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TETRAPRENYLTOLUQUINOLS FROM THE BROWN ALGA *CYSTOSEIRA STRICTA*

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Key Word Index—*Cystoseira stricta*; Cystoseiraceae; brown algae; tetraprenyltoluquinols.

Abstract—Four novel metabolites of mixed biogenesis have been isolated from the brown alga *Cystoseira stricta* and their structures determined by chemical and spectral methods.

INTRODUCTION

Among the many species belonging to the marine genus *Cystoseira* (order Fucales), one of the most widespread in the Mediterranean Sea is undoubtedly *Cystoseira stricta* which, at the level of the infralittoral zone, forms an almost continuous belt along the coasts facing this body of water. In the course of our continuing investigation of the marine flora of the central Mediterranean Sea, we made a number of collections of this alga at different stations of the Sicilian coasts.

A chromatographic comparison of the lipid extracts of these samples revealed a substantial identity of composition, at least qualitatively. A single sample, collected at Castelluccio (Syracuse, Sicily) showed a quite different chromatographic profile. A closer examination of the morphological characters of this alga allowed us to conclude that we had in hand a variety of this species, namely *C. stricta* var. *amentacea* Bory, originally described for the Aegean Sea but not reported previously for the Sicilian waters. In the present paper we wish to report on the chemical composition of the lipid fraction of this alga.

RESULTS AND DISCUSSION

A dichloromethane extract of the alga was repeatedly chromatographed to give four compounds, whose ^{13}C and ^{1}H NMR spectral properties (Tables 1 and 2) strongly suggested a common tetraprenyltoluquinol skeleton, frequently encountered in metabolites from *Cystoseira* and related genera [1–15].

The identity of one of the new compounds, **1**, $[\alpha]_D$ + 5.5°, $C_{27}H_{38}O_5$, was readily settled by comparison of its spectral properties with those of a metabolite (**7**) isolated previously from the congener alga *C. algeriensis*. The ^{13}C NMR spectrum of **1** (Table 1) differed from that of **7** (besides the obvious lack of aromatic OMe resonances) by the replacement of two methylene resonances (33.6 and 36.6 ppm) with two olefinic signals (122.6 and 153.6 ppm); moreover, the shift of the C-12 carbonyl from 216.7 in **7** to 205.9 in **1** established unambiguously that the additional double bond in **1** was conjugated. In the ^{1}H NMR spectrum of **1** (Table 2) the two signals associated to the C-13 and C-14 methylenes (δ 1.70 and 2.59, 2H each) of **7** were replaced by an AB system (δ 6.59 and 6.85, -CH=CH-). Taking into account the high-field position of the resonance of the vinyl methyl at C-3 in the ^{13}C NMR spectrum, which indicated an *E*-geometry of the relevant double bond, the novel compound was assigned structure **1**. The corresponding *Z* isomer (bifurcarenone) has been isolated

Table 1. ^{13}C NMR spectral data for compounds **1–4*** (75.5 MHz, $CDCl_3$, TMS as int. standard)

C	1	2	3	4
1′	149.8 *s*	150.1 *s*	149.6 *s*	149.4 *s*
2′	131.1 *s*[a]	134.5 *s*[a]	131.0 *s*[a]	131.9 *s*[a]
3′	115.4 *d*	116.4 *d*	115.4 *d*	115.5 *d*
4′	145.4 *s*	153.3 *s*	145.5 *s*	145.9 *s*
5′	113.1 *d*	113.5 *d*	113.4 *d*	114.0 *d*
6′	127.9 *s*[a]	132.6 *s*[a]	127.5 *s*[a]	127.8 *s*[a]
1	28.6 *t*	28.0 *t*	29.1 *t*	29.7 *t*
2	127.8 *d*	128.9 *d*	127.5 *d*	127.0 *d*[b]
3	125.8 *s*[a]	131.6 *s*[a]	125.2 *s*[a]	124.1 *s*[a]
4	56.6 *t*	57.6 *t*	56.2 *t*	55.2 *t*
5	209.6 *s*	210.3 *s*	204.1 *s*	199.7 *s*
6	46.4 *t*	46.5 *t*	46.8 *t*	124.0 *d*[b]
7	46.9 *s*	47.5 *s*	47.1 *s*	159.1 *s*
8	36.6 *t*[b]	36.9 *t*[b]	36.9 *t*[b]	41.0 *t*[c]
9	20.0 *t*	20.7 *t*	19.0 *t*	24.8 *t*
10	34.2 *t*[b]	34.6 *t*[b]	34.7 *t*[b]	32.2 *t*[c]
11	60.2 *s*	61.0 *s*	60.8 *s*	44.7 *d*
12	205.9 *s*	207.1 *s*	208.5 *s*	204.2 *s*
13	122.6 *d*	123.1 *d*	40.7 *t*	122.6 *d*[b]
14	153.6 *d*	154.7 *d*	59.9 *d*	152.9 *d*
15	71.2 *s*	71.7 *s*	58.4 *s*	71.1 *s*
16	29.3 *q*	29.9 *q*	24.5 *q*[c]	29.3 *q*
17	29.3 *q*	29.9 *q*	21.4 *q*[c]	29.3 *q*
18	21.1 *q*[c]	21.6 *q*[c]	20.6 *q*[d]	16.7 *q*[d]
19	20.2 *q*[c]	20.8 *q*[c]	19.9 *q*[d]	19.4 *q*
20	16.2 *q*[d]	16.8 *q*[d]	16.4 *q*[c]	16.5 *q*[d]
6′-Me	16.3 *q*[d]	16.6 *q*[d]	16.2 *q*[c]	16.2 *q*[d]
OMe	—	61.0 *q*	—	—

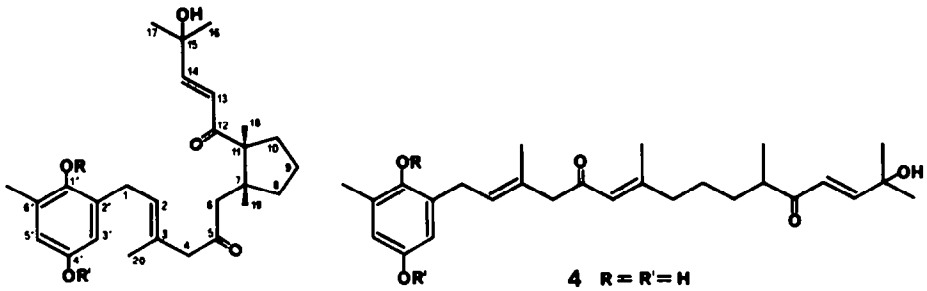
*Multiplicities were obtained with DEPT sequence for compound **1**, and by off-resonance decoupling experiments for **2–4**.

[a–d]Values with identical superscripts within a column can be interchanged.

Table 2. ^{1}H NMR spectral data for compounds 1–4* (300 MHz, $CDCl_3$, TMS as int. standard)

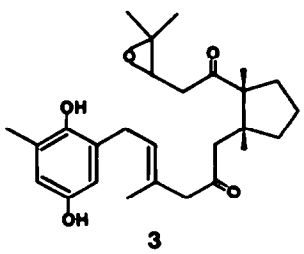
H	1	2	3	H	4
3′	6.50 } AB(3)*	6.59 } AB(3)	6.48 } AB(3)	3′ }	6.52 *s(br)*
5′	6.40 }	6.45 }	6.41 }	5′ }	
1	3.29 *d*(7)	3.47 *d*(6.5)	3.26 *d*(7.5)	1	3.33 *d*(7.5)
2	5.35 *t*(7)	5.45 *t*(6.5)	5.35 *t*(7.5)	2	5.37 *t*(7.5)
4	3.00 *s(br)*	3.05 *s(br)*	2.98 *s(br)*	4	3.13 *s*
6	2.44 } AB(16)	2.47 } AB(14)	2.46 } AB(16)	6	6.11 *s(br)*
	2.30 }	2.37 }	2.29 }		
8 }				8	2.05
9 }	1.9, 1.7, 1.5	2.1, 1.6, 1.3	1.9, 1.6, 1.4	9 }	1.35–1.70
10 }				10 }	
			{ 2.82 *dd*(18, 6)	11	2.71 *m*
13	6.59 } AB(16)	6.68 } AB(16)	{ 2.55 *dd*(18, 6)	13	6.39 } AB(16)
14	6.85 }	6.89 }	3.04 *t*(6)	14	6.94 }
16	1.30 *s*	1.31 *s*	1.25 *s*	16	1.39 *s*
17	1.28 *s*	1.28 *s*	1.17 *s*	17	1.39 *s*
18	1.15 *s*	1.20 *s*	1.09 *s*	18	1.11 *d*(7)
19	1.14 *s*	1.18 *s*	1.08 *s*	19	2.09 *s*
20	1.68 *s*	1.60 *s*	1.59 *s*	20	1.72 *s*
6′-Me	2.17 *s*	2.26 *s*	2.14 *s*	6′-Me	2.21 *s*
-OMe	—	3.68 *s*	—	OH	5.01 *s*
OH	7.36 *s*	8.02 *s*	—		
OH	5.18 *s*	—	—		

*Coupling constants (*J* in parentheses) are given in Hz; assignments were confirmed by decoupling experiments.



1 R = R′ = H
2 R = Me R′ = H
5 R = R′ = Me

4 R = R′ = H
6 R = R′ = $COCH_3$



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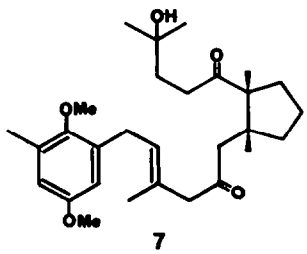

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previously from *Bifurcaria galapagensis* (Cystoseiraceae) [16].

A second compound (**2**) had the molecular formula $C_{28}H_{40}O_5$ and spectral features very close to those of *trans*-bifurcarenone, the main difference being a 3H singlet at δ3.68 indicating that **2** was a phenolic monomethyl ether of **1**. Location of the methoxyl in position 1′ instead of 4′ was deduced from the value of its chemical shift (δ61.0) in the ^{13}C NMR which agrees with those observed in a series of closely related compounds (OMe groups in position 1′ resonate near 55 ppm [17]). Confirmation of the proposed structure came from methylation of **2** with

methyl iodide in the presence of potassium carbonate which afforded a compound (**5**) indistinguishable (^{1}H NMR and MS) from that obtained by methylation of **1** in the same experimental conditions.

The third metabolite isolated from *C. stricta* var. *amentacea*, amentaepoxide (**3**), was an isomer of **1**. Its ^{1}H NMR spectrum closely resembled that of *trans*-bifurcarenone, but lacked the AB system due to the olefinic protons H-13 and H-14 which were replaced by a double doublet at δ2.82 and 2.55 (H-13), and a 1H triplet at δ3.04 (H-14). In the light of this, the novel metabolite was assigned structure **3**, which was also compatible with the ^{13}C NMR data. Definite proof of this structure was obtained by treatment of **3** with diisobutylaluminium hydride, which is known to isomerize epoxides into allylic alcohols. The main product of this reaction was identical in all respects (UV, IR spectra, MS, ^{1}H and ^{13}C NMR, [α]) to compound **1**.

The last metabolite, amentadione (**4**) $[\alpha]_D$ −0.44, had molecular formula $C_{27}H_{38}O_5$. Its UV spectrum was indicative of a hydroquinol while IR absorptions at 1685 and 1620 cm^{-1}, and resonances at δ199.7 and 204.2 in the ^{13}C NMR spectrum were consistent with the presence of two enone groups. Treatment of **4** with acetic anhydride in pyridine gave the diacetate **6**, which still had hydroxyl absorption in the IR spectrum, thus proving that the remaining oxygen atom of the empirical formula belonged to a tertiary alcohol function. Consideration of the ^{13}C NMR data of amentadione in conjunction with the molecular formula established the acylic nature of the terpenoid moiety and structure **4** was easily formulated through comparison of its ^{1}H and ^{13}C NMR data with those of a product previously isolated from *Cystoseira sauvageauana* [18], in which the tetraprenyl open chain lacks the C-13 double bond. Indeed, amentadione differed from this metabolite in the absence from its ^{1}H NMR spectrum of the resonances for two interrelated methylene triplets, which were replaced by an AB system (δ6.39 and 6.94, J = 16 Hz) associated with two *trans*-olefinic protons. Analogously, in the ^{13}C NMR spectrum, two resonances of sp^3-hybridized carbons were replaced by two sp^2-carbons.

Finally, we wish to recall here that in a previous paper [18] we have suggested the possible intermediacy of acyclic diketones like **4** in the biosynthesis of the monocyclic versions containing the cyclopentane ring, which could arise via C-11 to C-7 bond formation through a Michael reaction. The co-occurrence in *C. stricta* var. *amentacea* of both types of metabolites lends some support to this hypothesis.

EXPERIMENTAL

General. EIMS: direct inlet, 70 eV; ^{1}H and ^{13}C NMR: 300 and 75.5 MHz respectively.

Plant material. C. stricta var. *amentacea* Bory (voucher specimen deposited at the Herbarium of the Department of Botany, University of Palermo, Italy) was collected on rocks at about 1 m depth in October 1985 at Castelluccio (Syracuse, Sicily).

Extraction and purification. Shade-dried and ground alga (1.5 kg) was extracted (× 3) with CH_2Cl_2 at room temp. with continuous stirring. The extracts were pooled and evaporated to give a dark green oil (30 g) which was applied to a column (4 × 100 cm) of silica gel and eluted with increasing concentrations of Et_2O in hexane. Fractions of 200 ml were collected and those exhibiting similar TLC profiles combined.

Fractions 23–26 were pooled and subjected to PLC (LiChroprep Si-60) using as eluent $CHCl_3$–Et_2O (41:9) followed by $CHCl_3$–Et_2O (4:1) to give *trans*-bifurcarenone 1'-methyl ether (**2**) (150 mg, 0.01% dry wt of the alga) and amentaepoxide (**3**) (342 mg, 0.023% dry wt).

Fractions 27–30 were further chromatographed (LiChroprep Si-60, $CHCl_3$–Et_2O, 4:1) to give *trans*-bifurcarenone (**1**) (776 mg, 0.052% dry wt) and amentadione (**4**) (18 mg, 0.001% dry wt).

Compound **1**. Oily; $[\alpha]_{20}(\lambda)$: + 5.5° (589), + 5.7° (578), + 7.1° (546) (*c* 8 in EtOH); IR ν_{max}^{film} cm^{-1}: 3385, 1700, 1670, 1615; UV λ_{max}^{EtOH} nm(ε): 290 (4000), 228 (18300), 220 (19800); HRMS: [M]$^+$ 442.2711 (calc. for $C_{27}H_{38}O_5$ 442.2719); MS *m/z* (rel. int.): 442 (5), 424 (18), 406 (12), 251 (9), 233 (43), 205 (21), 191 (22), 177 (21), 175 (64), 161 (14), 150 (28), 137 (36), 121 (16), 113 (57), 109 (21), 95 (100), 85 (14), 81 (21), 69 (21), 67 (28), 55 (21), 43 (64), 41 (21).

Compound **2**. Oily; $[\alpha]_{20}(\lambda)$: + 5.3° (589), + 4.6° (578), + 5.9° (546) (*c* 1.15 in EtOH); IR ν_{max}^{film} cm^{-1}: 3400, 1710, 1675, 1615; UV λ_{max}^{EtOH} nm(ε): 285 (3750), 220 (22100); HRMS: [M]$^+$ 456.2870 (calc. for $C_{28}H_{40}O_5$ 456.2875); MS *m/z* (rel. int.): 456 (2), 438 (9), 287 (3), 274 (3), 251 (11), 233 (34), 205 (11), 190 (16), 189 (7), 177 (21), 175 (25), 151 (40), 150 (100), 137 (50), 135 (21), 123 (11), 121 (29), 113 (24), 109 (26), 107 (11), 105 (7), 97 (10), 96 (11), 95 (65), 93 (7), 91 (10), 83 (7), 81 (11), 79 (8), 77 (8), 69 (18), 67 (14), 55 (13), 43 (38), 41 (15).

Compound **3**. Oily; $[\alpha]_{20}(\lambda)$: + 14.6° (589), + 15.0° (578), + 20.2° (546) (*c* 1.25 in EtOH); IR ν_{max}^{film} cm^{-1}: 3400, 1690, 1605; UV λ_{max}^{EtOH} nm(ε): 291 (3800), 215 (10400); HRMS: [M]$^+$ 442.2712 (calc. for $C_{27}H_{38}O_5$ 442.2719); MS *m/z* (rel. int.): 442 (1), 424 (7), 406 (4), 274 (7), 256 (2), 233 (3), 216 (6), 215 (4), 205 (6), 190 (8), 178 (5), 177 (37), 176 (6), 175 (28), 151 (37), 150 (100), 149 (11), 138 (6), 137 (46), 136 (5), 135 (39), 123 (14), 121 (5), 119 (4), 113 (8), 109 (14), 107 (11), 105 (7), 97 (5), 96 (9), 95 (30), 93 (9), 91 (11), 83 (14), 81 (18), 79 (11), 77 (7), 69 (11), 67 (23), 65 (4), 55 (20), 53 (8), 43 (65), 41 (28).

Compound **4**. Oily; $[\alpha]_{20}(\lambda)$: −0.44° (589), −0.69° (578), −0.88° (546), −4.7° (436) (*c* 1.6 in EtOH); IR ν_{max}^{film} cm^{-1}: 3400, 1685, 1665, 1620; UV λ_{max}^{EtOH} nm(ε): 290 (3120), 227 (21100); HRMS: [M]$^+$ 442.2713 (calc. for $C_{27}H_{38}O_5$ 442.2719); MS *m/z* (rel. int.): 442 (26), 424 (16), 300 (6), 274 (13), 233 (13), 215 (12), 192 (23), 177 (100), 175 (81), 165 (13), 161 (13), 150 (74), 137 (86), 121 (12), 113 (12), 109 (19), 105 (12), 95 (28), 91 (10), 81 (23), 69 (18), 55 (17), 43 (42), 41 (19).

Methylation of **1** *and* **2**. **1** or **2** (20 mg each) dissolved in Me_2CO was subjected to methylation with MeI (0.5 ml) in the presence of K_2CO_3. The ppt was filtered off and the soln evaporated. The residue was purified by PLC (LiChropep Si-60, $CHCl_3$–Et_2O, 9:1) to give **5**, oily, HRMS: [M]$^+$ 470.3030 (calc. for $C_{29}H_{42}O_5$ 470.3032); ^{1}H NMR ($CDCl_3$, 80 MHz, TMS): δ6.66 and 6.36 (2H, AB system, J = 15 Hz, H-13 and H-14), 6.33 (2H, *bs*, H-3' and H-5'), 5.18 (1H, *t*, J = 7 Hz, H-2), 3.62 and 3.56 (6H, 2*s*, OCH_3), 3.26 (2H, *d*, J = 7 Hz, H-1), 2.94 (2H, *bs*, H-4), 2.20 (3H, *s*, 6'-Me), 1.65 (3H, *bs*, H-20), 1.31 (6H, 2*s*, H-16 and H-17), 1.12 (6H, 2*s*, H-18 and H-19).

Isomerization of **3**. To a soln of **3** (25 mg) in dry THF (1 ml) was slowly added, under a steam of N_2, a 20% soln (60 μl) of diisobuthylaluminium hydride. After stirring at 0° for 3 hr, the reaction was quenched by cautious addition of H_2O (2 ml) followed by 2M HCl (0.5 ml), and the mixture extracted (× 3) with Et_2O. The extracts were washed with aq. $NaHCO_3$, dried over Na_2SO_4 and evapd to give an oil from which the main component (11 mg) was isolated by PLC (LiChroprep Si-60, $CHCl_3$–Et_2O, 8:2). The physical properties (UV, IR, ^{1}H NMR, [α]) of this product were identical with those of the natural compound **1**.

Acetylation of **4**. Compound **4** (8 mg) was acetylated (Ac_2O–pyridine; overnight at room temp.) to give **6** (6 mg);

HRMS: $[M]^+$ 526.2927 (calc. for $C_{31}H_{42}O_7$ 526.2930); MS *m/z*: 526 $[M]^+$, 508 $[M-H_2O]^+$, 466 $[M—H_2O—CH_2CO]^+$; IR ν_{max}^{film} cm^{-1}: 3470, 1740, 1685, 1610.

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