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THREE FURTHER DOLABELLANE DITERPENOIDS FROM *DICTYOTA* SP.

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Key Word Index—*Dictyota*; Phaeophyceae; brown alga; diterpenes; dolabellane derivatives.

Abstract—A brown alga of the genus *Dictyota* has yielded three new dolabellane diterpenoids the structures of which were elucidated mainly by spectroscopic methods.

INTRODUCTION

Examination of *Dictyota* species has led to the isolation of a number of dolabellane diterpenoids. Recently we have described the isolation and structure of five dolabellane-based compounds from an unnamed *Dictyota* species [1, 2]. In a continuation of this work, we have now isolated from the less polar fractions of the chloroform extract of this same alga three minor congeners, the structures of which we have elucidated essentially by spectroscopic methods.

RESULTS AND DISCUSSION

Compound **1** was an optically active ($[\alpha]_D^{EtOH}$ +1.5°) secondary alcohol (IR $\nu_{max}^{CHCl_3}$ 3480 cm^{-1}; MS: 270 *m/z* [M $-H_2O$]$^+$; ^{13}C NMR: δ78.5, *d*, >CHOH; ^{1}H NMR: δ4.35, *dd*, *J* = 11.5 and 5 Hz, >CHOH). Mass spectral analysis established the molecular formula $C_{20}H_{32}O$. The ^{13}C NMR spectrum confirmed the presence of 20 carbon atoms, of which six were sp^2, thus implying that compound **1** was carbobicyclic. The mass spectral fragmentation pattern, which showed marked similarities with those of other metabolites isolated previously from the same source [1, 2], pointed to a dolabellane skeleton. In fact, hydrogenolysis of **1** gave a mixture of saturated $C_{20}H_{38}$ hydrocarbons indistinguishable by GLC (capillary column) from those obtained by hydrogenolysis of **2** [1]. When considered in conjunction with the probable biogenetic relationships among the *Dictyota* diterpenoids, these properties suggested that the compound possessed structure **1**. This was fully supported by the ^{13}C and ^{1}H NMR spectra.

The proton decoupled and off-resonance decoupled ^{13}C NMR spectra showed the presence, besides the hydroxymethine carbon and the six sp^2 carbons, of the following groups in the sp^3 region: four methyls, six methylenes, two methines and a single quaternary carbon. Assignments (Table 1) were made by comparing the data with those obtained for closely related compounds.

The ^{1}H NMR data of compound **1** were strongly reminiscent of those of the known diol **2**, apart from the replacement of the hydroxymethyl signal by a vinyl methyl singlet at δ1.71, and indicated the presence of a second vinyl methyl singlet (δ1.55), an angular methyl (δ1.20) and an isopropyllidene group (δ1.50, *s*, C-18 Me; 4.88 and 4.68, each 1H, *s* (*br*), H-20a and H-20b). The remaining resonances pertained to the bicyclic ring system; extensive decoupling experiments afforded conclusive structural information. The signal of the carbinolic methine at δ4.35 was the X part of an AMX system (C-2, C-3) which resembled that observed in the spectrum of **2**. A long-range coupling between H-3 and the olefinic proton at δ5.01 located the latter at position 5. Moreover, irradiation at δ5.01 not only simplified the signal of H-3 but at the same time modified the methyl resonance at δ1.71 (C-16). Additional decouplings identified the proton sequence H-5, H-6′, H-7, their multiplicity being only compatible with part structure C-5 through C-8. The

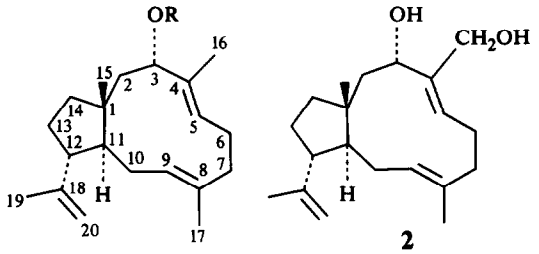
Table 1. ^{13}C NMR assignments for compounds **1**, **3** and **4** (20 MHz, $CDCl_3$, TMS as int. standard)*

C	2†	1	3	4
1	47.1 *s*	47.0 *s*	46.0 *s*	45.3 *s*
2	32.8 *t*	31.8 *t*	32.8‡ *t*	23.5‡ *t*
3	79.1 *d*	78.5 *d*	24.0‡ *t*	27.8‡ *t*
4	134.8‡ *s*	135.2‡ *s*	131.4§ *s*	140.7 *s*
5	137.8 *d*	130.5 *d*	137.9 *d*	150.5 *d*
6	24.2§ *t*	24.1§ *t*	24.0 *t*	29.3‡ *t*
7	39.7‖ *t*	39.8‖ *t*	40.0‖ *t*	39.7§ *t*
8	134.7‡ *s*	134.9‡ *s*	134.3§ *s*	133.2 *s*
9	126.8 *d*	126.5 *d*	126.0 *d*	126.4 *d*
10	28.2§ *t*	28.0§ *t*	28.8‡ *t*	27.8‡ *t*
11	51.4 *d*	51.3 *d*	50.9 *d*	50.5 *d*
12	42.6 *d*	42.2 *d*	42.8 *d*	43.0 *d*
13	41.8‖ *t*	41.7‖ *t*	42.4‖ *t*	44.0§ *t*
14	42.7‖ *t*	42.7‖ *t*	43.6‖ *t*	44.1§ *t*
15	23.4 *q*	23.3 *q*	23.5 *q*	23.5‖ *q*
16	57.9 *t*	10.0 *q*	60.9 *t*	190.3 *d*
17	15.5 *q*	15.5 *q*	15.5 *q*	15.6 *q*
18	146.2 *s*	146.0 *s*	147.1 *s*	146.8 *s*
19	24.8 *q*	24.9 *q*	25.7 *q*	23.6‖ *q*
20	112.0 *t*	111.6 *t*	111.9 *t*	112.1 *t*

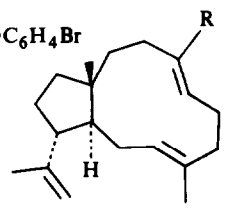
*Multiplicities were obtained by 'off-resonance' decoupling.

†Included for comparison.

‡§‖ Assignments may be reversed.



1 R=H
5 R=COC_6H_4Br



3 R=CH_2OH
4 R=CHO

methyl singlet at δ1.55 (C-17) was allylically coupled with the doublet associated with H-9, which was adjacent to a methylene whose protons resonate at δ1.66 (H-10) and 2.23 (H-10′). The multiplicity of the latter signal (*ddd*) required the bonding of the C-10 methylene to a methine (C-11).

This evidence led to the formulation of structure **1** for the novel algal metabolite exclusive of stereochemistry. The *trans* geometry of both C-4 and C-8 double bonds was deduced from the chemical shifts of the vinyl methyls (10.0 and 15.5 ppm) in the ^{13}C NMR spectrum [3]. The stereochemistry of the chiral centres, which were expected to be as depicted in **1** from a consideration of the known stereochemistry of its co-metabolites isolated previously, was confirmed as follows: (i) application of Nakanishi's method for the determination of the chirality of allylic secondary alcohols [4], which showed that the configuration at C-3 must be *S*; (ii) NOE experiments (see Experimental), which gave results analogous to those observed for compound **2**, whose relative stereochemistry and preferred conformation had been reported previously [1]. In particular, the observation that 1-Me is within NOE distance from H-3 but not H-11′ confirmed that the chirality at C-1 and C-11 was the same as in other dolabellane diterpenes isolated previously from *Dictyota* sp.; (iii) H-12 was *trans* to H-11′, as shown by the values of the coupling constants ($J_{11,12}$ = 6 Hz, $J_{12,13}$ = 12 Hz, $J_{12,13'}$ = 6 Hz) (which were identical to those found for the corresponding protons in compound **2**), and this allowed the assignment of the absolute stereochemistry to C-12.

The second compound to be reported here, **3**, $[\alpha]_D^{EtOH}$ +85°, was an isomer of **1**. The presence of the hydroxyl group (IR $\nu_{max}^{CHCl_3}$ 3480 cm^{-1}) as $-\dot{C}=\dot{C}-CH_2OH$ was deduced from the ^{13}C (δ60.9, *t*, $-CH_2OH$) and ^1H (δ4.17 and 3.91, AB system, $-\dot{C}=\dot{C}-\underline{CH_2}OH$) NMR data. The ^{13}C NMR spectrum included six sp^2 carbons, indicating **3** to be carbobicyclic. The dolabellane nature of the skeleton was again established by hydrogenolysis, which afforded the same hydrocarbon mixture as **1**. Ozonolysis followed by oxidative work-up gave laevulinic acid. This fact required two double bonds located at positions C-4 and C-8 with the allylic hydroxymethyl at C-4 or, alternatively, the double bonds placed at C-3 and C-7 and the hydroxymethyl at C-8. The latter possibility could be rejected on the basis of the following evidence. In the ^1H NMR spectrum of the related aldehyde **4** (*vide infra*) the vinyl proton at δ5.21 had a long-range coupling with the methyl singlet at δ1.49 and at the same time a vicinal coupling with a proton at δ2.09, whose multiplicity (Table 2) showed that it belonged to a methylene adjacent to a methine. The location of the functions in the eleven-membered ring having been settled, the structure of the rest of the molecule was deduced from a comparison of ^1H and ^{13}C NMR spectra with those of compound **2**. The *trans* geometry of the C-8 double bond was assigned on the basis of the chemical shift (δ15.5) of the vinyl group in the ^{13}C NMR spectrum, while the configuration of the C-4 double bond was inferred as *trans* from the relationship between **3** and aldehyde **4** (*vide infra*). As **3** co-occurs with **1**, we suggest that the stereochemical assignment at all the chiral centres must be identical for both metabolites.

The last *Dictyota* diterpenoid, $[\alpha]_D^{EtOH}$ −72°, had molecular formula $C_{20}H_{30}O$. The UV (λ_{max}^{EtOH} 242 nm, ε7500) and IR ($\nu_{max}^{CHCl_3}$ 1675 cm^{-1}) spectra indicated the presence of a conjugated carbonyl. In the light of this information and also the interpretation of the ^{13}C and ^1H NMR spectra, the unknown compound was tentatively assigned structure **4**. Confirmation was obtained by chemical correlation with **3** via sodium borohydride reduction. Conversely, manganese dioxide oxidation of **3** afforded an aldehyde identical with **4**. The *trans* configuration of the C-4 double bond resulted from the chemical shift of the aldehyde group in the ^{13}C (δ190.3) and ^1H (δ10.0) NMR spectra [2, 5], while the *trans* geometry of the second double bond in the eleven-membered ring was deduced from the chemical shift (δ15.6) of the C-8 Me in the ^{13}C NMR spectrum [3]. In view of the biological activities previously observed for dolabellane compounds [1, 6, 7], antimicrobial activity tests were carried out on the new metabolites **1** and **3**, and on the related compound **2**. Table 3 summarizes the results obtained.

EXPERIMENTAL

General procedure. Prep. liquid chromatography (prep. LC) was carried out on a Jobin-Yvon Miniprep LC using LiChroprep Si 60 as the stationary phase.

Plant material. The alga was collected at a depth of 4–5 m near Portopalo (SE coast of Sicily) in July 1982. A voucher specimen was deposited in the Herbarium of the Institute of Botany, Catania.

Extraction and isolation. The air-dried and ground alga (600 g), was extracted with $CHCl_3$ (× 3) at room temp. under continuous stirring. Evaporation of the $CHCl_3$ afforded a dark green oil (24 g) which was taken up in hexane–Et_2O (1:4). The soln was passed through a column of Florisil and the eluate evaporated. The residue (16 g) was subjected to open CC on silica gel using Et_2O–hexane (1:3) as the eluant. Fractions of 50 ml were collected and those exhibiting similar TLC profiles were combined. Fractions 5–7 were pooled and subjected to prep. LC (CH_2Cl_2–hexane, 1:3) to give 150 mg (0.02% dry wt) of 1 (*R*), 11 (*S*), 12 (*R*)-dolabell-4 (*Z*), 8 (*E*), 18-trien-16-al (**4**) as a yellow oil; $[\alpha]_D$ −72° (*c* 1), EtOH; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1675, 1450, 1370, 890; UV λ_{max}^{EtOH} nm: 242 (ε7500); MS *m/z*: 286.2288 $[M]^+$ (calc. for $C_{20}H_{30}O$, 286.2296), 271 $[M-Me]^+$, 244, 201, 187, 145, 131, 121, 95; ^{13}C and ^1H NMR: see Tables 1 and 2, respectively. Prep. LC (Et_2O–hexane, 1:9) of fractions 10-13 gave in order of

Table 2. ^{1}H NMR assignments for compounds **1**, **3** and **4** (400 MHz, $CDCl_3$, TMS as int. standard)*

H	2‡	1	3	4
2	1.59 (1H, *dd*)	1.40–1.70†	1.25–1.65†	1.20–1.70†
2′	1.39 (1H, *dd*)	1.29 (1H, *dd*, *J* = 11.5, 10)		
3	4.48 (1H, *dd*)	4.35 (1H, *dd*, *J* = 11.5, 5)	2.10–2.30†	1.90–2.50†
5	5.18 (1H, *dd*)	5.01 (1H, *dd*, *J* = 12, 2)	5.10 (1*H*, *dd*, J = 10.5, 3)	6.35 (1*H*, *dd J* = 12, 5)
6	2.15–2.30†	2.20–2.30†	1.74†	2.36†
6′	2.43 (1H, *dddd*)	2.34 (1H, *dddd*, *J* = 13, 12, 12, 5)	2.39 (1H, *dddd*, *J* = 13, 12, 12, 3)	3.09 (1H, *dddd*, *J* = 14, 12, 11.5, 4)
7	2.11 (1H, *ddd*)	2.09 (1H, *ddd*, *J* = 12, 12, 5)	2.08 (1H, *ddd*, *J* = 12, 12, 5)	2.19 (1H, *ddd*, *J* = 12, 11, 5, 4)
7′	2.15–2.30†	2.20–2.30†	2.10–2.30†	2.33†
9	5.16 (1H, *dd*)	5.15 (1H, *dd*, *J* = 11.5, 4.5)	5.16 (1H, *dd*, *J* = 10.5, 4)	5.21 (1H, *dd*, *J* = 12, 4)
10	1.63†	1.66†	2.10–2.30†	1.70†
10′	2.19 (1H, *ddd*)	2.23 (1H, *ddd*, *J* = 12, 11.5, 1.5)		2.09 (1H, *ddd*, *J* = 12, 11.5, 1.5)
11′	1.40–1.70†	1.40–1.70†	1.60–1.70†	1.20–1.70†
12	2.56 (1H, *ddd*)	2.56 (1H, *ddd*, *J* = 12, 6, 6)	2.67 (1*H*, *ddd*, J = 8, 7.5, 7.5)	2.75 (1*H*, *ddd*, J = 8, 7.5, 7.5)
13	1.40–1.70†	1.40–1.70†	1.60–1.70†	1.20–1.70†
14	1.40–1.70†	1.40–1.70†	1.25–1.65†	1.20–1.70†
15	1.11 (3H, *s*)	1.20 (3H, *s*)	1.05 (3H, *s*)	0.99 (3H, *s*)
16a	4.21 } 2H, AB system	1.71 (3H, *s*)	4.17 } 2H, AB system *J* = 11.5	10.0 (1H, *s*)
16b	4.05 }		3.91 }	
17	1.47 (3H, *s*)	1.55 (3H, *s*)	1.49 (3H, *s*)	1.49 (3H, *s*)
19	1.68 (3H, *s*)	1.50 (3H, *s*)	1.71 (3H, *s*)	1.67 (3H, *s*)
20a	4.90 (1H, *s*, *br*)	4.88 (1H, *s*, *br*)	4.83 (1H, *s*, *br*)	4.83 (1H, *s*, *br*)
20b	4.67 (1H, *s*, *br*)	4.68 (1H, *s*, *br*)	4.68 (1H, *s*, *br*)	4.74 (1H, *s*, *br*)

*Assignments were aided by spin decoupling.
†Overlapped with other signals.
‡Included for comparison.

Table 3. Antimicrobial activity of compounds **1**–**3**

Micro-organism	Compound 1	2	3
Staphylococcus aureus	+	−	++
Serratia marcescens	+	−	++
Candida albicans	−	−	−
Saccaromyces cerevisiae	−	−	−
Aspergillus niger	+	+	−
Mucor racemus	+++	+	++
Fusarium sp.	−	−	−

increasing polarity: (a) 180 mg (0.03% dry wt) of 16-hydroxy-1 (*R*), 11 (*S*), 12 (*R*)-dolabell-4 (*E*), 8 (*E*), 18-triene (**3**) as colourless oil; $[\alpha]_D$ +85° (*c* 1, EtOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3610, 3480, 1650, 1450, 1380, 1000, 895; MS *m/z*: 288.2445 $[M]^+$ (calc. for $C_{20}H_{32}O$, 288.2453), 273 $[M-Me]^+$, 270 $[M-H_2O]^+$, 257, 255 $[M-Me-H_2O]^+$, 245, 277, 201, 187, 173, 160, 147, 135, 121, 119, 107, 95, 93; ^{13}C and ^{1}H NMR, see Tables 1 and 2, respectively. (b) 190 mg (0.03% dry wt) of 3 (*S*)-hydroxy-1 (*R*), 11 (*S*), 12 (*R*)-dolabell-4 (*E*), 8 (*E*), 18-triene (**1**) as a pale yellow oil; $[\alpha]_D$ +1.5° (*c* 1, EtOH); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3480, 1645, 1450, 1380, 1000, 895; MS *m/z* 288.2448 $[M]^+$ (calc. for $C_{20}H_{32}O$, 288.2453), 273 $[M-Me]^+$, 270 $[M-H_2O]^+$, 257, 255 $[M-Me-H_2O]^+$, 245, 227, 221, 187, 173, 159, 147, 137, 121, 119, 117, ^{13}C and ^{1}H NMR, see Tables 1 and 2 respectively. ^{1}H NOEDS (Nuclear Overhauser Enhancement Difference Spectra) were carried out at 270 MHz, in degassed $CDCl_3$ solns. The following estimated internuclear distances (Å) based on the NOEDS were obtained using Dreiding models: C-1 Me/H-3 (2.0); C-1 Me/H-9 (2.5); H-5/H-3 (2.3); H-5/H-9 (2.5); C-8 Me/H-6′ (2.9).

Catalytic hydrogenolysis of **1** *and* **3**. Compound **1** (20 mg) in EtOH (3 ml) were hydrogenated over 10% Pd–C (7 mg) at room temp. and pressure for 24 hr. The catalyst was removed by filtration and the solvent removed under reduced pressure leaving an oil whose MS showed a molecular ion at *m/z* 278 $[C_{20}H_{38}]^+$. This material was analysed by capillary-GLC (Carbowax 20 M, 25 m, 180°, carrier gas N_2, 1 ml/min) and gave four peaks, which could not be separated from those of a mixture of saturated hydrocarbons obtained in a parallel run by hydrogenolysis of **2** [1]. Analogous results were obtained on hydrogenolysis of **3** (20 mg).

Application of Nakanishi's method to **1**. An excess (30 mg) of *p*-bromobenzoyl chloride was added to a soln of **1** (30 mg) in CH_2Cl_2 (3 ml) and C_5H_5N (1 ml), and the mixture refluxed for 5 hr. After partitioning with H_2O the organic phase was concentrated *in vacuo* and chromatographed on a silica gel column (Et_2O–hexane, 1:99). The *p*-bromobenzoate (**5**) was obtained as a white crystalline solid (40 mg); $[\alpha]_D$ −44° (*c* 1, EtOH); UV λ_{max}^{hexane} 244 nm (ε22 800); MS *m/z* 470. A soln of **5** (10^{-4} M, hexane) was used to record the CD spectrum from 300 to 210 nm (1 cm cell). In the region of the UV maximum the spectrum shows a negative CD ellipticity ($\Delta\varepsilon = -34.2$) indicating an *S* configuration of the chiral centre at C-3.

Ozonolysis of **3**. Compound **3** (5 mg) in EtOH (4 ml) was ozonized at −70° for 5 min. After removal of excess O_3, perhydrol (30%, 0.1 ml) and aq. KOH (10%, 0.5 ml) were added and the soln kept at room temp. for 1 hr. The soln was acidified

(dil. HCl) and exhaustively extracted with Et_2O. The solvent was evaporated and the residue methylated with MeOH–HCl. Methyl laevulinate was identified by capillary-GLC (SE-30, 25 m, 150°, N_2 as carrier, flow 1 ml/min).

MnO_2 *oxidation of* **3** *to produce* **4**. MnO_2 (70 mg) was added to a soln of **3** (50 mg) in Et_2O (4 ml) and the suspension was stirred for 1 hr. The ppt was filtered off and the soln was evapd to give 40 mg of an aldehyde, identified by comparison of its physical properties ($[\alpha]_D$, UV, IR, MS, NMR) with those of the natural product **4**.

$NaBH_4$ *reduction of* **4** *to produce* **3**. $NaBH_4$ (10 mg) was added to a soln of **4** (50 mg) in EtOH (4 ml) and the mixture was kept at room temp for 20 min. After addition of H_2O (10 ml), excess reagent was destroyed by addition of dil. HCl and the organic material was extracted with Et_2O ($\times 3$). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo* to yield 40 mg of an oil, homogeneous by TLC, whose properties ($[\alpha]_D$, IR, MS, NMR) were identical with those of **3**.

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