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A HYDROXYLATED DITERPENOID SUBSTITUTED QUINOL FROM THE BROWN ALGA *CYSTOSEIRA ELEGANS*

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Key Word Index—*Cystoseira elegans*; Cystoseiraceae; brown alga; marine diterpenoids; tetraprenyl substituted quinols.

Abstract—A new metabolite has been isolated from the brown alga *Cystoseira elegans* and characterized as (2*E*, 6*E*, 14*E*)-1-(1′-hydroxy-4′-methoxy-6′-methylphenyl)-5,12-dihydroxy-13-one-3,7,11,15-tetramethyl hexadeca-2,6,14-triene by spectral analysis and chemical degradation.

We recently described the isolation and characterization of the new hydroxylated tetraprenyl substituted quinols, **1–4**, from the brown alga *Cystoseira elegans* collected along the Catalan coasts (June 1980) [1, 2]. We now report the isolation and characterization from this alga of a new diterpenoid substituted quinol of a similar mixed biogenesis.

Silica gel column chromatography of the methanol extract of the alga gave a fraction containing **5** as an impure oil. This fraction was further purified by HPLC to give **5** as an optically active oil (molecular composition $C_{28}H_{42}O_5$ by high resolution mass spectrometry). The spectral features of **5** (see Table 1 and Experimental) resembled those of compound **4** and indicated similar structural features. The main differences consisted of the presence of an α,β-unsaturated ketone ($\nu_{C=O}$ 1670, $\nu_{C=C}$ 1615 cm^{-1}) as indicated in the ^{13}C NMR spectrum ($\nu_{C=O}$ δ200.96), and in the low field ^1H NMR resonances at δ2.00, 2.25 (Me) and 6.15 (olefinic proton). The mass spectra of compound **5** and its acetate were characterized by a base peak at *m/z* 83.05007 as in eleganolone [3], indicative of a carbonyl group included in a CO–CH $=CMe_2$ constellation. The mass spectrum contained ions of significant intensity in agreement with the fragmentation patterns of such a linear structure.

Compound **5** was assigned as the more plausible one by studies of the ^1H and ^{13}C NMR features, including decoupling experiments [2, 4–6]. Mass spectral data and ^1H NMR data of the acetylated compound allowed confident assignments of the hydroxyl functions at C-5 and C-12.

The presence of an α-ketol was confirmed by oxidative cleavage [7] with alkaline hydrogen peroxide of the corresponding methylated compound, giving a carboxylic acid which was methylated with diazomethane to yield the corresponding methyl ester. This ester was investigated by ^1H NMR and was clearly similar, with the same stereochemistry at C-11 ($\delta_{C-H} = 2.30$), to the compound obtained from **4** by the same reaction [2].

The diterpenoid substituted quinol composition is independent of the size of the algae (e.g. it was in the largest algae > 40 cm that we found **5** for the first time) and the period of harvesting. All these results were expected by us for compounds playing a part in the chemical defence of *C. elegans*. However, we have found some *C. elegans* with eleganolone but without diterpenoid substituted quinols at other sites of harvesting, making for the moment these algae the only Cystoseiraceae which are able to biosynthesize acyclic diterpenoids or diterpenoids of mixed biogenesis. In fact, eleganolone seems to be present in the youngest seaweed and to be subject to important variations. Thus acyclic diterpenoids are found during March/April and not after this short period.

EXPERIMENTAL

Isolation of (2E,6E,14E)-1-(1′-*hydroxy*-4′-*methoxy*-6′-*methyl*-*phenyl*)-5,12-*dihydroxy*-13-*one*-3,7,11,15-*tetramethyl hexadeca*-2,6,14-triene (**5**). Freshly collected *C. elegans* (Banyuls-sur-Mer, France, June 1980) was frozen, ground to a fine powder with a blender in the presence of MeOH–H_2O (7:3) and extracted (× 3) with MeOH. The extracts were left overnight at −30° in order to precipitate the lipids. After filtration, the MeOH was evapd and the aq. phase extracted with Et_2O. After combination of the solvents and evapn, 1 g extract was obtained and this was applied to an open column of silica gel. The column was eluted with a solvent gradient from hexane to Et_2O. Compound **5** was eluted with hexane–Et_2O (3:2) and was subsequently purified by HPLC (40% EtOAc in isooctane).

5: $[\alpha]_D$ + 3.2° (*c* 1.86; MeOH); IR ν_{max}^{film} cm^{-1}: 3420, 1670, 1615; UV λ_{max}^{MeOH} nm (ε): 225 (12 400); ^1H and ^{13}C NMR see Table 1; HRMS: *m/z* 440.2934 $[M-H_2O]^+$ calc. for $C_{28}H_{40}O_4$, 440.29266; *m/z* (rel. int.): 440.2934 $[M-H_2O]^+$ (15.4), 257.1534 $[C_{17}H_{21}O_2]^+$ (4.1), 229.1125 $[C_{15}H_{17}O_2]^+$ (10.7), 217.1224 $[C_{14}H_{17}O_2]^+$ (9.0), 204.1135 $[C_{13}H_{16}O_2]^+$ (4.5), 191.1072 $[C_{12}H_{15}O_2]^+$ (93.9), 189.09129 $[C_{12}H_{13}O_2]^+$ (37.2), 177.0892 $[C_{11}H_{13}O_2]^+$ (4.8), 151.07579 $[C_9H_{11}O_2]^+$ (76.12), 150.06799 $[C_9H_{10}O_2]^+$ (13.8), 149.02395 $[C_9H_9O_2]^+$ (5.5), 123.0807 $[C_8H_{11}O]^+$ (7), 107.0859 $[C_8H_{11}]^+$ (12.7), 95.0862 $[C_7H_{11}]^+$ (11.2), 83.05007 $[C_5H_7O]^+$ (100), 79.0552 $[C_6H_7]^+$ (10.1), 77.0394 $[C_6H_5]^+$ (7.5).

Methylation of compound **5**. To a soln of **5** (40 mg) in dry

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for compound **5** (^{1}H: 360 MHz, TMS as internal standard; ^{13}C: 50 MHz, TMS as internal standard)

Position No.	^{13}C	^{1}H ($CDCl_3$)	^{1}H (C_6D_6)	^{1}H (acetate in $CDCl_3$)
		3.43 *dd* (16, 8)	3.37 *dd* (16, 7.5)	
1	30.5 *t*	3.33 *dd* (16, 6)	3.22 *dd* (16, 6)	3.13 *d* (17)
2	125.5 *d*	5.42 *t* (*br*) (7)	5.40 *t* (*br*) (7)	5.26 *t* (*br*) (7)
3	138.6 *s*			
4	47.9 *t*	‖	2.19 *dd* (14, 9)	
			2.07 *dd* (14, 4.5)	11
5	66.08 *d*	4.53 *ddd* (8.5, ?, ?)	4.41 *ddd* (8.5, 9, 4.5)	5.65 (8.7, ?, ?)
6	128.8 *d*	5.15 *d* (*br*) (8.5)	5.11 *d* (*br*) (8.5)	5.08 *d* (*br*) (8.7)
7	134.2 *s*			
8	39.5 *t*	1.94 *m*	1.80 *m*	‖
9	25.4 t	‖	‖	‖
10	29.4 *t*	‖	‖	‖
11	36.6 *d*	‖	‖	‖
12	80.7 *d*	3.70 *d* (6)	3.33 *d* (6)	4.85 *d* (4.5)
13	200.96 *s*			
14	130.8 *d*	6.15 *s*	5.78 *s*	6.12 *s*
15	159.2 *s*			
16	28.0 *q*	2.00 *s*	1.65 *s*	1.95§ *s*
17	21.4 *q*	2.25 *s*	2.08 *s*	2.16§ *s*
18	17.1 *q*	1.12 *d* (8)	1.14 *d* (8)	0.96 *d* (7)
19	16.4* *q*	1.68 *s*	1.44 *s*	1.68 *s*
20	16.4* *q*	1.85 *s*	1.46 *s*	1.71 *s*
1′	146.5 *s*			
2′	125.6† *s*			
3′	114‡ *d*	6.60 *d*	6.68 *d* (2.9)	6.59 *d* (3)
4′	153.2 *s*			
5′	113‡ *d*	6.56 *d*	6.63 *d* (2.9)	6.56 *d* (3)
6′	127.6† *s*			
7′	16.3* *q*	2.24 *s*	2.17 *s*	2.30 *s*
–OMe	55.6 *q*	3.77 *s*	3.43 *s*	3.75 *s*
–OH		4.92 and 4.10	3.96 and 3.92	2.13,§ 2.11,§ 1.94§ (COMe)

Figures in parentheses, *J* (Hz); multiplicities obtained by off-resonance decoupling experiments.

*, †, ‡, §, Assignments may be reversed.

‖Overlapped with other signals.

Me_2CO (4 ml) was added MeI (0.2 ml) and dry K_2CO_3 (400 mg). The mixture was refluxed for 3 hr, diluted with H_2O and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried ($MgSO_4$) and evapd to dryness. The resulting product was subjected to HPLC (20% EtOAc in TMP) to give the pure dimethyl substituted quinol product.

Oxidative cleavage. To 0.4 ml of a methanolic soln (0.022 mM) of the methylated compound **5** (20 mg) was added 0.1 ml 2 M NaOH, 0.09 mmol EDTA and 0.12 ml 0.5 M H_2O_2. After 2 hr, the soln was extracted with Et_2O. After solvent evapn, the acidic products were treated with CH_2N_2 and the resulting ester was purified on HPLC (10% EtOH in TMP). The main compound was rapidly assigned by comparison of its ^{1}H NMR spectrum with that of the product obtained from **4** with the same reaction [2].

Acetylation of compound **5**. Prepared from **5** by standard procedure. IR (film) cm^{-1}: 1740, 1720, 1680, 1615; ^{1}H NMR see Table 1; HRMS: *m/z* 524.31269 $[M-HOAc]^+$ calc. for $C_{32}H_{44}O_6$, 524.31379; *m/z* (rel. int.): 524.31269 (3), 482.3022 $[M-HOAc-CH_2CO]^+$ (5.9), 191.10732 (32.2), 189.0917 (17.4), 175.1488 (4.70), 166.06298 (9.57), 151.07578 (21.86), 150.0683 (5.02), 125.09668 (4.42), 107.0860 (4.8), 95.0858 (5.41), 83.0501 (100), 77.0397 (3).

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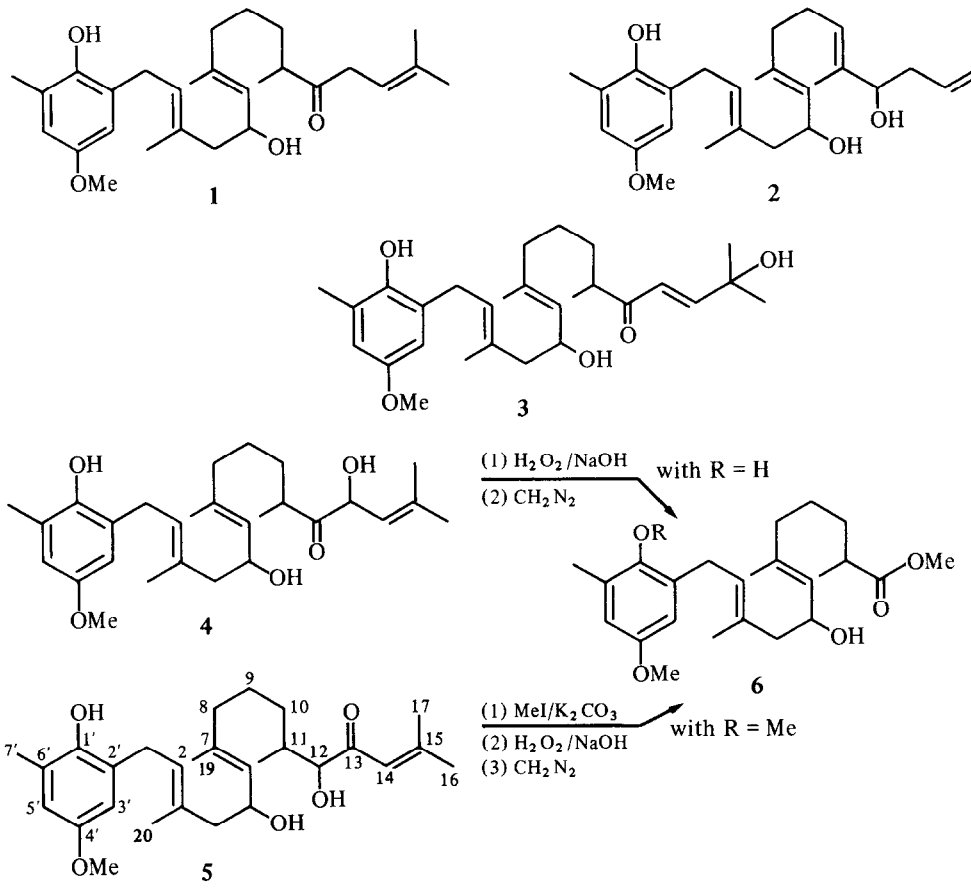

Scheme 1.

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