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NOVEL METABOLITES FROM THE MARINE GENUS *CYSTOSEIRA* - APPLICATION OF TWO-DIMENSIONAL ^{1}H-^{13}C CORRELATION TO THE STRUCTURE ELUCIDATION

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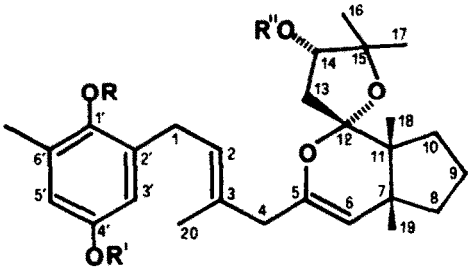
Abstract - Three novel metabolites of mixed biogenesis have been isolated from brown algae of the genus *Cystoseira*. Their structures have been determined by chemical transformations and spectral analysis, including 2D NMR spectroscopy.

In the course of our previous investigations on Mediterranean seaweeds for compounds with potential pharmacological activity, we have reported the isolation, from species belonging to the genus *Cystoseira*, of a number of tetraprenyl-toluquinol derivatives in which the terpenoid component has been variously functionalized to give open-chain, mono- and polycyclic structures.[1-4] The present paper deals with the structure determination of three novel representatives of this class of metabolites.

Amentol **1**, $C_{27}H_{38}O_5$ (*m/z* 442.2715, calc. 422.2719), was isolated from *Cystoseira stricta* var. *amentacea* Bory as an optically active oil, $[\alpha]_D = +7.3°$. It shows in the IR spectrum a band consistent with the presence of hydroxyl(s) (film, 3390 cm^{-1}), while the UV spectrum has absorptions at 290 (ε=3300) and 220 nm (ε=10400) indicative of a hydroquinol chromophore. The ^{13}C NMR spectrum (Table 1) contains, besides the resonances for a tetrasubstituted aromatic ring, six quaternary carbons, three methines, six methylenes and six methyls. The ^{1}H NMR spectrum (Table 1) of **1** closely resembles that of a previously isolated *Cystoseira* metabolite, cystoketal **2**,[5] the main difference consisting in the absence of the AB pattern associated with the olefinic protons in the dihydrofuran ring, which is replaced by an ABX system [δ 2.37, dd (J=13 and 7.5 Hz), 2.10, dd (J=13 and 7.5 Hz) and 4.33, t (J=7.5 Hz); the last signal was shifted to δ 5.13 in the triacetate **3**] assignable to a $-CH_2-CHOH-$ fragment. These data suggested structure **1** (devoid of stereochemistry) for amentol, in which location of the secondary hydroxyl at C-14 rather then C-13, more plausible on biogenetic grounds, resulted from the presence of nOe interaction between the geminal methyls at C-15 and

Table 1. ^{1}H and ^{13}C NMR data for amentol (1) and amentol 1'-methyl ether (4) (see Experimental section for details)

Position	Amentol (1)			Amentol monomethyl ether (4)	
	δ_C	δ_H(J)	H/C long-range correlation	δ_C	δ_H(J)
1'	146.4s		H-3', H-5', Me-6'	150.6s	
2'	127.8s		2H-1	132.1s	
3'	114.2d	6.50d (3)	H-5'	114.3d	6.47d (3)
4'	148.9s		H-3', H-5'	151.8s	
5'	115.6d	6.53d (3)	H-3', Me-6'	115.6d	6.56d (3)
6'	125.8s		Me-6'	133.9s	
1	30.1t	H_a 3.42dd (16, 8) H_b 3.19dd (16, 6)	H-3'	27.9t	3.33d (7.5)
2	123.9d	5.36dd (8, 6)	2H-4, 3H-20	125.0d	5.33t (7.5)
3	135.6s		2H-4, 3H-20	135.9s	
4	45.3t	2.69s	H-6, 3H-20	45.2t	2.65s
5	145.1s		2H-4, H-6	145.9s	
6	109.5d	4.33s	2H-4, 3H-19	109.1d	4.26s
7	43.3s		3H-18, 3H-19	43.3s	
8	40.4t	1.50-1.80m	3H-19	40.5t	1.45-1.75m
9	20.4t	1.55m	2H-10	20.4t	1.50m
10	36.2t	1.30m, 1.80m	3H-18	36.2t	1.30-1.80m
11	46.3s		H-6, 3H-18, 3H-19	46.3s	
12	109.1s		3H-18	108.9s	
13	41.7t	H_a 2.37dd (13, 7.5) H_b 2.10dd (13, 7.5)		41.7t	H_a 2.20 (overlap.) H_b 2.06dd (12, 7.5)
14	77.3d	4.33t (7.5)	H_b-13, 3H-16, 3H-17	77.4d	4.36t (7.5)
15	83.4s		3H-16, 3H-17	83.1s	
16	28.2q	1.22s	3H-17	28.2q	1.22s
17	22.3q	1.13s	3H-16	22.2q	1.12s
18	19.5q	1.00s		19.3q	0.98s
19	22.9q	1.10s		23.0q	1.08s
20	15.4q	1.72s	2H-4	15.4q	1.76s
Me-6'	16.1q	2.16s	H-5'	16.2q	2.22s
OMe				60.5q	3.66s

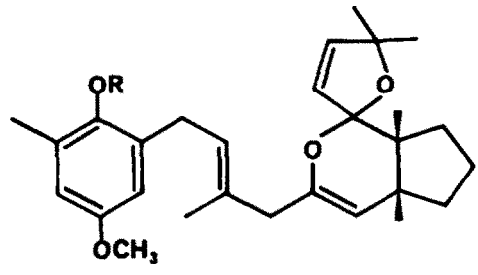


1 R = R' = R'' = H

3 R = R' = R'' = $COCH_3$

4 R = CH_3; R' = R'' = H

5 R = R' = CH_3; R'' = H



2 R = H

6 R = CH_3

H-14, but not the C-13 methylene protons (Table 2). Chemical correlation with cystoketal **2** supported the proposed structure; indeed, methylation of the phenolic hydroxyls gave the dimethyl ether **5**, which on subsequent dehydration afforded a compound (**6**) indistinguishable (UV, IR, MS, NMR, $[\alpha]$) from the methylation product of cystoketal. This result, in addition, settled the relative stereochemistry of all the chiral centres in **1**, but the hydroxymethine carbon, which was deduced from the observation that H-14 and 18-Me are within nOe distance. Therefore, stereostructure **1** appeared conclusively determined for amentol. However, a recent report by French workers on the isolation from *C. stricta* and closely related species (*C. mediterranea*, *C. tamariscifolia*) of a metabolite, cystoseirol A,[6] which possesses the very same ^{1}H NMR spectrum as that of a semisynthetic compound obtained from cystoketal by chemical conversion into the corresponding chromane[5] (significant differences are however observed in the ^{13}C NMR and UV spectra) although it contains a totally different, rearranged diterpenoid moiety, prompted a structure elucidation of **1** independent from chemical interrelation with **2**. One-bond ^{1}H-^{13}C correlation (Table 1) permitted the assignment of the protonated carbons in the ^{13}C NMR spectrum of **1**, while ^{1}H-^{13}C long-range correlation identified all the quaternary carbons and established bond connectivities across them, allowing to define the carbon skeleton. In particular, C-3 was seen to correlate with 2H-4 and 3H-20, C-5 with 2H-4 and H-6, C-7 with 3H-18 and 3H-19, C-11 with H-6, 3H-18 and 3H-19, C-12 with 3H-18, and C-15 with 3H-16 and 3H-17. Correlation of C-14 with H_b-13, 3H-16 and 3H-17 secured the location of the secondary hydroxyl. The *E* configuration of the C-2 double bond was indicated by the value of the resonance of the relevant vinyl methyl (15.4 ppm) in the ^{13}C NMR spectrum of **1**, while the relative stereochemistry of the chiral centres was deduced from the nOe data (Table 2).

Since in the meanwhile we had the occasion to reisolate a sufficient amount of cystoketal, we measured two-dimensional heteronuclear spectra, both one-bond and long-range, also for this compound. They were in full agreement with the previously proposed structure and permitted unambiguous assignment of all the ^{13}C resonances (see Experimental section).

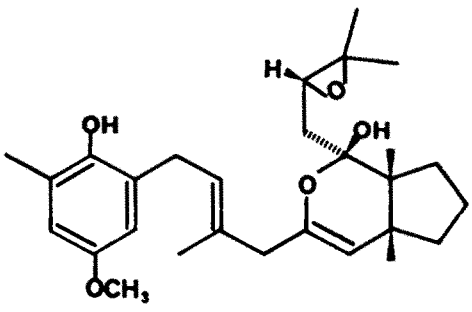
Another of the new metabolites isolated from *C. stricta* var. *amentacea*, **4**, $[\alpha]_D$ = +2.4°, oily, had the molecular formula $C_{28}H_{40}O_5$ (HREIMS). Its spectral properties [λ_{max}^{EtOH} (ε) 287 (2400) and 222 nm (10500); ν_{max}^{film} 3400, 1700, 1615 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1] indicated a structure closely related to amentol (**1**), with a methoxyl replacing one of the phenolic hydroxyls. The value of the resonance of the methoxyl in the ^{13}C NMR spectrum of **4** (60.5 ppm), considered in comparison with mo-

Table 2. Results of nOe experiments on amentol (**1**) and strictaepoxide (**7**)

Amentol (1)		Strictaepoxide (7)	
Signal irradiated	Signal enhanced	Signal irradiated	Signal enhanced
18-Me (δ 1.00s)	H-14 (δ 4.33t) H_b-13 (δ 2.10dd)	18-Me (δ 1.05s)	OH-12 (δ 4.09s) H_b-13 (δ 1.76dd)
19-Me (δ 1.10s)	H-6 (δ 4.33s) H_b-13 (δ 2.10dd)	19-Me (δ 1.18s)	H-6 (δ 4.40s) OH-12 (δ 4.09s)
16-Me (δ 1.13s)	H-14 (δ 4.33t)	16-Me (δ 1.28s)	H_b-13 (δ 2.09dd)
17-Me (δ 1.22s)	H-14 (δ 4.33t)	17-Me (δ 1.22s)	H-14 (δ 3.17dd)
		H-14 (δ 3.17dd)	H_a-13 (δ 2.07dd) 17-Me (δ 1.22s)
		H_a-13 (δ 2.07dd)	H_b-13 (δ 1.76dd) H-14 (δ 3.17dd)

Table 3. ^{1}H and ^{13}C NMR data for strictaepoxide **7** (see Experimental section for details)

Position	δ_C	δ_H(J)	H/C long-range correlation
1'	146.9s		H-3', H-5', Me-6'
2'	127.3s		2H-1, OH-1'
3'	113.2d	6.51d (3)	H-5', 2H-1
4'	153.0s		H-3', H-5', OMe-4'
5'	114.2d	6.55d (3)	H-3', Me-6'
6'	125.7s		Me-6', OH-1'
1	31.0t	H_a 3.43dd (16, 8) H_b 3.26dd (16, 7)	H-3'
2	124.0d	5.42dd (8, 7)	2H-4, 3H-20
3	135.5s		2H-4, 3H-20
4	45.0t	H_a 2.78 H_b 2.69	H-6, 3H-20
5	145.0s		2H-4, H-6
6	109.8d	4.40s	2H-4, 3H-19
7	43.4s		H-6, 3H-18, 3H-19
8	40.5t	1.50-1.70m	3H-19
9	20.0t	1.55m	
10	36.1t	1.35m, 1.85m	3H-18
11	47.9s		H-6, OH-12, 3H-18, 3H-19
12	102.5s		OH-12, 2H-13, 3H-18
13	33.8t	H_a 2.07dd (14, 3.5) H_b 1.76dd (14, 8.5)	OH-12, H-14
14	60.5d	3.17dd (8.5, 3.5)	H_b-13, 3H-16, 3H-17
15	57.9s		H_b-13, 3H-16, 3H-17
16	18.9q	1.28s	3H-17
17	24.2q	1.22s	3H-16
18	19.1q	1.05s	
19	22.9q	1.18s	H-6 (H-8)
20	15.7q	1.79s	2H-4
Me-6'	16.2q	2.18s	H-5'
OMe	55.5q	3.71s	
OH-1'		5.25s	
OH-12		4.09s	



7

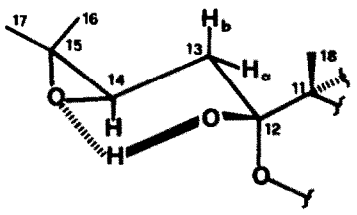


Fig. 1

del compounds, allowed this group to be placed at C-1' rather than C-4' (in compounds of this type, OMe groups in position 4' resonate near 55 ppm).[7] In accordance with this structural hypothesis, methylation of **4** with methyl iodide in the presence of potassium carbonate gave a product (**5**) identical in all respects to that obtained by methylation of **1**.

The third novel compound, strictaepoxide (**7**), was isolated from *C. stricta* (Mont.) Sauv. as an optically active clear liquid, $[\alpha]_D$= -5.1°. HREIMS established the molecular formula $C_{28}H_{40}O_5$. The compound displayes UV bands at 289 (ε=3600) and 220 nm (ε=12200), and IR absorption for hydroxyl at 3430 cm^{-1}. The ^{1}H and ^{13}C NMR spectra of **7** (Table 3) were strongly reminiscent of amentol (**1**), the main difference being significant upfield shifts for H-14 (from δ 4.33 in **1** to 3.17

in **7**), and for C-14 and C-15 (from 77.3 and 83.4 ppm in **1** to 60.5 and 57.9 ppm in **7**, respectively). These data suggested that C-14 and C-15 were embodied in an oxirane ring and therefore strictaepoxide was formulated as **7**. One-bond and long-range shift correlations (Table 3) definitely proved this structure. Concerning the stereochemistry, the *E* geometry of the C-2 double bond was desumed from the chemical shift (δ 15.7) of the methyl at C-3, while the relative configuration of the chiral centres at C-7, C-11 and C-12 was inferred from nOe data (Table 2), which require the angular methyls and the hemiketal hydroxyl to be on the same face of the molecule. Configuration at the remaining chiral centre (C-14) was assigned as drawn on the basis of the nOe interrelations of H-14 with H_a-13 and 17-Me, and of H_b-13 with 16-Me and 18-Me, assuming for the distal isoprenoid unit a preferred conformation (Fig. 1) stabilized by hydrogen bonding (the chemical shift of the OH-12 signal in the ^{1}H NMR spectrum of **7** is in fact independent from concentration).

EXPERIMENTAL

General methods. EIMS were determined at 70 eV on a Kratos MS-50S apparatus. UV spectra were recorded on a Perkin-Elmer mod. 330 and IR spectra on a Perkin-Elmer mod. 684 spectrophotometers. NMR spectra were measured in $CDCl_3$ solution on a Bruker WM-250 instrument operating at 250 and 62.5 MHz for ^{1}H and ^{13}C, respectively. Chemical shifts are quoted in ppm (δ) relative to TMS. Multiplicity of ^{13}C NMR resonances were determined by DEPT experiments. Long-range heteronuclear correlations were performed with maximum polarization for 7.5 Hz, leading to ^{2}J (geminal coupling) and ^{3}J (vicinal coupling) spots in the same spectrum.[8] Optical rotations were determined with a Perkin-Elmer 141 polarimeter using a 10 cm microcell. Preparative liquid chromatography (PLC) were carried out on a Jobin-Yvon LC Miniprep.

Plant material. Cystoseira stricta (Mont.) Sauv. was collected on rocks at about 1 m depth in March 1985 at Acicastello, Catania, Sicily. *C. stricta* var. *amentacea* Bory was harvested in March 1985 at Castelluccio, Syracuse, Sicily, on rocks at about 1 m depth. Voucher specimens are deposited at the Herbarium of the Department of Botany, Palermo, Italy.

Extraction and purification. Shade-dried and ground plant material (1 kg) was extracted x3 with CH_2Cl_2 at room temp. with continuous stirring. The pooled extracts were evaporated to give a dark green oil, which was chromatographed on an open column of Si gel (4 x 120 cm) using increasing concentrations of Et_2O in hexane as solvent system. The appropriate fractions were further purified by PLC; from *C. stricta* var. *amentacea* were obtained amentol **1** (260 mg) and amentol 1'-methyl ether **4** (335 mg), while *C. stricta* gave strictaepoxide **7** (1.2 g).

Amentol **1**. Oily, $[\alpha]_{20}(\lambda)$ +7.3° (589), +7.2° (578), +8.5° (546) (*c* = 1.6 in EtOH); MS *m/z* (%): 442 (0.5), 424 (13), 274 (23), 256 (3), 235 (5), 217 (9), 205 (3), 191 (3), 190 (6), 177 (65), 175 (16), 150 (100), 137 (49), 135 (23), 123 (8), 121 (7), 109 (16), 107 (8), 95 (23), 91 (8), 83 (13), 81 (13), 69 (23), 55 (16), 43 (44), 41 (18); ^{1}H and ^{13}C NMR see Table 1.

^{13}C NMR assignment for cystoketal **2**. 2D heteronuclear correlation experiments, both one-bond and long-range, allowed to assign unambiguously the ^{13}C signals in the NMR spectrum ($CDCl_3$, TMS) of cystoketal as follows: δ 153.1s (C-4'), 146.8s (C-1'), 146.2s (C-5), 139.9d (C-14), 135.7s (C-3), 127.4s (C-2'), 126.5d (C-13), 125.7s (C-6'), 123.5d (C-2), 115.1s (C-12), 114.1d (C-5'), 112.9d (C-3'), 109.3d (C-6), 87.8s (C-15), 55.6q (-OMe), 46.2s (C-7), 45.0t (C-4), 43.1s (C-11), 40.5t (C-8), 36.1t (C-10), 30.6t (C-1), 28.7q (C-16), 26.2q (C-17), 22.6q (C-19), 20.3t (C-9), 20.1q (C-18), 16.1q (Me-6'), 15.7q (C-20).

Amentol 1'-methyl ether **4**. Oily, $[\alpha]_{20}$ (λ) +2.4° (589), +2.4° (578), +2.6° (546), +2.9° (436) (*c* = 5.7 in EtOH); HREIMS: M$^+$ 456.2872 (calc. for $C_{28}H_{40}O_5$ 456.2875); MS *m/z* (%): 456 (3), 438 (34), 228 (6), 274 (5), 256 (3), 233 (34), 205 (19), 191 (22), 190 (28), 177 (19), 175 (28), 168 (94), 151 (75), 150 (100), 137 (75), 135 (43), 123 (62), 121 (31), 109 (31), 107 (16), 95 (50), 91 (12), 81 (22), 71 (31), 69 (31), 55 (22), 43 (62), 41 (31); ^{1}H and ^{13}C NMR see Table 1.

Strictaepoxide **7**. Oily, $[\alpha]_{20}$ (λ) -5.1° (589), -5.4° (578), -6.6° (546) (*c* = 5.6 in EtOH); HREIMS: M$^+$ 456.2870 (calc. for $C_{28}H_{40}O_5$ 456.2875); MS *m/z* (%): 456 (13), 438 (7), 420 (7), 251 (7), 233 (20), 206 (15), 205 (11), 192 (8), 191 (48), 190 (8), 189 (24), 175 (9), 151 (28), 150 (38), 137 (24), 135 (12), 123 (12), 113 (75), 109 (10), 96 (10), 95 (100), 93 (8), 91 (10), 85 (9), 81 (15), 71 (14), 69 (17), 67 (17), 55 (17), 43 (88), 41 (28); ^{1}H and ^{13}C NMR see Table 3.

Acetylation of **1** *to give* **3**. Amentol **1** was acetylated overnight at room temp. with Ac_2O-Py. Purification by PLC (LiChroprep Si-60, 25-40 μm, Et_2O-C_6H_{14}, 18:82) gave pure **3**; HREIMS: M$^+$ 568.7110 (calc. for $C_{33}H_{44}O_8$ 568.7102); ^{1}H NMR: δ 6.82 and 6.79 (AB system, each 1H, d, J=3 Hz, H-5' and H-3'), 5.31 (1H, t, J=7 Hz, H-2), 5.13 (1H, dd, J=7 and 4.5 Hz, H-14), 4.26 (1H, s, H-6), 3.19 (2H, d, J=7 Hz, H-1), 2.65 (2H, bs, H-4), 2.46 (1H, dd, J=14 and 7 Hz, H_a-13), 2.32 (3H, s, CH_3CO-), 2.26 (3H, s, CH_3CO-), 2.18 (1H, dd, J=14 and 4.5 Hz, H_b-13), 2.14 (3H, s, Me-6'), 2.06 (3H, s, CH_3CO-), 1.63 (3H, s, H-20), 1.31 and 1.14 (each 3H, 2s, H-16 and H-17), 1.10 and 1.00 (each 3H, 2s, H-18 and H-19).

Methyl iodide methylation. MeI (0.1 ml) and K_2CO_3 were added to a solution of compound (10 mg) in Me_2CO and the mixture refluxed for 3 hr. The ppt was filtered off, the solution evaporated and the residue was further purified by PLC. Methylation of **1** and **4** gave the same compound (**5**), while **2** afforded **6**. *Compound* **5**: ^{1}H NMR (80 MHz, $CDCl_3$, TMS): δ 6.43 and 6.34 (AB system, each 1H, d, J=3 Hz, H-5' and H-3'), 5.20 (1H, t, J=7.5 Hz, H-2), 4.12 (1H, s, H-6), 4.05 (1H, t, J=7.5 Hz, H-14), 3.63 and 3.57 (each 3H, 2s, 2 x OMe), 3.25 (2H, d, J=7.5 Hz, H-1), 2.56 (2H, s, H-4), 2.20 (3H, s, Me-6'), 2.20 (1H, overlapped, H_a-13), 2.05 (1H, dd, J=12 and 7.5 Hz, H_b-13), 1.62 (3H, s, H-20), 1.20 (3H, s, H-16), 1.10 (3H, s, H-17), 1.05 (3H, s, H-19), 0.98 (3H, s, H-18). *Compound* **6**: ^{1}H NMR: δ 6.58 and 6.54 (AB system, each 1H, d, J=3 Hz, H-5' and H-3'), 6.01 and 5.61 (AB system, each 1H, d, J=5.5 Hz, H-14 and H-13), 5.38 (1H, t, J=7 Hz, H-2), 4.31 (1H, s, H-6), 3.73 and 3.66 (each 3H, 2s, 2 x OMe), 3.34 (2H, m, H-1), 2.72 and 2.63 (AB system, each 1H, d, J=15 Hz, H_a-4 and H_b-4), 2.27 (3H, s, Me-6'), 1.70 (3H, s, H-20), 1.32 and 1.28 (each 3H, 2s, H-16 and H-17), 1.13 (3H, s, H-19), 0.88 (3H, s, H-18).

Dehydration of **5** *to give* **6**. A solution of **5** (10 mg) in benzene (10 ml) was refluxed for 2 hr in the presence of Florisil (400 mg). After filtration and evaporation of the solvent, the residue was purified by PLC to give a product indistinguishable from **6** obtained by methylation of cystoketal **2**.

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