12), 5.11 (1H, *br d*, *J* = 8.8 Hz, H-6), 5.21 (1H, *br t*, *J* = 7 Hz, H-2), 5.50 (1H, *br t*, *J* = 7.6 Hz, H-10), 5.66 (1H, *ddd*, *J* = 8.8, 7.3 and 6.1 Hz, H-5), 6.43 (1H, *dt*, *J* = 2.6, and 1.8 Hz, H-3'), 6.55 (1H, *dq*, *J* = 2.6 and 1.7 Hz, H-5'); ^1H and ^{13}C NMR in C_6D_6: Table 3.

HPLC analysis of compounds **3** *and* **4**. The method previously described for the determination of sterols and diterpenoids from Cystoseiraceae [1] was used. In this study, the internal standard was replaced by 4-hydroxybenzaldehyde and UV detection was used.

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