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INHERITANCE OF CHEMICAL CONSTITUENTS IN ALGAE: TETRAPRENYLTOLUQUINOLS OF *CYSTOSEIRA ELEGANS* × *C. ALGERIENSIS*

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Key Word Index—*Cystoseira elegans*, *C. algeriensis*, *C. elegans* × *C. algeriensis*, Cystoseiraceae; brown algae, hybridization, tetraprenyltoluquinols

Abstract—The chemical composition of a *Cystoseira* species that had been previously described as *C. algeriensis*, together with its morphological and anatomical features, establish that it is the naturally occurring hybrid *C. elegans* × *C. algeriensis*. The structures of two new tetraprenyltoluquinols isolated from *C. elegans* is also reported

INTRODUCTION

The taxonomy of the marine genus *Cystoseira*, comprising no less than 40 species mostly occurring along the Mediterranean and the contiguous Atlantic coasts, is still a matter of debate, possibly on account of presently active speciation, intraspecific variation and interspecific hybridization [1–3]

In previous papers [4, 5], we described the isolation of several novel secondary metabolites (**1–6**) from a Mediterranean alga classified as *Cystoseira algeriensis*. However, some discrepancies between its exomorphic characters and those reported in the literature [6] raised doubts as to the correctness of the classification Repeated in-the-field observations allowed us to establish that the alga in question always occurs in association with *C. elegans* Sauv. and *C. algeriensis* J. Feld., and that the general appearance of individual plants is intermediate between these two species, occasionally resembling more closely one or other of them These observations suggested that the alga could be the natural hybrid *C. elegans* × *C. algeriensis*. Since studies of the chemistry of secondary metabolites have been used to establish the existence in nature of hybrids that had only been suspected on morphological grounds (in general, the chemical composition of a hybrid tends to be the sum of that of its two parents) [7–14], we investigated the utilization of chemical markers as a criterion for validation of the apparent algal hybrid.

RESULTS AND DISCUSSION

Morphological and anatomical attributes of the parental species and the hybrid

In Table 1 are summarized the distinguishing features of the hybrid and the two parents

Cystoseira algeriensis clearly differs from *C. elegans* in overall habit, appearance of tophules (smooth or verrucose-tuberculate, never spinulate), secondary and tertiary laterals (stiff and spine-like) and receptacles (slender, never compact). Conceptacles are located in or near the basal part of the appendages, never in the axial part.

Putative hybrids vary considerably in external morphology, some resembling more closely one or other of the parents, in general, they have very verrucose-tuberculate tophules with few spines, secondary and tertiary laterals flexuous and spine-like, conceptacles in the axes of branches, and slander and/or compact receptacles

In a cross-section of the axis, the size of the cells of the meristoderm of the hybrid is comparable to that of *C. algeriensis*, while those of the cortex and medulla are respectively intermediate between, and approximately half of, those of the parent species. The anatomy of the tophules is similar in both the 'pure' species and in the hybrid In the latter, a remarkable increase of the number of conceptacles is observed in a cross-section of the receptacles, while the separation between the conceptacles is reduced merely to a diaphragm. An interesting recessive character of the hybrid is the absence of branching of the antheridial hairs, which is instead observed in both the parent species. Shape and size of conceptacles and ratio length-diameter (*ca* 2), as well as size of antheridia are comparable in the hybrid and *C. elegans*

Chemical attributes of the parental species and the hybrid

HPLC examination of 10 samples of *C. algeriensis* collected over a one-year period showed that the known tetraprenyltoluquinols **1**, **2**, **5** and **6** were consistently present, although quantitative differences in their ratios were observed Similarly, the constant presence in *C. elegans* of four metabolites of the same class was observed; two of these were identified with the known **3** and **4**, while the remaining two were easily assigned structures **7** and **8** by comparison of their spectral properties (see Experimental) with those of closely related compounds

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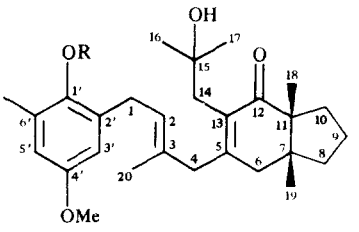
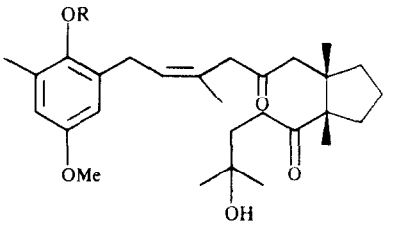


1 R = Me
2 R = H



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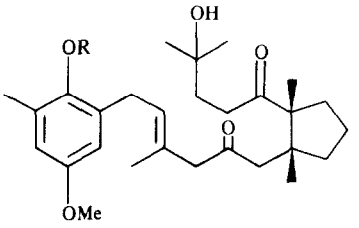

4 R = Me
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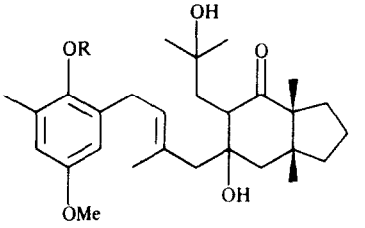

5 R = Me
6 R = H

HPLC analyses of 15 samples of the putative hybrid revealed that the profile of secondary metabolites in the lipid fraction is essentially a composite of that of the parent species, as compounds **1**–**8** were always present

In conclusion, the chemical characters validates the natural hybrid between *C elegans* and *C algeriensis*, suspected from consideration of morphological and anatomical features, this result extends to algae the utilization of chemical markers as a criterion for interspecific hybridization Tetraprenyltoluquinols, a class of metabolites of mixed biogenesis widespread in the family Cystoseiraceae, appear promising candidates as species-specific markers in the study of natural hybridization within the genus *Cystoseira*

EXPERIMENTAL

MS direct inlet, 70 eV, ^{1}H and ^{13}C NMR 80 and 20.1 MHz respectively

Plant material The individuals from 'pure' populations serving as standard types for the morphological and chemical analyses, all of comparable size and developmental stage, were obtained from the following localities *C elegans*, near Capo Passero, Sicily and *C algeriensis*, off a rocky islet (Isola dei Porri), 6 miles south-west of Pozzallo, Sicily Collections of putative hybrids of *C elegans* × *C algeriensis* on the basis of their exomorphic features were made from an intermediate locality (Punta delle Formiche) Voucher specimens are deposited in the Herbarium of the Department of Botany, Palermo, Italy

Chromatography TLC silica gel F_{254} (Merck), detection by spraying with 1% $Ce(SO_4)_2$ in 1 M H_2SO_4 or by UV light (254 nm), Prep LC LiChrosorb Si-60, 25–40 μ, HPLC Hypersil 5 μ column (4 × 200 mm), solvent systems (1 ml/min flow rate) *i*-PrOH–heptane (3 97), Et_2O–hexane (2 3), UV monitor (290 nm)

Isolation of compounds Shade dried alga (300 g) was extracted (× 3) with $CHCl_3$ at room temp with continuous stirring The extracts were pooled and evapd to give a dark green oil that was applied to an open column (2 × 80 cm) of silica gel The column was eluted with increasing concentrations of Et_2O in hexane Fractions of 50 ml were collected and those exhibiting similar TLC profiles combined Further purification of crude compounds and/or separation of mixtures were carried out using PLC or HPLC

Chromatographic examination of plant extracts Individual plants were freeze-dried, ground and extracted (x3) with $CHCl_3$ at room temp with continuous stirring Evaporation of the solvent gave a residue that was dissolved in Et_2O and purified on a Sep-Pak Florisil cartridge (Waters Associates) The eluates were used for TLC and HPLC *C algeriensis* yielded, in order of increasing polarity, the known tetraprenyltoluquinols **1**, **2**, **5** and **6**, identified by comparison of their spectral properties with those of reference samples, available from previous work [5], while *C elegans* afforded two known (**3** and **4**) and two unknown metabolites The structures of the latter were easily-established as **7** and **8** from their spectral properties

Compound **7** $[\alpha]_{20}(\lambda)$ +12 5 (589), +12 1 (578), +13 7 (546), IR ν_{max}^{film} cm^{-1} 3450, 1705, 1697, 1610, UV λ_{max}^{EtOH} nm (ε) 288 (3600), 218 (12250), HRMS [M]$^+$ 458 3029 (calc for $C_{28}H_{42}O_5$ 458 3032), MS *m/z* (rel int), 458 (13), 440 (80) 422 (10), 235 (40), 205 (34), 191 (90), 189 (60), 175 (30), 151 (65), 139 (100), 97 (58), 95 (90), 69 (60), 67 (31), 55 (28), 43 (35), 41 (40), ^{1}H NMR (80 MHz, $CDCl_3$, TMS). δ6 43 (2H, *br s*, H-3′ and H-5′), 5 27 (1H, *t*, J = 7 5 Hz, H-2), 3 58 (3H, *s*, OMe), 3 15 (2H, *d*, J = 7 5 Hz, H-1), 2 93 (2H, *s*, H-4), 2 62 and 2 31 (2H, AB system, J = 16 Hz, H-6), 2 52 (2H, *t*, J = 7 Hz, H-13), 2 20 (3H, *s*, 6′-Me), 1 70 (2H, H-14, overlapped), 1 65 (3H, *s*, H-20), 1 22 (6H, 2*s*, H-16 and H-17), 1 02 (3H, *s*, H-19), 0 95 (3H, *s*, H-18), ^{13}C NMR (20 1 MHz, $CDCl_3$) δ217 4*s* (C-12), 209 8*s* (C-5), 153 2*s* (C-4′), 147 3*s* (C-1′), 130 5*s*, 127 3*s*, 127 0*s* (C-2′, C-6′, C-3), 126 4*d* (C-2), 114 7*d*, 113 3*d* (C-3′, C-5′), 70 3*s* (C-15), 60 7*s* (C-11), 55 9*q* (OMe) 49 5*t* (C-4),

Table 1 Comparison of morphological features of *C elegans* × *C. algeriensis* and its parent species

Character	*C elegans* × *C algeriensis*	*C elegans*	*C algeriensis*
Habit			
— Tophules	Spinulate and verrucose-tuberculate	Spinulate	Smooth or slightly verrucose-tuberculate
— Secondary and tertiary laterals	Acute and flexuous	Cylindric and flexous	Acute and stiff
— Conceptacles	In the axial part of the branches	In the axial part of the branches	In the basal part of branches
— Receptacles	Compact	Compact	Slender or slightly compact
Size of cells in cross section (μm)			
— Tophule			
— Meristoderm	13 × 15	12 × 17	14 × 16
— Cortex	48 × 60	52 × 57	40 × 58
— Medulla	36 × 40	38 × 47	34 × 49
— Axis			
— Meristoderm	13 × 16	17 × 21	13 × 16
— Cortex	28 × 21	18 × 24	47 × 54
— Medulla	18 × 23	38 × 44	38 × 46
Size in cross section (μm)			
— Conceptacles	315 × 362	320 × 347	232 × 264
— Antheridia	19 × 33	13 × 36	9 × 18
— Oogonia	32 × 89	63 × 102	50 × 68
Number of conceptacles in the cross section of receptacles	7	2	6

48 6*t* (C-6), 46 3*s* (C-7), 36 6*t*, 36 2*t*, 36 2*t*, 33.5*t* (C-8, C-10, C-13, C-14), 31 0*t* (C-1), 29 6*q*, 29 6*q* (C-16, C-17), 24 5*q* (C-20), 22 0*q*, 21 6*q* (C-18, C-19), 19 6*t* (C-9), 16 6*q* (Me-6′).

Compound **8**. $[\alpha]_{20}(\lambda)$: +19 1° (589), +19 8° (578), +22 9° (546); IR ν_{max}^{film} cm^{-1} 3450, 1705, 1695, 1605, UV λ_{max}^{EtOH} nm (ε). 288 (3140), 220 (10 700), HRMS. $[M]^+$ 458 3820 (calc. for $C_{28}H_{42}O_5$ 458 3032), MS *m/z* (rel int) 458 (8), 440 (67), 422 (12), 235 (25), 205 (33), 191 (83), 189 (58), 175 (25), 151 (62), 139 (100), 97 (62), 95 (96), 69 (58), 67 (71), 55 (29), 43 (33), 41 (42); ^{1}H NMR (80 MHz, $CDCl_3$, TMS) δ6 43 (2H, *br s*, H-3′ and H-5′), 5.27 (1H, *t*, *J* = 7 5 Hz, H-2), 3 65 (3H, *s*, OMe), 3 27 (2H, *d*, *J* = 7 5 Hz, H-1), 3 00 (2H, *s*, H-4), 2 65 and 2.34 (2H, AB system, *J* = 17 Hz, H-6), 2 52 (2H, *t*, *J* = 7 Hz, H-13), 2 17 (3H, *s*, 6′-Me), 1.70 (2H, H-14, overlapped), 1 69 (3H, *s*, H-20), 1 19 (6H, 2*s*, H-16 and H-17), 1 02 (3H, *s*, H-19), 0 90 (3H, *s*, H-18), ^{13}C NMR (20.1 MHz, $CDCl_3$ δ217 4*s* (C-12), 209 7*s* (C-5), 153 5*s* (C-4′), 146 9*s* (C-1′), 131 2*s*, 128 1*s*, 127 8*s* (C-2′, C-6′, C-3), 126.3*d* (C-2), 114.4*d*, 113 3*d* (C-3′, C-5′), 70 3*s* (C-15), 60 7*s* (C-11), 55.8*q* (OMe), 55 8*t* (C-4), 48 2*t* (C-6), 46 2*s* (C-7), 36 6*t*, 36 6*t*, 36 1*t*, 33 5*t* (C-8, C-10, C-13, C-14), 30.7*t* (C-1), 29 5*q*, 29.5*q* (C-16, C-17), 21.9*q*, 21 4*q* (C-18, C-19), 19 5*t* (C-9), 16 8*q*, 16 4*q* (C-20, Me-6′)

Histology The pertinent portions of the algae, taken from plants of comparable life stage and size, were processed by the usual alcohol dehydration, paraffin infiltration series. Cross sections 20 μm thick were mounted on slides and stained with safranin For each section, the size of 10 cells was measured and the mean values are shown in Table 1 The size of conceptacles refers to cross-sections of completely developed and mature receptacles

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