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PRENYLATED *O*-METHYLTOLUQUINOLS FROM *CYSTOSEIRA STRICTA*

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Key Word Index—*Cystoseira stricta*; Phaeophyta; Cystoseiraceae; tetraprenyltoluquinols.

Abstract—The lipid fraction of the brown alga *Cystoseira stricta* contains, in addition to five known tetraprenyltoluquinols, three previously unreported compounds belonging to the same class of metabolites.

INTRODUCTION

Species of the algal genus *Cystoseira* are known to accumulate a variety of tetraprenylated quinols [1–4]. *C. stricta* (Mont.) Sauv., an alga widespread in the Mediterranean Sea, has recently been reported to elaborate a novel class of metabolites of mixed biogenesis, cystoseirols [5], whose terpenoid component possesses an oxabicyclo[5, 4, 1]dodecane system. The uniqueness of this structure, the biogenetic origin of which is far from obvious, drew our attention to the alga in question. So far, work by our group on Sicilian collections of the alga has resulted in the isolation and characterization of two new tetraprenyltoluquinols with a regular diterpenoid moiety [6, 7].

The present paper describes further work on the lipid fraction from *C. stricta*, which has led to the identification of other components, including three previously unreported metabolites.

RESULTS AND DISCUSSION

Figure 1 shows a typical chromatographic pattern of the lipid fraction from *C. stricta* after preliminary purification on Florisil. Examination of individual plants collected over a 4-month period at different stations off the eastern coast of Sicily showed only moderate variations in the relative areas of peaks whose number remained unchanged. Five of the peaks present in the chromatograms have been identified, on the basis of their chromatographic and spectral characteristics and by comparison with authentic samples with previously described compounds. They are, in order of increasing polarity: the chromane derived from cystoketal (epimeric mixture at C-3, possibly an artifact of isolation) [8], cystoketal itself [8], strictaepoxide [6], strictaketal [7] and balearone [9].

In addition to the known compounds, three novel metabolites were isolated and their structure determined.

The less polar unknown substance, *iso*cystoketal (**1**), is an optically active oil $[\alpha]_D$ 24.9°, elemental composition $C_{28}H_{38}O_4$ (HREIMS), whose spectral properties are very similar to the isomeric cystoketal. In particular, the virtual identity of their mass fragmentation patterns (neglecting differences in the relative intensities of peaks) is indicative of a stereochemical, rather than structural, difference. The nature of this difference was clarified by comparison of the ^{13}C NMR spectrum of **1** (Table 1) with that of cystoketal; an upfield shift of the C-4 resonance (from 45.0 ppm in cystoketal to 37.1 ppm in **1**) and an opposite shift of the C-20 resonance (from 15.7 ppm in cystoketal to 23.0 ppm in **1**), while the rest of the spectrum is essentially unaffected, revealed a change in the configuration of the C-2 double bond from *E* in cystoketal to *Z* in *iso*cystoketal, which was therefore formulated as **1**. Its ^1H NMR spectrum (Table 2) is also in full agreement with the proposed structure since, in comparison with that of cystoketal, it shows an upfield shift of the resonance of the vinyl methyl at C-3 (from δ1.76 to 1.71) and a concurrent downfield shift of the AB system associated with the C-4 methylene

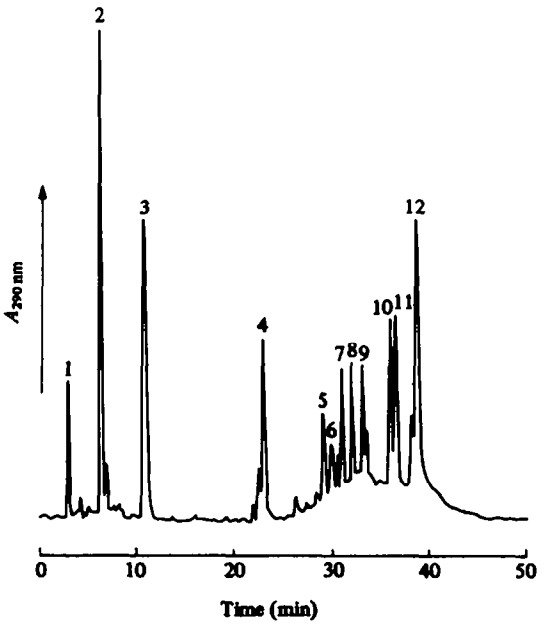
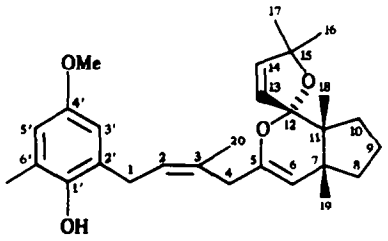


Fig. 1. HPLC elution profile of the dichloromethane extract from *C. stricta*. Peaks: 1 = cystoketal chromane; 2 = *iso*cystoketal; 3 = cystoketal; 4 = strictaepoxide; 7 = *iso*strictaketal; 10 = *iso*balearone; 11 = strictaketal; 12 = balearone; 5,6,8,9 = unknown tetraprenyltoluquinols.



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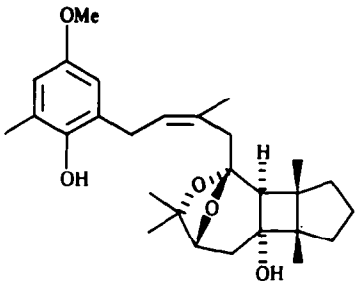

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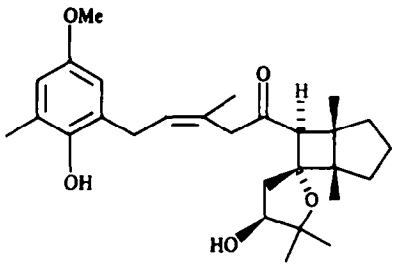

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Table 1. ^{13}C NMR assignments for compounds 1–3 (75.7 MHz, $CDCl_3$, TMS as int. standard)*

C	1	2	3
1′	146.7 *s*[a]	146.8 *s*	146.2 *s*
2′	127.9 *s*[b]	127.8 *s*[a]	126.8 *s*[a]
3′	112.9 *d*	112.9 *d*	112.8 *d*
4′	153.0 *s*	152.5 *s*	152.4 *s*
5′	114.2 *d*	114.3 *d*	114.2 *d*
6′	124.6 *s*[b]	126.1 *s*[a]	126.0 *s*[a]
1	30.5 *t*	31.1 *t*	30.6 *t*
2	126.2 *d*	126.6 *d*	127.2 *d*
3	133.9 *s*	131.1 *s*	129.4 *s*
4	37.1 *t*[c]	37.6 *t*[b]	48.6 *t*
5	145.8 *s*[a]	108.2 *s*	207.2 *s*
6	109.6 *d*	57.1 *d*	61.8 *d*
7	46.4 *s*	43.1 *s*	46.2 *s*
8	40.5 *t*	42.3 *t*	41.0 *t*
9	20.4 *t*	24.5 *t*	24.2 *t*
10	36.2 *t*[c]	36.0 *t*[b]	35.8 *t*[b]
11	43.2 *s*	52.6 *s*	52.8 *s*
12	115.5 *s*	69.5 *s*	81.4 *s*[c]
13	126.2 *d*	32.3 *t*	35.6 *t*[b]
14	140.1 *d*	80.6 *d*	78.0 *d*
15	88.0 *s*	79.9 *s*	80.0 *s*[c]
16	28.5 *q*[d]	28.4 *q*[c]	27.4 *q*[d]
17	26.3 *q*[d]	22.4 *q*[c]	22.2 *q*[d]
18	20.1 *q*[e]	19.1 *q*[d]	19.0 *q*[e]
19	22.7 *q*[e]	17.0 *q*[d]	17.2 *q*[e]
20	23.0 *q*	26.3 *q*	24.6 *q*
6′-Me	16.3 *q*	16.5 *q*	16.8 *q*
OMe	55.6 *q*	55.6 *q*	55.6 *q*

*Multiplicities were obtained by off-resonance decoupling experiments.

[a–e]Values with the same superscript within a column can be interchanged.

(from δ2.74 and 2.69 (J = 15 Hz) to 2.97 and 2.81 (J = 14 Hz)).

The other unknown compounds, *iso*strictaketal (**2**), a crystalline substance $[\alpha]_D$ 1.3°, and *iso*balearone (**3**), oily, $[\alpha]_D$ 38.8°, both have elemental composition $C_{28}H_{40}O_5$ (HREIMS). Consideration of their ^{13}C and ^1H NMR spectra (Tables 1 and 2) and other spectral properties, mainly mass fragmentation (see Experimental), showed that they are the *Z*-2-isomers of strictaketal and balearone, respectively.

Definite confirmation of the structure assigned to the novel compounds and proof of the relative stereochemistry of the chiral centres was obtained by photoisomerization of compounds **1**–**3**, which gave in each case a compound identical in all respects, including optical rotation, to the corresponding *E*-2-isomer.

Finally, four further compounds (Fig. 1, peaks 5, 6, 8, 9) have been isolated in pure condition and their spectral features recorded. These compounds, currently under investigation, certainly belong to the same class as the metabolites identified, but none of them has physical properties identical with those of any cystoseirols or related compounds. Since the compounds isolated comprise over 95% of the total quinol fraction, the remaining percentage being associated with a number of very small peaks, we can conclude confidently that Sicilian samples of *C. stricta* do not contain significant amounts of rearranged tetraprenyltoluquinols.

EXPERIMENTAL

General. Mp (Kofler block) is uncorr; MS: direct inlet, 70 eV; ^1H and ^{13}C NMR: 300 MHz and 75 MHz respectively. Chemical shifts relative to TMS.

Plant material. Twelve individual plants of *C. stricta* were sampled at various stations along the eastern coast of Sicily, from Taormina to Capo Passero, in the period March–June 1985, freeze-dried and kept at −20° until required. A batch collection was made in March 1985 at Aci Castello, Catania, Sicily, on rocks in the intertidal zone. A voucher specimen has been deposited at the Herbarium of the Institute of Botany, Catania, Sicily.

HPLC analyses of plant extracts. Freeze-dried material (about 1 g) was taken from an individual plant and extracted (× 3) with CH_2Cl_2. The solvent was removed *in vacuo*, the residue dissolved in Et_2O (5 ml) and passed through a Florisil cartridge (Sep-Pak) which was eluted with Et_2O (15 ml). The eluate was evaporated to dryness and the residue was dissolved in a known volume of 2% Et_2O in hexane; this soln was used for HPLC analyses. Column:

Table 2. ^{1}H NMR spectral data for compounds 1–3 (300 MHz, TMS as int. standard)*

H	1	H	2	H	3
3′	6.57 *d* (3)	3′	6.45 *d* (3)	3′	6.48 *d* (3)
5′	6.51 *d* (3)	5′	6.51 *d* (3)	5′	6.56 *d* (3)
1	3.40 *dd* (16, 7.5) 3.29 *dd* (16, 7.5)	1	3.35 *dd* (16, 8) 3.19 *dd* (16, 8)	1	3.28 *dd* (16, 8) 3.13 *dd* (16, 8)
2	5.26 *t* (7.5)	2	5.27 *t* (8)	2	5.43 *t* (8)
4	2.97, 2.81 AB (14)	4	2.65, 2.27 AB (14)	4	3.14 *s*
6	4.39 *s*	6	1.79 *s*	6	3.08 *s*
8, 9, 10	1.9, 1.7, 1.4 *m*	8, 9	1.63–1.20 *m*	8, 9, 10	1.9, 1.7, 1.4 *m*
		10	2.11 *m* 1.32 *m*	13	2.96 *dd* (12, 6) 1.91 *dd* (12, 8)
13	6.00, 5.68 AB (5.5)	13	2.40 *dd* (16, 6) 1.76 *d* (16)	14	3.94 *dd* (8, 6)
14		14	4.08 *d*	16	1.08 *s*
16	1.25 *s*	16	1.19 *s*	17	1.18 *s*
17	1.28 *s*	17	1.54 *s*	18	1.00 *s*
18	0.90 *s*	18	1.93 *s*	19	0.93 *s*
19	1.16 *s*	19	1.12 *s*	20	1.69 *s*
20	1.71 *s*	20	1.82 *s*	6′-Me	2.26 *s*
6′-Me	2.22 *s*	6′-Me	2.19 *s*	OMe	3.74 *s*
OMe	3.74 *s*	OMe	3.70 *s*	OH	6.05 *s*
		OH	6.45 *s*		

*Coupling constants (*J* in parentheses) are given in Hz.

Hypersil (particle size 5 μm, 250 × 3.5 mm i.d.) at 25°; mobile phase, (A) hexane, (B) Et_2O; gradient, 0–7 min 2–10% B, 10–15 min 10% B, 15–25 min 10–30% B, 25–40 min 30% B; flow rate 1 ml/min. The eluent was detected at 290 nm. Figure 1 was obtained using these conditions. Preliminary identification of compounds was based on co-chromatography with reference samples, while definite confirmation was obtained by comparison (UV, IR, MS, ^{1}H NMR, [α]) of the isolated compounds (column; Whatman, Partisil M9 10/25, gradient elution as above) with authentic specimens.

Extraction and isolation of constituents. Shade-dried and ground plant material (1.5 kg) was extracted (× 3) with CH_2Cl_2 at room temp. with continuous stirring. The extracts were pooled and the solvent removed to give a dark green viscous mass (70 g), which was chromatographed on silica gel (4 × 120 cm). The column was eluted with increasing concentrations of Et_2O in hexane. Fractions of 200 ml were taken and the elution monitored by TLC.

Fractions 35–38 were pooled and subjected to PLC (LiChroprep Si-60, C_6H_{12}-Et_2O, 9:1) to give pure **1** (250 mg, 0.0033% dry wt of the alga); $[\alpha]^{20}$ (λ): +24.9° (589), +26.8° (578) +30.9° (546), +58.0° (436) (*c* 0.54 in EtOH); IR ν_{max}^{film} 3520, 1690, 1610 cm^{-1}; UV λ_{max}^{EtOH} nm (ε): 288 (3800), 220 (14300); HREIMS: $[M]^+$ 438.2765 (calc. for $C_{28}H_{38}O_4$ 438.2769); EIMS *m/z* (rel. int.): 438 (26), 420 (6), 289 (6), 288 (35), 233 (6), 205 (11), 191 (35), 189 (20), 151 (44), 150 (base), 149 (29), 137 (76), 135 (29), 109 (13), 105 (6), 104 (12), 95 (20), 91 (9), 69 (9), 57 (25), 56 (3), 55 (10), 43 (24), 41 (12).

Evapn. of fractions 50–55 gave a semicrystalline residue which was further purified by PLC (LiChroprep Si-60, C_6H_{12}–Me_2CO 19:1) followed by recrystallization from C_6H_{12}–Me_2CO (1:1) to give pure **2** (350 mg, 0.0046% dry wt), mp 94–95°; $[\alpha]^{20}$ (λ): +1.3° (589), +1.5° (578), +2.1° (546), +9.1° (436), +30.4 (365) (*c* 0.94 in EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}; 3440, 1605; UV λ_{max}^{EtOH} nm (ε): 289 (3900), 220 (10700); HREIMS: $[M]^+$ 456.2870 (calc. for $C_{28}H_{40}O_5$ 456.2875); EIMS *m/z* (rel. int.): 456 (14), 438 (22), 420 (5), 301 (31), 251 (14), 231 (17), 206 (12), 192 (14), 191 (74), 190 (20), 189 (32), 176 (17), 168 (38), 155 (14), 151 (65), 150 (base), 137 (43), 113 (20), 111 (15), 109 (22), 96 (34), 95 (51), 81 (22), 71 (22), 69 (23), 67 (22), 59 (10), 55 (25), 43 (45), 41 (31).

Fractions 60–64 were evaporated to give a viscous residue (3.5 g) which was subjected to PLC (LiChroprep Si-60, C_6H_{12}-*i*-PrOH 97:3) to give **3** (350 mg, 0.0046% dry wt), oily, $[\alpha]^{20}$ (λ): +38.3°(589), +40.7° (578), +48.1° (546), +101° (436) (*c* 1.2 in EtOH); IR ν_{max}^{film} cm^{-1}; 3420, 1700, 1610; UV λ_{max}^{EtOH} nm (ε): 289 (3400), 220 (11000); HREIMS: $[M]^+$ 456.2870 (calc. for $C_{28}H_{40}O_5$ 456.2875); EIMS *m/z* (rel. int.): 456 (6), 438 (19), 420 (8), 288 (27), 233 (16), 205 (12), 191 (45), 189 (21), 168 (34), 151 (43), 150 (base), 137 (40), 109 (14), 95 (19), 69 (15), 67 (13), 43 (23), 41 (13).

Photoisomerization. A 10^{-4} M EtOH soln of compound in a standard spectrophotometric cell was deoxygenated with a stream of N_2 and subjected to 30 min irradiation with a 450 W Hanovia lamp, equipped with a pyrex filter. HPLC separation (Hypersil 5 μm) of the reaction mixture gave the following results. *Iso*cystoketal **1** (hexane–Et_2O, 19:1): starting material (39%), cystoketal chromane (44%), cystoketal (17%). *Iso*strictaketal **2** (hexane–Et_2O, 7:3): starting material (53%), strictaketal (47%). *Iso*balearone **3** (hexane–Et_2O, 13:7): starting material (24%), balearone (8%) and in addition two peaks both having retention time shorter than balearone (total area 68%). That these were the expected C-3 epimers of the corresponding chromane was not investigated further.

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