Pergamon

0031-9422(95)00750-4

Phytochemistry, Vol. 41, No. 5, pp. 1367–1371, 1996

0031-9422/96 $15.00 + 0.00

MERODITERPENES FROM THE BROWN ALGA *CYSTOSEIRA AMENTACEA* VAR. *STRICTA* COLLECTED OFF THE FRENCH MEDITERRANEAN COAST

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(*Received in revised form* 13 *September* 1995)

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

Abstract—Two new cystoketal derivatives demethoxy cystoketal chromane and cystoketal quinone have been isolated from the brown alga *Cystoseira amentacea* var. *stricta* collected off the French Riviera coast. These meroditerpenes were characterized by spectral methods and chemical reactions. They were also quantitatively analysed and the results were discussed with available data in the literature on the same species collected from Sicily (Italy).

INTRODUCTION

In the course of our continuing phytochemical investigation of the marine family Cystoseiraceae [1–5], we have recently investigated the lipid extract from *Cystoseira amentacea* var. *stricta*, a widespread Mediterranean seaweed which was collected off the French Riviera coast from Sausset les Pins near Marseille to Saint-Raphaël. The same species collected off the Sicilian coast has been widely studied [6–12]. Many tetraprenyltoluquinols have been isolated, either with a regular diterpenoid moiety, such as: balearone (**1**) [6], cystoketal (**2**) [7], cystoketal chromane (**3**) [7], amentol (**4**) [8], strictaepoxide (**5**) [8], strictaketal (**6**) [9], isocystoketal (**7**) [10], isostrictaketal (**8**) [10], isobalearone (**9**) [10], (2*E*)-bifurcarenone (**10**) [11], amentaepoxide (**11**) [11] and amentadione (**12**) [11], or with a rearranged one, such as: neobalearone (**13**) [12] and 2-epi-neobalearone (**14**) [12]. Similarly, this species collected near Nice (French Riviera) contains the rearranged meroditerpenes: mediterraneols [13, 14], e.g., mediterraneol A (**15**) and cystoseirols [15, 16], e.g., cystoseirol D (**16**).

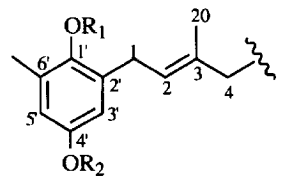
To our knowledge, no data have been reported about the metabolites from *C. amentacea* var. *stricta* collected on the western part of the French Riviera. In this paper, we describe the isolation and structure elucidation of two new meroditerpenes (**17**, **18**) from this alga which were present together with previously studied compounds **2** and **3** in the lipid extract. Their quantitative determination is also reported.

RESULTS AND DISCUSSION

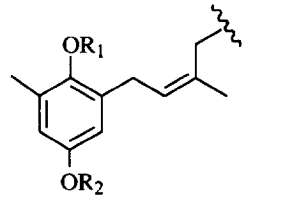
HPLC of the less polar fraction of the lipid extract of *C. amentacea* var. *stricta* collected on the western part of the French Riviera (0.7% dry wt of the alga in March 1992) revealed the presence of two minor components (**17**, **18**) in addition to sterols and the previously described meroditerpenes **2** and **3** [7].

Compound **17**, $C_{27}H_{36}O_4$ (HR-mass spectrometry), was obtained as an epimeric mixture at C-3. The IR spectrum revealed the presence of a phenolic function (3400, 1680 and 1610 cm^{-1}), while UV absorption at 296 nm (ε 3000) was indicative of a chromane chromophore. In the same way, the mass spectrum indicated a chromane ring with fragments at *m/z* 177 (74%), 150 (base) and 137 (36%) and suggested that **17** could be a chromane derivative of **2**.

The most significant variations in the ^1H NMR spectrum of **17** in comparison with that of **2** (Table 1) are the absence of the methoxyl signal at δ 3.75, the replacement of the ABX system corresponding to the protons at C-1 and C-2 by two 2 H multiplets at δ 1.70 and 2.51, the shift of the C-4 methylene signal from a doubly-allylic to an allylic position (δ 2.37) and the upfield shift of the Me-3 signal (δ 1.44). The ^{13}C NMR data (Table 2) confirmed the close relationship with cystoketal (**2**) at the level of C-5 to C-19 in the diterpenoid chain [7]. The observed doubleting of the signals belonging to the methylene carbons C-2 (31.5 and 31.8 ppm) and C-4 (44.4 and 44.6 ppm) and to the methyl carbon C-20 (25.5 and 25.7 ppm) is caused by stereoisomerism about the chiral



a - R_1 = H R_2 = H
b - R_1 = H R_2 = CH_3



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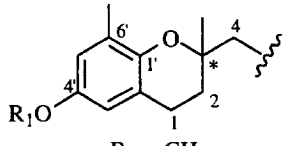
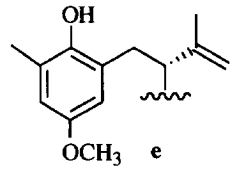


c - R_1 = CH_3
d - R_1 = H



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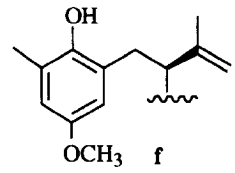

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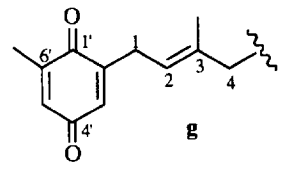
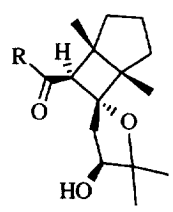
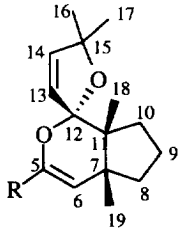


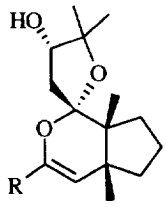
g



1 R = b **13** R = e
9 R = b' **14** R = f



2 R = b **7** R = b'
3 R = c **17** R = d
18 R = g



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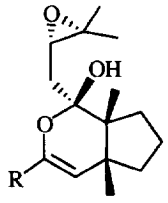

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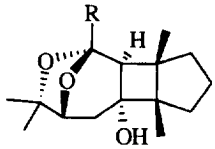
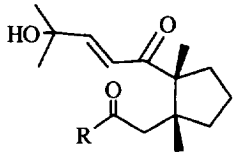
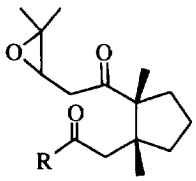


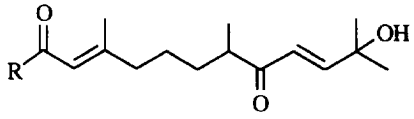
6 R = b **8** R = b'



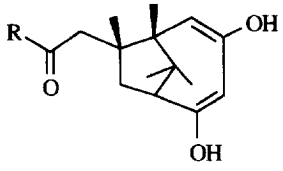
10 R = a



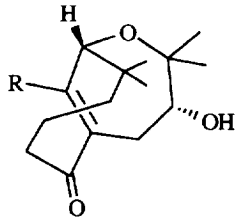
11 R = a



12 R = a



15 R = a



16 R = a

centre at C-3. All these data confirmed that **17** was the 4'-demethoxy chromane derivative of **2**. The assignments of the carbon and proton signals were confirmed by means of homonuclear (COSY ^1H–^1H) and heteronuclear (HCCORR) 2D NMR experiments. In particular, the methyl groups were precisely located leading us to revise their positions in ref. [7] (Tables 1 and 2). Moreover, the relative stereochemistry of the chiral centres at C-7, C-11 and C-12 was deduced from a homonuclear ^1H–^1H ROESY experiment which showed the C-18 and C-19 angular methyls, and the olefinic proton at C-13 to be on the same face of the molecule, as for **2** [7]. This deduction was based on the correlations observed between the C-18 methyl protons and H-19, H-13, H-16, respectively, and between the C-19 methyl protons and H-18, H-13, respectively.

To further confirm this structural hypothesis, compound **17** was treated with methyl iodide in the presence of potassium carbonate. This methylation gave a substance identical in all respects with cystoketal chromane (**3**) [7].

Table 1. ^{1}H NMR spectral data of compounds **2**, **17** and **18** (TMS as int. standard)*

H	**2** 250 MHz† ($CDCl_3$)	**17** 400 MHz (C_6D_6)	**17** 400 MHz ($CDCl_3$)	**18** 200 MHz (C_6D_6)	**18** 200 MHz ($CDCl_3$)
3′	6.58 } AB (3)	6.45 } AB (3)	6.55 } AB (3)	6.60 *dt* (2.6, 1.8)	6.45 *dt* (2.6, 2)
5′	6.51 }	6.35 }	6.42 }	6.20 *dq* (2.6, 1.7)	6.54 *dq* (2.6, 2)
1	3.24 *dd* (16, 6.3) 3.43 *dd* (16, 7.7)	2.51 *m*	2.71 *m*	3.10 *d* (7.3)	3.13 *d* (7)
2	5.38 *t* (7)	1.70 *m* 1.83 *m*	1.72 *m*	5.30 *t* (7.3)	5.16 *t* (7)
4	2.69 } AB (15) 2.74 }	2.37 *s*	2.29 *brs*	2.80 *s*	2.65 } AB (15) 2.75 }
6	4.35 *s*	4.30 *s*	4.28 *s*	4.40 *s*	4.30 *s*
8	1.57 *m*	1.49 *m* 1.67 *m*	1.67 *m*	2.2 *m* 2.3 *m*	1.55 *m*
9	1.69 *m*	1.70 *m*	1.70 *m*	2.3 *m*	1.67 *m*
10	1.37 *m*	1.27 *m*	1.40 *m*	2.1 *m*	1.37 *m*
13	6.02 } AB (5.5)	5.63 } AB (5.7)	6.01 } AB (5.6)	5.75 *brs*	6.05 } AB (5.5)
14	5.65 }	5.43 }	5.60 }		5.69 }
16	1.32 *s*	1.32 *s*	1.35 *s*	1.47 *s*	1.43 *s*
17	1.28 *s*	1.31 *s*	1.30 *s*	1.40 *s*	1.37 *s*
18	0.89 *s*‡	1.17 *s*	1.13 *s*	1.30 *s*	1.17 *s*
19	1.15 *s*‡	0.91 *s*	0.88 *s*	1.00 *s*	1.11 *s*
20	1.76 *s*	1.44 *s*	1.39 *s*	1.60 *s*	1.63 *s*
Me-6′	2.21 *s*	2.27 *s*	2.15 *s*	1.65 *s*	1.56 *s*
OMe-4′	3.75 *s*	—	—	—	—
OH	4.88 *s*	4.65 *s*	3.68	—	—

*Chemical shifts are δ values; coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling and 2D NMR experiments (COSY ^{1}H–^{1}H, HCCORR).

†^{1}H NMR data of ref. [7] added for comparison.

‡These values could be exchanged.

Compound **18**, $C_{27}H_{34}O_4$ (HR-mass spectrometry), was an optically active oil. UV absorption at 263 nm ($\varepsilon = 16465$) indicated a *p*-benzoquinone moiety [17], which was confirmed by IR bands at 1656, 1650 and 1614 cm^{-1}, and by MS fragments at *m/z* 175 and 137. In the ^{1}H NMR spectrum (Table 1) H-5′ appeared at δ6.20 as a *dq* ($J = 2.6$, 1.7 Hz) due to long-range couplings with H-3′ and 6′-Me, while H-3′ (δ 6.60) 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 δ5.30 leading to a *d* ($J = 7.3$ Hz) at δ 3.10.

The ^{13}C NMR spectrum of this compound (Table 2) showed peaks corresponding to 27 carbon atoms (in agreement with the mass spectrometry data). Their multiplicities were determined by DEPT sequence as six methyl groups, five methylenes, six sp^2 methynes (–CH=C) and ten quaternary carbons: four sp^3 (two of them being oxygen-bonded at δ 88.0 and 115.4), two aromatics, two olefinics (one of them being oxygen-bonded at δ 145.3) and two carbonyls corresponding to the benzoquinone moiety (δ 187.5). The comparison with NMR data of the side chain of compound **2** [7] together with the information given by 2D homo- and heteronuclear NMR experiments led us to propose that **18** was the quinone derivative of **2** and to name this new metabolite: cystoquinone. The signals of the side chain affected by the presence of the benzoquinone moiety were C-1, C-2, C-3 (^{13}C NMR, Table 2) and H-1, H-2, H-20, H-13, H-14 (^{1}H NMR, Table 1), respectively.

It must be pointed out that the natural precursor of compounds **2**, **3**, **17** and **18** might be the 4′-demethoxy-cystoketal which has not yet been described in the literature.

Quantitative analysis–chemotaxonomy

To complete the phytochemical study of *C. amentacea* var. *stricta* collected on the western part of the French Riviera, we have determined the concentration of the less polar meroditerpenoids from its lipid extract. For this purpose, the alga was collected from Marseille (Sausset les Pins) to Saint-Raphaël (Le Trayas) at separate locations. The different collections were treated and extracted in an identical fashion and each ether extract was analysed by normal-phase HPLC (ethyl acetate-isooctane, 1:4).

In each collection studied, compounds **2**, **3**, **17** and **18** were present showing that **2** (cystoketal), the main compound previously isolated from a Sicily collection [7] can be regarded as a chemotaxonomic marker of the species. We think that the study of meroditerpenoids contained in the more polar fractions of the lipid extract must now be undertaken and the results compared with the data available in the literature to verify the possible presence of any other chemical markers.

Table 2. ^{13}C NMR spectral data of compounds **2**, **17** and **18** (TMS as int. standard)

C	2 (62.5 MHz)† $CDCl_3$		**17** (100 MHz) C_6D_6	**17** (100 MHz) $CDCl_3$		**18** (50 MHz) C_6D_6	**18** (50 MHz) $CDCl_3$	
1′	146.5	C	146.1	145.8	C	187.5	188.0	C=O
2′	136.0	C	121.3	121.1	C	147.9	148.3	C
3′	114.0	CH	116.3	114.3	CH	132.5	132.4	CH
4′	153.3	C	149.1	151.9	C	187.5	187.9	C=O
5′	113.0	CH	113.2	110.8	CH	133.2	133.3	CH
6′	127.0	C	128.0	127.0	C	146.8	146.2	C
1	30.8	CH_2	22.9	22.3	CH_2	27.8	27.7	CH_2
2	123.8	CH	31.5/31.8	30.8/31.1	CH	120.7	119.0	CH
3	126.0	C	75.7	75.4	C	136.9	138.3	C
4	45.1	CH_2	44.4/44.6	43.7/44.0	CH_2	45.5	40.1	CH_2
5	147.1	C	151.9	146.5	C	145.3	147.0	C
6	109.0	CH	111.2	111.0	CH	109.4	109.5	CH
7	43.2	C	43.4	43.0	C	43.5	43.2	C
8	40.5	CH_2	40.9	40.3	CH_2	40.9	40.4	CH_2
9	20.4	CH_2	20.7	20.1	CH_2	20.8	20.4	CH_2
10	36.1	CH_2	36.5	35.9	CH_2	36.5	36.2	CH_2
11	46.3	C	46.3	46.1	C	46.6	46.2	C
12	115.0	C	115.3	114.9	C	115.4	115.1	C
13	140.0	CH	139.9	139.7	CH	140.1	140.0	CH
14	126.8	CH	126.9	126.1	CH	127.3	127.0	CH
15	88.0	C	88.2	87.9	C	88.0	88.0	C
16	26.3‡	CH_3	22.9	22.5	CH_3	23.1	22.8	CH_3
17	28.7	CH_3	28.8	28.6	CH_3	28.9	28.8	CH_3
18	20.2‡	CH_3	26.5	26.2	CH_3	26.4	26.3	CH_3
19	22.7‡	CH_3	20.3	20.0	CH_3	20.3	20.3	CH_3
20	16.5	CH_3	25.5/25.7	24.4/24.7	CH_3	26.4	16.2	CH_3
Me-6′	16.2	CH_3	16.5	16.0	CH_3	15.6	—	CH_3
OMe-4′	53.6	CH_3	—	—	—	—	—	—

†^{13}C NMR data of ref. [7] added for comparison.
‡These values could be exchanged.

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 Hz. Final purification of all metabolites was achieved by HPLC on silica gel (Intersphere Si-60, 5 μm), with RI monitoring.

Plant material. C. amentacea Bory var. *stricta* Montagne was collected in March 1992 at Sausset les Pins (Marseille, France) for isolation of compounds **2**, **3**, **17**, **18** and from Marseille to Saint-Raphaël at separate locations (Sausset les Pins, Le Brusc, Toulon, Carqueiranne, Cap Cartaya, Saint-Aygulf, Boulouris, Le Trayas) for the geographical variation study. 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 (255 g) was ground and extracted with Et_2O at room temp. After filtration and evaporation of solvent, 1.785 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 compounds **2**, **3**, **17** and **18** were eluted with hexane–Et_2O (4:1). They were subsequently purified by semi-prep normal phase HPLC (EtOAc–isooctane, 1:4) to give 382 mg **2**, 230 mg **3**, 120 mg **17** and 40 mg **18**. The known compounds **2** and **3** were identified by comparison of their spectral properties (IR, UV, mass spectrometry, ^1H and ^{13}C NMR) and optical rotations with those of reference samples available from previous work on *C. amentacea* var. *stricta* collected off the Sicilian coast [7].

Compound **17**. Oil; IR ν_{max}^{film} cm^{-1}: 3400, 2930, 1680, 1610, 1470, 1380, 1340, 1270, 1220, 1106, 1080, 1050, 980, 930, 854; UV λ_{max}^{EtOH} nm (ε): 208 (22360), 296 (3000); HRMS: $[M]^+$ 424.2617 (calc. for $C_{27}H_{36}O_4$, 424.2613); EIMS (70 eV) *m/z* (rel. int.): 424 (36), 275 (11), 274 (42), 256 (7), 216 (10), 190 (13), 177 (74), 175 (19), 150 (100), 151 (41), 137 (36), 135 (23), 109 (16), 95 (24), 81 (16), 67 (21), 55 (19); ^1H and ^{13}C NMR: Tables 1 and 2.

Methylation of compound **17**. 38.8 g of **17** dissolved in Me_2CO (5 ml) were subjected to methylation with MeI (1 ml) in the presence of K_2CO_3 (0.5 g). The ppt. was filtered off and the soln evaporated. The residue was purified by semi-prep HPLC (EtOAc–isooctane, 1:9) to give 10 mg of a pure compound identical in all respects with **3** [7].

Compound **18**. Oil; $[\alpha]_D^{25} = 11.2°$ (CH_2Cl_2; *c* 1.3); IR ν_{max}^{film} cm^{-1}: 3000, 1656, 1650, 1614, 1450, 1380, 1294, 1194,

1106, 1082, 1048, 980, 912, 856, 798; UV λ_{max}^{EtOH} nm (ε): 215 (9697), 263 (16465); HRMS: $[M]^+$ 422.2460 (calc. for $C_{27}H_{34}O_4$, 422.2457); EIMS (70 eV) *m/z* (rel. int.): 422 (3), 221 (17), 207 (21), 177 (18), 175 (11), 150 (28), 137 (21), 109 (21), 96 (46), 81 (40), 69 (48), 55 (43), 43 (100); ^1H and ^{13}C NMR: Tables 1 and 2.

HPLC analysis of compounds **2**, **3**, **17** *and* **18**. The method previously described for the determination of sterols and diterpenoids from Cystoseiraiceae [1] was used with RI monitoring and 2-methylbutan-2-ol as internal standard.

Acknowledgement—The authors wish to thank Dr M. Pellegrini (University of Marseille II, France) for the classification of plant material.

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