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NOVEL ACYCLIC DITERPENES FROM THE BROWN ALGA *CYSTOSEIRA CRINITA*

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Key Word Index—*Cystoseira crinita*; Cystoseiraceae; brown algae; acyclic diterpenes; (2*E*,10*E*)-1,6-dihydroxy-7-methylene-13-keto-3,11,15-trimethylhexadeca-2,10,14-triene; (2*E*,5*E*,10*E*)-1,7-dihydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene; (2*E*,10*E*)-1-hydroxy-6,13-diketo-7-methylene-3,11,15-trimethylhexadeca-2,10,14-triene.

Abstract—Three acyclic diterpenes have been isolated from the brown alga *Cystoseira crinita* and characterized as (2*E*,10*E*)-1,6-dihydroxy-7-methylene-13-keto-3,11,15-trimethylhexadeca-2,10,14-triene, (2*E*,5*E*,10*E*)-1,7-dihydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene and (2*E*, 10*E*)-1-hydroxy-6,13-diketo-7-methylene-3,11,15-trimethylhexadeca-2,10,14-triene.

Four acyclic diterpenes have been isolated so far from members of the family Cystoseiraceae (brown algae): crinitol (**1**) from *Cystoseira crinita* [1], eleganolone (**2**) from *C. elegans* [2], epoxyeleganolone (**3**) and eleganediol (**4**) from *Bifurcaria bifurcata* [3]. The isolation and structural elucidation of three novel acyclic diterpenes from *C. crinita* are reported in the present paper.

Open column Si gel chromatography of the chloroform extract of the dried alga, using hexane with increasing amounts of ether as the eluant, followed by HPLC of the more polar fractions gave pure **5a**, **6a** and **7**.

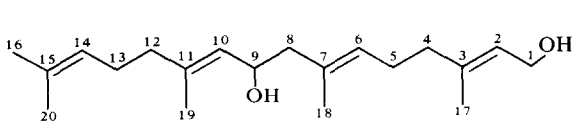
The first of the new diterpenes, **5a**, $C_{20}H_{32}O_3$, $[\alpha]_D^{EtOH} + 96.5°$, had an IR spectrum which showed hydroxyl and conjugated carbonyl absorptions. The consecutive losses of two molecules of water in the MS, and the formation of a diacetate (**5b**) established the hydroxylic nature of two oxygen atoms. A base peak at *m/z* 83, also observed in the MS of eleganolone, indicated that the carbonyl group was included in a $-COCH=C(Me)_2$ moiety. When **5a** was hydrogenated catalytically it gave the known 2,6,10,14-tetramethylhexadecan-4-one (**8**). This result demonstrated the nature of the carbon skeleton and at the same time indicated that both hydroxyls must be allylic. The ^1H NMR spectrum of **5a** was similar to that of eleganolone (Table 1), apart from the replacement of a vinyl methyl signal at δ 1.60 by two 1 H singlets at 4.87 and 5.06, attributable to the protons of a terminal methylene, and of a vinyl proton at 5.10 by a hydroxymethine triplet at 4.06. The above evidence, coupled with the ^{13}C NMR data (Table 2) led unambiguously to structure **5a**, in which the *trans*(*E*) configuration of the double bonds at C-2 and C-10 was indicated by the chemical shifts of the methyls at C-3 and C-11 in the ^{13}C NMR spectrum (16.58 and 16.39 ppm, respectively) [4], while the stereochemistry of the chiral centre at C-6 was established as *S* by application of Mislow's method [5].

Another of the new diterpenes (**6a**) isolated from *C. crinita* had a molecular formula $C_{20}H_{32}O_3$ and possessed a ketone function in a $-COCH=C(Me)_2$ moiety (ν_{max} 1680 and 1610 cm^{-1}, λ_{max} 242 nm, *m/z* 83). Two hydroxyl groups were revealed by the spectral properties (ν_{max} 3440 cm^{-1}, *m/z* 302 $[M^+ - H_2O]$ and 284 $[M^+ - 2H_2O]$); they were both allylic, as deduced by their facile elimination in the course of catalytic reduction, which led to the saturated ketone **8**. Furthermore, one hydroxyl resisted acetylation, showing it to be tertiary. Comparison of the ^1H NMR of **6a** with that of eleganolone (Table 1) showed that a vinyl methyl at δ 1.60 was replaced by a tertiary methyl at 1.26 deshielded by an adjacent oxygen function. Moreover, the olefinic region of the spectrum, which integrated for one proton more than eleganolone, included the AB part of an ABX_2 system whose X_2 part appeared as a doublet at 2.72. Taken together, the above properties and the ^{13}C NMR data permit the formulation of the new hydroxyketone as **6a**. The stereochemistry of the C-5 double bond was deduced to be *E* from the value of $J_{5,6}$ (15.5 Hz).

The third novel diterpene (**7**) had the molecular formula $C_{20}H_{30}O_3$. It contained a primary hydroxyl group (ν_{max} 3420 cm^{-1}, *m/z* 300 $[M^+ - H_2O]$; δ 4.12, *t*, $-CH_2OH$) and two conjugated carbonyls (ν_{max} 1675 cm^{-1}, 199.56 and 201.63 ppm), one of them in a $-COCH=C(Me)_2$ unit (*m/z* 83). Inspection of the ^1H and ^{13}C NMR data (Tables 1 and 2) in comparison with those of the congeners pointed to structure **7** for the new diterpene. This structure was confirmed by partial $NaBH_4$ reduction which gave optically inactive **5a**.

EXPERIMENTAL

Extraction and isolation. Cystoseira crinita (10 kg), collected in March 1979 near Catania, Sicily, was air-dried and ground to a fine powder with a blender. The dried alga (1100 g) was extracted



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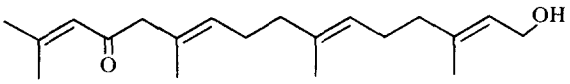

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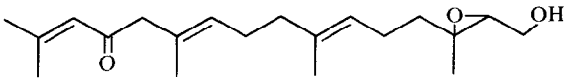

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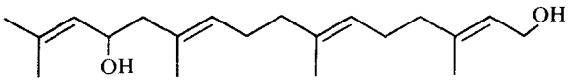

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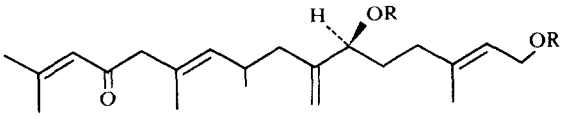

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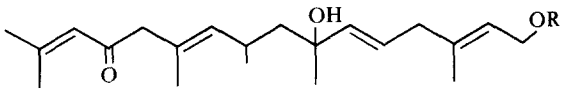
5b R = Ac



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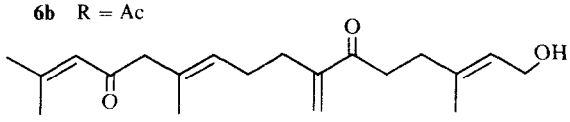

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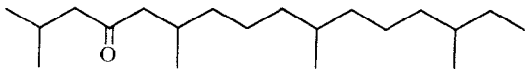

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Table 1. ^{1}H NMR spectral data of **2**, **5a**, **6a** and **7***

	2†	**5a**	**6a**	**7**
H-1	4.15 *d* (6.5)	4.14 *d* (6.5)	4.13 *d* (6.5)	4.12 *d* (6.5)
H-2	5.40 *t* (6.5)	5.43 *t* (6.5)	5.40 *t* (6.5)	5.36 *t* (6.5)
H-4	2.04	2.06‡	2.72 *d*‖	2.29 *t* (7.0)
H-5	2.04	1.65‡	5.55‖	2.84 *t* (7.0)
H-6	5.10 *t* (6.5)§	4.06 *t* (6.0)		
H-8	2.04	2.07‡	1.61‡	2.24‡
H-9	2.04	2.22 *m*	2.08‡	2.15‡
H-10	5.21 *t* (6.5)§	5.24 *t* (6.5)	5.22 *t* (6.5)	5.18 *t* (6.5)
H-12	3.03 *s*	3.04 *s*	3.02 *s*	3.01 *s*
H-14	6.10 *s*	6.09 *s*	6.06 *s*	6.30 *s*
H-16	1.87 *s*	1.88 *s*	1.86 *s*	1.88 *s*
H-17	1.66 *s*	1.67 *s*	1.66 *s*	1.67 *s*
H-18	1.60 *s*	4.87 *s* 5.06 *s*	1.26 *s*	5.70 *s* 5.96 *s*
H-19	1.60 *s*	1.62 *s*	1.59 *s*	1.59 *s*
H-20	2.12 *s*	2.14 *s*	2.12 *s*	2.12 *s*

* Run in $CDCl_3$ with TMS as internal standard on a 270 MHz instrument; chemical shifts are δ values; coupling constants (J in parentheses) are given in Hz; assignments were confirmed by decoupling.

† Added for comparison.

‡ Obscured by other signals.

§ Assignments are tentative.

‖ Protons at C-4, C-5 and C-6 form an ABX_2 system; $J_{AB} = 15.5$, $J_{AX} + J_{BX} = 5.4$ Hz.

Table 2. ^{13}C NMR spectral data of compounds **2**, **5a**, **6a** and **7***

	2†	5a	6a	7
C-1	59.39 *t*	59.45 *t*	59.38 *t*	59.46 *t*
C-2	124.43 *d*	124.19 *d*	124.67 *d*	124.26 *d*
C-3	139.37 *s*	139.50 *s*	137.72 *s*	138.65 *s*
C-4	39.66 *t*	33.76 *t*	42.31 *t*	34.07 *t*
C-5	26.42 *t*	35.77 *t*	125.52 *d*	36.26 *t*
C-6	124.07 *d*	75.06 *d*	138.45 *d*	201.63 *s*
C-7	135.30 *s*	151.95 *s*	72.92 *s*	148.79 *s*
C-8	39.41 *t*	31.28 *t*	42.31 *t*	30.97 *t*
C-9	26.90 *t*	27.69 *t*	28.22 *t*	27.15 *t*
C-10	123.22 *d*	123.41 *d*	123.27 *d*	123.34 *d*
C-11	129.84 *s*	130.39 *s*	130.06 *s*	130.81 *s*
C-12	55.51 *t*	55.33 *t*	55.19 *t*	55.39 *t*
C-13	199.87 *s*	199.70 *s*	199.49 *s*	199.56 *s*
C-14	129.67 *d*	129.17 *d*	129.46 *d*	128.62 *d*
C-15	155.77 *s*	155.97 *s*	155.70 *s*	155.84 *s*
C-16	20.71 *q*	20.77 *q*	20.63 *q*	20.77 *q*
C-17	16.03 *q*	16.58 *q*	16.26 *q*	16.52 *q*
C-18	16.03 *q*	110.23 *t*	23.12 *q*	124.26 *q*
C-19	16.39 *q*	16.39 *q*	16.38 *q*	16.52 *q*
C-20	27.69 *q*	27.69 *q*	27.56 *q*	27.69 *q*

*Run at 20.1 MHz, $CDCl_3$, ppm from TMS; multiplicities were obtained by off-resonance decoupling experiments; assignments are based on extrapolation from reported spectra [3].

† Added for comparison.

3 × with $CHCl_3$ and the extract was concd under vacuum to give a dark green oil (75 g). The crude extract was applied to a column (5 × 120 cm) of Si gel. The column was eluted with a solvent gradient system from hexane to Et_2O. Fractions of 250 ml were collected and those exhibiting similar TLC profiles were combined. Fractions 6–89 contained previously isolated compounds [1,6] and were not investigated further. Fractions 90–96 were pooled and subjected to HPLC with 5% isopropanol in hexane to give pure (2*E*,10*E*)-1-hydroxy-6,13-diketo-7-methylene-3,11,15-trimethylhexadeca-2,10,14-triene (7, 200 mg, 0.018% dry wt); $\nu_{max}^{CCl_4}$ cm^{-1}: 3420, 1675, 1620; λ_{max}^{EtOH} nm: 235 ($\varepsilon = 20\ 425$); MS *m/z*: 318.2189 (M^+ calc. for $C_{20}H_{30}O_3$, 318.2195), 300, 123, 83, 43 (base). For ^1H and ^{13}C NMR see Tables 1 and 2, respectively. HPLC of fractions 110–119 gave (2*E*,10*E*)-1,6-dihydroxy-7-methylene-13-keto-3,11,15-trimethylhexadeca-2,10,14-triene (**5a**, 275 mg, 0.025% dry wt) and (2*E*,5*E*,10*E*)-1,7-dihydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene (**6a**, 495 mg, 0.045% dry wt).

(2*E*,10*E*)-1,6-dihydroxy-7-methylene-13-keto-3,11,15-trimethylhexadeca-2,10,14-triene (**5a**). Oily $[\alpha]_D + 96.5°$ (*c* 1 in EtOH); $\nu_{max}^{CCl_4}$ cm^{-1}: 3470, 1685, 1615, 900; λ_{max}^{EtOH} nm: 242 ($\varepsilon = 15\ 168$); MS *m/z*: 320.2345 (M^+ calc. for $C_{20}H_{32}O_3$, 320.2351), 302, 284, 204, 83 (base), 55. For ^1H and ^{13}C NMR see Tables 1 and 2, respectively. Compound **5b** (Ac_2O–pyridine). Oily, $\nu_{max}^{CCl_4}$ cm^{-1}: 1735, 1685, 1620, 1235; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 2.1 (6H, *s*, MeCOO—), 4.61 (2H, *d*, $J = 6.5$ Hz, —CH_2OAc). (2*E*,5*E*,10*E*)-1,7-dihydroxy-13-keto-3,7,11,15-tetramethylhexadeca-2,6,10,14-tetraene (**6a**). Oily, $[\alpha]_D - 16.1°$ (*c* 1 in EtOH); $\nu_{max}^{CCl_4}$ cm^{-1}: 3440, 1680, 1610; λ_{max}^{EtOH} nm: 242 ($\varepsilon = 11\ 914$); MS *m/z*: 320.2342 (M^+ calc. for $C_{20}H_{32}O_3$, 320.2351), 302, 284, 83 (base), 55, 43. For ^1H and ^{13}C NMR see Tables 1 and 2, respectively. Compound **6b** (Ac_2O–pyridine). Oily, $\nu_{max}^{CCl_4}$ cm^{-1}: 3490, 1730, 1680, 1610, 1225; ^1H NMR (60 MHz, $CDCl_3$, TMS): δ 2.1 (3 H, *d*, MeCOO—), 4.65 (2H, *d*, $J = 6.5$ Hz, —CH_2OAc).

Catalytic hydrogenation of **5a**. A soln of **5a** (25 mg) in 2 ml EtOH was hydrogenated at room temp. under atmos. pres. in the presence of 4 mg Pd–C for 18 hr. After filtration of the catalyst, the filtrate was concd under vacuum to yield 22 mg 2,6,10,14-tetramethylhexadecan-4-one (**8**), identified by comparison of its physical properties (MS, NMR, IR) with those reported in the literature [7]. This experiment was repeated with compound **6a** to give the same ketone **8**.

Sodium borohydride reduction of **7** *to give* **5a**. $NaBH_4$ (10 mg) was added to a soln of **7** (50 mg) in EtOH (3 ml) and mixture was stirred for 20 min. After addition of H_2O the organic matter was extracted 3 × with Et_2O. Evapn of the solvent left a residue (48 mg) which was subjected to HPLC to give optically inactive **5a** (9 mg), identified by comparison of the physical properties (IR, UV, NMR, MS) with those of the natural compound.

Application of the Mislow method to **5a**. **5a** (100 mg) was treated with pyridine (300 mg) in dry Et_2O (5 ml) and added under continuous stirring to a soln of *p*-Me—C_6H_4—SOCl (700 mg) in Et_2O (5 ml) at −15°. Conventional work-up [5,8] led to the isolation of a mixture of diastereomers which by reaction with methyl magnesium iodide gave a preponderance of (+)-(*R*)-methyl-*p*-tolyl sulphoxide.

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