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FIVE NOVEL TETRAPRENYLHYDROQUINOLS FROM THE BROWN ALGA *CYSTOSEIRA ALGERIENSIS*

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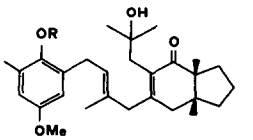
Key Word Index—*Cystoseira algeriensis*; Cystoseiraceae; brown algae; tetraprenylhydroquinols.

Abstract—Five novel metabolites of mixed biogenesis have been isolated from the brown alga *Cystoseira algeriensis*. They are tetraprenylhydroquinols and their structures have been determined by spectral analysis and chemical correlations.

INTRODUCTION

In a search for bioactive compounds from marine sources, we recently began to investigate the comparative chemistry of Mediterranean species of the genus *Cystoseira* (Phaeophyta). As a result of these studies, a variety of tetraprenylhydroquinol derivatives have been isolated and characterized [1–5]. Structurally related compounds have also been obtained from seaweeds belonging to other genera of the family Cystoseiraceae [6–11] and from members of the family Sargassaceae [12, 13].

The recent examination of *Cystoseira algeriensis* Feldm. has led to the isolation of a compound, cystalgerone, whose structure, with relative stereochemistry only, could be assigned as **1** [4]. Further investigation of the lipids from this species has now led to the isolation of five new metabolites (**2**–**6**), the structures of which are reported here.



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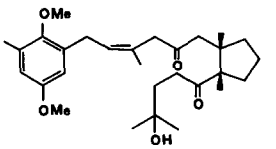

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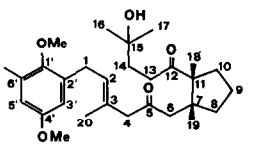

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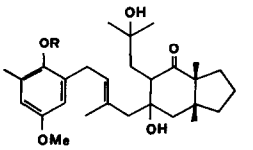

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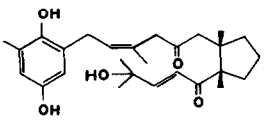

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RESULTS AND DISCUSSION

The chloroform extract of the alga was submitted to open column chromatography on silica gel, using increasing proportions of ether in hexane as the eluent, to afford several enriched fractions. Repeated chromatography and, whenever possible, final recrystallization, led to the isolation of the individual components.

The most abundant of the new compounds, **4**, $[\alpha]_D^{20} = +25°$, a liquid, had molecular formula $C_{29}H_{44}O_5$ and possessed an alcohol (ν_{OH} 3435 cm^{-1}) and two ketone (ν_{CO} 1712 and 1695 cm^{-1}; ^{13}C NMR: δ209.7 and 216.7) functions. UV absorptions at 225 and 283 nm (ε = 14 000 and 3100) suggested a hydroquinol chromophore. The ^1H NMR spectrum presented signals for two aromatic methoxyls (δ3.74 and 3.68), a methyl group on a benzene ring (δ2.27) and a benzylic methylene (δ3.39, *d*, *J* = 7.5 Hz), besides the resonance of two *meta*-coupled (*J* = 3 Hz) aromatic protons at δ6.56 and 6.59. These data suggested the presence of the partial structure depicted in **A**, which was confirmed by an intense ion at *m/z* 165 in the mass spectrum of **4**. The benzylic doublet was coupled with the vinyl proton at δ5.40 (*t*, *J* = 7.5 Hz) which was in turn allylically coupled with the vinyl methyl at 1.74. The spectrum also contained two AB quartets centred at δ3.10 (*J* = 13 Hz) and 2.75 (*J* = 15.5 Hz) assigned respectively to C-4 and C-6 methylenes, a 2H-triplet (*J* = 7.5 Hz) at 2.59 coupled with a 2H-signal at 1.70 (overlapped with other resonances) assigned respectively to the C-13 and C-14 methylenes, two methyl singlets on oxygen-bonded carbon (1.26, C-16 and C-17), two methyl singlets at 0.94 and 1.06 (C-18 and C-19) and complex signals totalling six protons at 1.4, 1.7 and 1.9 (C-8, C-9 and C-10). Data given above and evidence gained from the mass spectrum (see Experimental) suggested for the new compound structure **4** (devoid of stereochemistry). The ^{13}C NMR spectrum (Table 1) also agreed with the proposed structure in which the *E*-geometry of the side chain double bond was indicated by the chemical shift (δ16.4) of the vinyl methyl [14]. This structural hypothesis

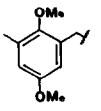
Table 1. ^{13}C NMR spectra of compounds 1–6*

Pos.	1†	2	3	4	5	6
C-1′	150.5 *s*	146.5 *s*	150.7 *s*	150.9 *s*	150.2 *s*	146.5 *s*
C-2′	134.9 *s*	127.5 *s*	134.8 *s*	134.0 *s*	134 7 *s*	127.5 *s*
C-3′	112.7 *d*	113.2 *d*	113.0 *d*	113.0 *d*	112 6 *d*	113.2 *d*
C-4′	155.4 *s*	153.6 *s*	156.0 *s*	156.0 *s*	155.4 *s*	153.4 *s*
C-5′	113.7 *d*	114.2 *d*	114 4 *d*	114 4 *d*	113.6 *d*	114.2 *d*
C-6′	131.7 *s*	125 1 *s*	132.2 *s*	132.4 *s*	133.6 *s*	125.2 *s*
C-1	29.2 *t*	29.8 *t*	28.8 *t*	28.7 *t*	28.6 *t*	28.7 *t*
C-2	125 5 *d*	125.6 *d*	127.4 *d*	128.3 *d*	128.4 *d*	127.9 *d*
C-3	131.5 *s*	132.5 *s*	130.1 *s*	130.4 *s*	131 6 *s*	135.3 *s*
C-4	40.1 *t*‡	45.3 *t*‡	48.7 *t*	55.9 *t*	47.4 *t*	47 6 *t*
C-5	154.0 *s*	154 5 *s*	208.6 *s*	209.7 *s*	80.5 *s*	80.3 *s*
C-6	42.5 *t*‡	41.6 *t*‡	48.6 *t*	48.3 *t*	41.7 *t*	42.4 *t*
C-7	44.6 *s*	44 6 *s*	46.1 *s*	46.1 *s*	45.6 *s*	45.8 *s*
C-8	34.8 *t*§	35.0 *t*§	36.7 *t*‡	36.7 *t*‡	34.4 *t*‡	34.7 *t*‡
C-9	18.8 *t*	18.9 *t*	19.6 *t*	19.5 *t*	19 1 *t*	19.2 *t*
C-10	28.6 *t*§	29.5 *t*§	36.1 *t*‡	36.0 *t*‡	35.4 *t*‡	35.6 *t*‡
C-11	54.9 *s*	55.1 *s*	60.7 *s*	60.6 *s*	58.1 *s*	58 2 *s*
C-12	208.5 *s*	208.6 *s*	217.0 *s*	216.7 *s*	213 2 *s*	214 7 *s*
C-13	138.3 *s*	134.1 *s*	36.5 *t*‡	36.6 *t*‡	54.4 *d*	54.2 *d*
C-14	40.2 *t*‡	39.1 *t*‡	33.6 *t*‡	33.6 *t*‡	28.4 *t*‡	30.2 *t*‡
C-15	70.9 *s*	71.4 *s*	70.1 *s*	70 1 *s*	70.6 *s*	71.0 *s*
C-16	28 9 *q*	29.8 *q*	29.6 *q*	29.6 *q*	28.5 *q*	29.0 *q*
C-17	30.8 *q*	30.4 *q*	29.6 *q*	29.6 *q*	30.7 *q*	30 8 *q*
C-18	22.5 *q*	22 7 *q*	21.9 *q*	22 0 *q*	22 9 *q*	23 2 *q*
C-19	21.0 *q*	21.0 *q*	20.5 *q*	21 4 *q*	22.2 *q*	22.5 *q*
C-20	17.9 *q*	16.4 *q*	24.3 *q*	16.7 *q*	17.9 *q*	18.3 *q*
OMe	60.5 *q*	—	60.3 *q*	60 8 *q*	60.2 *q*	—
OMe	55 3 *q*	55.8 *q*	55.6 *q*	55.6 *q*	55.2 *q*	55.7 *q*
6′-Me	16 3 *q*	16.4 *q*	16.5 *q*	16 4 *q*	16.2 *q*	16 2 *q*

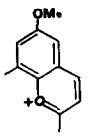
*^{13}C NMR spectra were recorded in $CDCl_3$ at 100 MHz (compounds 1 and 5) and 20.1 MHz (compounds 2, 3, 4 and 6); TMS as internal standard; multiplicities were obtained for spectra at 100 MHz with DEPT sequence and for those at 20.1 MHz by off-resonance decoupling experiments.

†Added for comparison

‡, §Interchangeable



(A)



(B)

was confirmed by base treatment of 4 which gave, through an aldol condensation and subsequent dehydration, a product indistinguishable from 1. Furthermore, the identity of the optical rotations of the semisynthetic and natural product allowed the assignment of the (relative) stereochemistry to the chiral centres at C-7 and C-11.

Another of the novel compounds isolated from *C. algeriensis*, 3, $[\alpha]_D^{20} = +12°$, was a liquid, $C_{29}H_{44}O_5$. Its spectral properties [λ_{max}^{EtOH} nm (ε): 220 (13 900) and 285 (2900); $\nu_{max}^{CCl_4}$ cm^{-1}: 3490 (OH), 1712 (CO), 1697 (CO), 1602, 1595; ^{1}H NMR and ^{13}C NMR see Tables 1 and 2] strikingly resembled those of 4 and indicated the presence of the same structural groups. Moreover, the isomers 3 and 4 exhibited almost identical mass spectra. A single characteristic difference observed in the ^{13}C NMR spectra was the downfield shift of the vinyl methyl from δ 16.7 in 4 to 23.3 in 3, which is consistent with a *Z*-geometry of the side-chain double bond [14]. The relative stereochemistry at the chiral centres has been assumed to be the same as in 4 on the basis of the observation that in the ^{13}C NMR spectrum of 3 the values of the chemical shift for carbons C-7, C-11, C-18 and C-19 are nearly identical to those of the corresponding carbons in the isomeric metabolite (Table 1). Compounds 3 and 4 are closely related to bifurcarenone 7, recently isolated from *Bifurcaria galapagensis* (Cystoseiraceae) [6].

The third compound isolated, 5, mp 105–107°, $[\alpha]_D^{20} = +3°$, had the molecular formula $C_{29}H_{44}O_5$ and its IR spectrum revealed both hydroxyl (3370 cm^{-1}) and non-conjugated carbonyl (1710 cm^{-1}) absorption. In addition, the UV gave λ_{max}^{EtOH} nm (ε): 283 (2200) and 220 (9500). The ^{13}C NMR spectrum (Table 1) showed similarities with cystalgerone (1), the most significant difference being the replacement of two sp^2-hybridized carbons by a hydroxyl-bearing quaternary carbon at δ 80.5 and a methine carbon at 54.4. In the ^{1}H NMR spectrum (Table 2) the C-13 proton appeared as a triplet (J = 5.6 Hz) at δ 3.32, a value

Table 2. ^{1}H NMR spectra of compounds 1–6*

Pos.	1†	2	3	4	5	6
H-3′	6.52 } AB (3)	6.47 } AB (3)	6.54 } AB (3)	6.56 } AB (3)	6.52 } AB (3)	6.46 } AB (3)
H-5′	6.57 }	6.52 }	6.58 }	6.59 }	6.56 }	6.50 }
H-1	3.37 *d* (7.5)	3.37 *d* (7.5)	3.24 *d* (7.5)	3.39 *d* (7.5)	3.39 *m*‡	3.32 *m*§
H-2	5.37 *t* (7.5)	5.34 *t* (7.5)	5.36 *t* (7.5)	5.40 *t* (7.5)	5.32 *t* (7.5)	5.27 *t* (7.5)
H-4	3.06 } AB (14.5)	3.04 } AB (13.5)	3.14 s	3.11 s	2.49 } AB (14.5)	2.49 } AB (15)
	2.97 }	2.96 }			2 13 }	2.14 }
H-6	2.45 } AB (18.5)	2.43 } AB (18)	3.02 } AB (16)	3.04 } AB (15.5)	2.15 } AB (14.5)	2.13 } AB (15)
	2.22 }	2.18 }	2.38 }	2.47 }	2.04 }	2.06 }
H-13	—	—	2.52 *t* (6.5)	2.59 *t* (7.5)	3.32 *t* (5.6)	3.29 *t* (6)
H-14	2.74 } AB (14.5)	2.72 } AB (13.5)	*ca* 1.70	*ca* 1.70	2.20 *dd* (14.5–5.6)	2.18 *dd* (15–6)
	2.47 }	2.45 }			1.75 *dd* (14.5–5.6)	1.73 *dd* (15–6)
H-16	1.13 *s*	1.13 *s*	1.17 *s*	1.26 *s*	1.26 *s*	1 24 *s*
H-17	1.22 *s*	1.22 *s*	1.17 *s*	1.26 *s*	1 30 *s*	1.27 *s*
H-18	0.81 *s*	0.81 *s*	0.92 *s*	0 94 *s*	0.70 *s*	0.68 *s*
H-19	1.04 *s*	1.04 *s*	1.03 *s*	1.06 *s*	1.19 *s*	1.16 *s*
H-20	1.67 *s*	1.69 *s*	1.67 *s*	1.74 *s*	1.84 *s*	1.85 *s*
OMe	3.68 *s*	3.71 *s*	3.55 *s*	3.68 *s*	3.67 *s*	3.70 *s*
OMe	3.74 *s*	—	3.63 *s*	3.74 *s*	3.74 *s*	—
6′-Me	2.27 *s*	2.22 *s*	2.20 *s*	2.27 *s*	2.26 *s*	2.18 *s*

*^{1}H NMR spectra were recorded in $CDCl_3$ at 400 MHz (compounds **1** and **5**) and 270 MHz (compounds **2**, **3**, **4** and **6**); TMS as internal standard; coupling constants, (*J*) in parentheses, are given in Hz; assignments were confirmed by decoupling.

†Added for comparison.

‡AB part of an ABX system, modified on irradiation at δ5.32 (X part) into an AB system, δ3.41 and 3.37, J = 14 Hz.

§AB part of an ABX system, modified on irradiation at δ5.27 (X part) into an AB system, δ3.34 and 3 30, J = 14 Hz

in accord with a methine vicinal to a carbonyl group, coupled with an isolated methylene group (C-14, *dd*, δ2.20 and 1.75, J = 14.5 and 5.6 Hz). The data above and consideration of the mass spectrum (see Experimental) led to formulation of the new algal metabolite as **5**. The final assignment of the structure and relative configuration at C-7 and C-11 was achieved by chemical correlation. When **5** was subjected to dehydration ($POCl_3$ in pyridine or Florisil in refluxing benzene–ether) cystalgerone (**1**) was obtained. Furthermore, compound **5** by heating at 200° was in part dehydrated to give **1** and in part converted, through a thermally-induced retroaldol reaction, into **4**. In order to determine the stereochemistry at C-5 and C-13, attempts are currently being made to obtain crystals suitable for X-ray diffraction analysis.

Compound **2** was obtained as crystals, mp 112–113°, $[\alpha]_D^{20} = +42°$, $C_{28}H_{40}O_4$, and had an IR spectrum which showed hydroxyl (3450 cm^{-1}) and conjugated carbonyl (1665 cm^{-1}) absorptions. The UV spectrum exhibited bands at 287 nm (ε = 2800), 246 (ε = 7200) and 222 (ε = 9500). ^{13}C and ^{1}H NMR spectra (Tables 1 and 2) suggested a structure closely related to cystalgerone (**1**) with a hydroxyl function replacing one of the aromatic methoxy groups. The presence in the mass spectrum of **2** of a fragment ion at m/z 189, which can be explained by the oxonium structure **B** [8], and the chemical shift of the methoxyl (δ55.8) in the ^{13}C NMR spectrum allowed the hydroxyl to be placed at the C-1′ instead of the C-4′ position [9]. In accordance with this structural hypothesis, treatment of compound **2** with methyl iodide in the presence of potassium carbonate gave a substance identical in all respects, including optical rotation, with cystalgerone (**1**).

The last compound isolated from *C. algeriensis*, **6**, mp 172–173°, $[\alpha]_D^{20} = +4°$, had the molecular formula $C_{28}H_{42}O_5$. An inspection of its spectral properties [λ_{max}^{EtOH} nm (ε): 215 (9300) and 289 (2900); $\nu_{max}^{CHCl_3}$ cm^{-1}: 3410 (OH), 1700 (CO), 1600 (aromatic); ^{13}C and ^{1}H NMR: see Tables 1 and 2] indicated that this substance bears to **5** the same relationship as **2** does to **1**. In the event, when **6** was methylated as above, compound **5** was obtained. This result firmly established the structure of **6**.

EXPERIMENTAL

General All mps were taken on a Kofler block and are uncorr. MS analyses were performed with a direct inlet system at 70 eV. IR spectra were determined on a Perkin–Elmer mod. 684 and UV spectra on a Perkin–Elmer mod. 330 spectrophotometers. ^{1}H NMR spectra were measured at 400 MHz and at 270 MHz on Bruker AM-400 and Bruker WP-270 FT instruments, respectively ^{13}C NMR spectra were run at 100 MHz and 20.1 MHz on Bruker AM-400 and WP-80 FT instruments, respectively Chemical shifts are quoted in ppm (δ) relative to TMS. Optical rotations were determined with a Perkin–Elmer 141 polarimeter Preparative liquid chromatography (PLC) and high performance liquid chromatography (HPLC) were carried out on Jobin-Yvon MiniPrep LC and Varian 5020 instruments.

Plant material. Cystoseira algeriensis Feldman (voucher specimen deposited at the Herbarium of the Institute of Botany, Palermo, Italy) was collected on rocks at about 1 m depth in November 1982 near Pachino, Sicily.

Extraction and isolation of constituents Shade dried and ground alga (2 kg) was extracted × 3 with $CHCl_3$ at room temp. with continuous stirring. The extracts were pooled and evaporated to give a dark green oil (28 g) The crude extract was applied to an open column (4 × 120 cm) of silica gel. The column

was eluted with increasing concentrations of Et_2O in hexane. Fractions of 200 ml were collected and those exhibiting similar TLC profiles combined. After eluting cystalgerone (**1**), the new compounds were eluted from the column in the order: **2**, **3**, **4**, **5** and **6**.

Fractions 70–73 were pooled and subjected to preparative TLC (LiChroprep Si-60, C_6H_{12}–Me_2CO, 85.15). Crystallization from hexane gave pure **2** (52 mg, 0.0026% dry wt); mp 112–113°, $[\alpha]_{20}^{\lambda}$: +42° (589), +44° (578), +50° (546) (*c* 1.2; EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}. 3450, 1665, 1605, UV λ_{max}^{EtOH} nm (ε): 287 (2800), 246 (7200), 222 (9500); HRMS. $[M]^+$ 440.2935 (calc. for $C_{28}H_{40}O_4$ 440.2926), MS *m/z* (rel. int.): 440 (5), 422 (100), 417 (21), 272 (35), 271 (38), 257 (28), 229 (26), 191 (82), 189 (39), 152 (28), 151 (80), 147 (36), 105 (24), 95 (18), 91 (33), 55 (19), 43 (44), 41 (30)

Fractions 76–85 were evaporated to give an oily residue (1.65 g) which was subjected to preparative HPLC (Porasil, hexane–*i*-PrOH, 98.2) to give **3** (172 mg, 0.0086% dry wt) and **4** (1.4 g, 0.07%).

Compound **3**, oily, $[\alpha]_{20}^{\lambda}$. +12° (589), +14° (546), +26° (436), +42° (365) (*c* 1.3, EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3490, 1712, 1697, 1602, 1595; UV λ_{max}^{EtOH} nm (ε): 220 (13 900), 285 (2900); HRMS. $[M]^+$ 472.3179 (calc. for $C_{29}H_{44}O_5$ 472.3188), MS *m/z* (rel int): 472 (25), 454 (25), 247 (33), 235 (8), 219 (25), 205 (16), 189 (33), 165 (100), 152 (33), 139 (75), 135 (75), 109 (25), 97 (33), 95 (83), 91 (41), 81 (25), 79 (25), 69 (41), 67 (33), 55 (50), 43 (91), 41 (83).

Compound **4**, oily, $[\alpha]_{20}^{\lambda}$: +25° (589), +26° (578), +31° (546), +58° (436), +113° (365) (*c* 1.1, EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3435, 1712, 1695, 1605, 1595; UV λ_{max}^{EtOH} nm (ε): 225 (14 000), 283 (3100); HRMS $[M]^+$ 472.3163 (calc. for $C_{29}H_{44}O_5$ 472.3188); MS *m/z* (rel. int.). 472 (3), 454 (65), 247 (36), 235 (61), 219 (36), 205 (36), 189 (21), 165 (100), 152 (60), 138 (91), 135 (51), 109 (18), 97 (76), 95 (91), 91 (15), 81 (18), 79 (12), 69 (54), 67 (15), 55 (24), 43 (24), 41 (30).

Evaporation of fractions 90–94 gave a semicrystalline residue which was subjected to preparative TLC (Et_2O–CH_2Cl_2, 1:9) followed by recrystallization from hexane–Et_2O (50:50) to give pure **5** (730 mg, 0.036% dry wt), mp 105–107°; $[\alpha]_{20}^{\lambda}$: +3° (589), +4° (578), +6° (546), +20° (436), +68° (365) (*c* 1.5; EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}. 3370, 1710, 1610; UV λ_{max}^{EtOH} nm (ε): 220 (9500), 283 (2200); HRMS: $[M]^+$ 472.3167 (calc. for $C_{29}H_{44}O_5$ 472.3188); MS *m/z* (rel int.) 472 (0.8), 454 (15), 436 (77), 421 (15), 396 (31), 271 (15), 235 (54), 231 (31), 219 (31), 205 (46), 165 (100), 139 (23), 135 (69), 111 (23), 109 (23), 97 (31), 95 (61), 91 (23), 81 (23), 79 (15), 69 (31), 55 (31), 43 (38), 41 (31).

Compound **6** was obtained from fractions 95–98 by preparative TLC (hexane–Et_2O, 3:7) followed by HPLC (CH_2Cl_2–*i*-PrOH, 97.3) and finally crystallization from Et_2O (30 mg, 0.0015% dry wt). Pure **6** had mp 171–173°; $[\alpha]_{20}^{\lambda}$. +4° (589), +4° (578), +5° (546), (*c* 1.2, EtOH); IR $\nu_{max}^{CCl_4}$ cm^{-1}. 3410, 1700, 1600; UV λ_{max}^{EtOH} nm (ε): 215 (9300), 289 (2900), HRMS. $[M]^+$ 458.3019 (calc. for $C_{28}H_{42}O_5$ 458.3032); MS *m/z* (rel int.). 458 (0.5), 440 (19), 422 (44), 407 (20), 379 (5), 333 (9), 271 (16), 235 (31), 206 (22), 205 (21), 191 (64), 189 (25), 175 (15), 152 (21), 151 (57), 147 (20), 139 (41), 111 (21), 109 (16), 105 (15), 97 (41), 95 (100), 91 (20), 81 (20), 69 (27), 67 (17), 59 (19), 55 (26), 43 (37), 41 (37).

Dehydration of **5** *to give* **1**. $POCl_3$ (2 ml) was added to a soln of **5** (100 mg) in pyridine (5 ml) and the mixture was stirred at 0° for 30 min. The soln was then diluted with H_2O (5 ml) and extracted with Et_2O. Evaporation of the solvent left a residue which was subjected to preparative TLC (Et_2O–hexane, 3.7) to afford 25 mg of a compound identical in all respects (mp, $[\alpha]$, UV, IR, ^1H NMR) to **1**. Better yields were obtained with the following alternative procedure. compound **5** (100 mg) was dissolved in C_6H_6–Et_2O (2.1, 30 ml), Florisil (activated at 120°, 2 g) was added and the mixture refluxed with stirring for 6 hr. The filtered soln was evaporated and the residue purified by preparative TLC to give **1** (70 mg).

Thermal treatment of **5** *to give* **1** *and* **4**. Compound **5** (15 mg) was heated in a sealed tube under N_2 at 200° for 10 min. The product was separated by preparative TLC (LiChroprep, Et_2O–hexane, 35:65) into two components, **1** (5 mg) and **4** (6 mg).

Treatment of **4** *with base to produce* **1**. A 10% aq. soln of KOH (3 ml) was added to a soln of **4** (100 mg) in EtOH (5 ml). After 30 min H_2O (5 ml) was added and the soln extracted with Et_2O. The organic solvent was evaporated and the residue purified by preparative TLC (LiChroprep, Et_2O–hexane, 40:60) to give pure **1** (30 mg) indistinguishable (mp, $[\alpha]$, UV, IR, ^1H NMR) from the natural compound.

Methylation of **2** *to give* **1**. MeI (0.05 ml) and K_2CO_3 were added to a soln of **2** (5 mg) in Me_2CO and the mixture refluxed for 3 hr. The ppt was filtered off and the soln was evaporated. The crystalline residue was purified by preparative TLC (LiChroprep Si-60, C_6H_{12}–Me_2CO, 85:15) to give **1** (4 mg), identified by comparison of the physical properties (mp, $[\alpha]$, IR, UV, ^1H NMR) with those of reference sample.

Methylation of **6** *to give* **5** Methylation as above of **6** (5 mg) and preparative TLC (LiChroprep Si-60, Et_2O–CH_2Cl_2, 1.9) of the crude product gave **5** (3 mg), identified by comparison of the physical properties (mp, $[\alpha]$, IR, UV, ^1H NMR) with those of a reference sample.

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