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STRUCTURE AND CONFORMATION OF TWO NEW DOLABELLANE-BASED DITERPENES FROM *DICTYOTA* SP.

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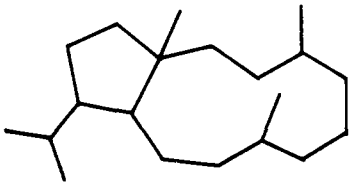
ABSTRACT.—Two new dolabellane derivatives (**5** and **6**) have been isolated from a *Dictyota* sp. and their structure, including absolute configuration, has been determined on the basis of spectral studies and chemical correlations.

In the last decade, brown seaweeds of the family Dictyotaceae have been intensively investigated. As the result of this effort, several compounds possessing biological activities, including some dolabellane (**1**) derivatives, have been discovered (1-8). Previously, we reported the isolation from *Dictyota* sp. of the three new dolabellane-based diterpenoids **2-4**, whose structure and relative stereochemistry could be established; one of them, namely compound **2**, exhibits significant cytotoxic activity *in vitro* against KB cells (8). Further investigation of this alga has now led to the isolation of two additional members (**5** and **6**) of the same class of compounds.



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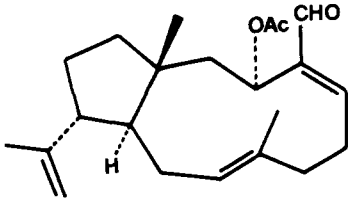

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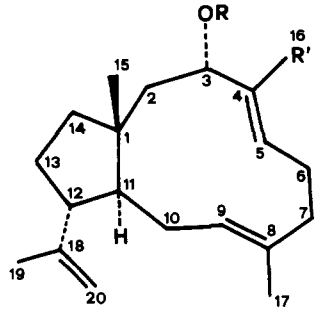

2 R=Ac, R′=CH_2OH
3 R=H, R′=CH_2OH
4 R=Ac, R′=CHO
5 R=H, R′=CH_2OAc
7 R=COC_6H_4Br, R′=CH_2OAc

The first compound isolated, **5** (0.08% dry weight of the alga), was a liquid, $[\alpha]^{25}D = +43.9°$, $C_{22}H_{34}O_3$, and possessed hydroxyl (3600, 3480 cm^{-1}) and acetoxy (1725, 1245 cm^{-1}) groups, confirmed by alternative losses of 18 and 60 amu from the molecular ion. These data led to the suspicion that it might be 3-hydroxy-16-acetoxy-dolabell-4(E),8(E),18-triene, a compound obtained previously (8), along with the isomer **2**, by partial acetylation of diol **3**. Comparison of the natural product with semisynthetic **5** showed they were identical. Assignments of pmr and cmr resonances, not reported before, are given in Tables 1 and 2.

The absolute stereochemistry at C-3 was established by application of Nakanishi's

TABLE 1. Pmr Data for Compounds **5** and **6**[a]

Position	**5**	**6**
2	1.60-1.70[b]	1.44 (1H, dd, J=11,4)
2′	1.24 (1H, dd, J=10,6)	2.26 (1H, dd, J=12.5, 11)
3	4.45 (1H, dd, J=11, 6)	5.63 (1H, dd, J=12.5, 4)
5	5.34 (1H, dd, J=11, 2.5)	6.74 (1H, dd, J=13, 5)
6	2.20-2.34[b]	2.47[b]
6′	2.47 (1H, dddd, J=14, 12, 11, 5)	2.92 (1H, dddd, J=13, 12, 12, 4.5)
7	2.20-2.34[b]	1.95 (1H, ddd, J=13, 12, 4)
7′	2.12 (1H, ddd, J=12, 12, 5.2)	2.35-2.45[b]
9	5.19 (1H, dd, J=11, 4.5)	5.49 (1H, dd, J=11, 4)
10	1.60-1.70[b]	1.89 (1H, ddd, J=12, 12, 4)
10′	2.20 (1H, ddd, J=12, 11, 1.5)	2.14 (1H, ddd, J=12, 11, 1.5)
11′	1.40-1.75[b]	1.35-1.67[b]
12	2.56 (1H, ddd, J=12, 6, 6)	2.64 (1H, ddd, J=13.5, 4, 4)
13	1.40-1.75[b]	1.35-1.67[b]
14	1.40-1.75[b]	1.35-1.67[b]
15	1.12 (3H, s)	1.19 (3H, s)
16a 16b	4.58 4.46 (2H, AB system, J=12)	9.46 (1H,s)
17	1.55 (3H, s)	1.59 (3H, s)
19	1.67 (3H, s)	1.49 (3H, s)
20a	4.89 (1H, bs)	4.88 (1H, bs)
20b	4.68 (1h, bs)	4.66 (1H, bs)
-OAc	2.06 (3H, s)	1.99 (3H, s)

[a]Pmr spectra were recorded at 400 MHz, $CDCl_3$. Assignments were aided by spin-decoupling experiments. TMS was used as internal standard: chemical shifts are δ values. J values are reported in Hz.

[b]Overlapped with other signals.

method for the determination of the chirality of allylic alcohols (9,10). Compound **5** was treated with *p*-bromobenzoyl chloride yielding derivative **7**, which at λ max (hexane) 240 nm showed a negative Cotton effect, indicating a negative chirality of the exciton and, therefore, an *S*-configuration at C-3. This result determined the whole stereostructure as depicted in **5** and, in addition, made it possible to deduce the absolute stereochemistry of the chemically related compounds **2-4**.

The second compound, **6**, was isolated (0.25% dry weight of the alga) as an optically active crystalline solid, $[\alpha]^{25}D = -13.9°$, mp 72-73°. Its high resolution ms gave the formula as $C_{22}H_{32}O_3$. Loss of 60 amu from the parent ion and ir absorptions at 1725 and 1245 cm^{-1} suggested the presence of an acetoxy group. An intense ir band at 1690 cm^{-1} and uv absorption at λ max (EtOH) 224 nm (ε = 10,100) were indicative of an α,β-unsaturated adlehyde group. These inferences were corroborated by cmr (193.3, d, -*C*HO; 170.3, s, -*C*OCH$_3$; 21.0, q, -COCH$_3$; 77.2, d, -*C*HOAc) and pmr (9.46, 1H, s, -*CH*O; 6.74, 1H, dd, -C*H*=C(-CHO)-; 1.99, 3H, s, -COC*H*$_3$; 5.63, 1H, dd, -CH=C(-C*H*OAc)-) data which showed, in addition, the acetoxyl to be secondary and in allylic position. The pmr and cmr spectra of **6** (Tables 1 and 2) were remarkably similar to those of its isomer **4** and indicated the presence of the same structural groups. The most significant differences concerned the values of the chemical shift of the aldehyde group in the cmr and pmr spectra (193.3 ppm and 9.46 δ in **6** as compared with 190.2 ppm and 9.95 δ in **4**) indicating (11) that **6** and **4** were related to each other as geometrical isomers at the C-4 double bond, which is *cis* instead of *trans* in the new metabolite. This was supported by a 12% nOe between the aldehydic proton and H-5 and firmly established by photochemical isomerization of **6**, which gave a product identical in all respects (including optical rotation) to **4**. Compound **6** is unique, among the dolabel-

TABLE 2. Pmr Data for Compounds **5** and **6**[a]

Position	**5**		**6**	
1	46.8	s	45.6	s
2	32.5	t	32.3[b]	t
3	77.3	d	77.2	d
4	134.9[b]	s	139.2[c]	s
5	137.7	d	158.4	d
6	24.7[c]	t	28.0[b]	t
7	39.5[d]	t	34.8	t
8	134.6[b]	s	136.1[c]	s
9	126.8	d	125.1	d
10	27.9[c]	t	28.1[b]	t
11	51.3	d	51.8	d
12	42.6	d	39.1	d
13	41.6[d]	t	42.5[d]	t
14	42.5[d]	t	43.0[d]	t
15	23.1	q	23.0	q
16	58.5	t	193.3	d
17	15.6	q	20.2	q
18	145.7	s	145.2	s
19	24.8	q	23.5	q
20	111.9	t	111.4	t
$-COCH_3$	171.5	s	170.3	s
$-COCH_3$	21.0	q	21.0	q

[a]Cmr spectra were recorded at 20.1 MHz, $CDCl_3$, ppm from TMS. Multiplicities were obtained by 'off-resonance' decoupling. Assignments were based on comparison to models.

[b-d]Values with identical superscript within each column may be intermixed.

lane derivatives until now isolated from marine sources (5, 8, 12-15), in possessing a *cis*-configuration of a double bond in the ring system. Therefore, the possibility was considered that **6** could be an artifact derived from **4** during the isolation process. However, when a small sample of the alga was extracted immediately after collection and the extract subjected to hplc analysis, the two metabolites were present in a 1:1.2 ratio of **4** to **6**.

The preferred conformation of **5** and **6** were studied by nuclear Overhauser enhancement difference spectroscopy (NOEDS). While **5** adopts a conformation (Figure 1a) not sensibly different from that found previously for metabolites **2-4**, in compound **6**, in view of the different geometry of C-4 double bond, H-3, H-6, H-9, and C-1 Me are on the same face of the molecule while H-5, C-8 Me, and the aldehydic proton are on the opposite face, as represented in Figure 1b.

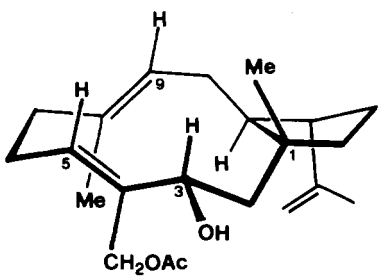

FIGURE 1a. Preferred conformation for compound **5**.

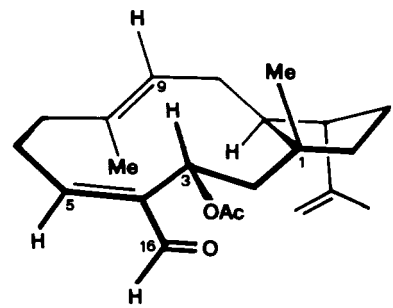

FIGURE 1b. Preferred conformation for compound **6**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler apparatus and are uncorrected. Mass spectra were recorded on an AEI MS 902 instrument at 70 eV (direct introduction of sample). Ir spectra were run in $CHCl_3$ solutions on a Perkin-Elmer Model 684 spectrophotometer. Uv spectra were obtained on a Perkin-Elmer Model 330 instrument. Cd spectrum of **7** was recorded on a Cary Model 60 CD/ORD apparatus, in hexane solution. Pmr spectra were run on Brücker AM-400 (400 MHz) and Brücker WP-250 (250 MHz) FT spectrometers. ^{1}H-NOEDS (nuclear Overhauser enhancement difference spectra) were carried out at 250 MHz, in degassed $CDCl_3$ solutions. A Brücker WP-80 (20.1 MHz) was used to record cmr spectra. Both pmr and cmr spectra were obtained in $CDCl_3$ solutions, using TMS as internal standard; chemical shifts are measured in δ (ppm). Optical rotations were determined with a Perkin-Elmer Model 141 instrument (1 dm tubes). Tlc was carried out using glass-packed, pre-coated silica gel F_{254} plates (Merck). Spot detection was obtained by spraying with 10% solution of $Ce(SO_4)_2$ in 2N H_2SO_4, or by uv light (254 nm). Preparative liquid chromatography (Plc) was effected on Jobin-Yvon Prep-10 and Miniprep liquid chromatographs; hplc was performed on a Varian 5000 liquid Chromatograph, equipped with a uv variable wavelength detector (Varian UV 50). All solvents were spectral grade or distilled prior to use. For hplc work, 'Baker Analyzed HPLC Reagents' were used.

PLANT MATERIAL.—*Dictyota* sp. was collected by SCUBA at a depth of 4-5 m near Portopalo (south-east coast of Sicily) in July 1982. A specimen was deposited in the Herbarium of the Institute of Botany, Catania.

EXTRACTION AND ISOLATION OF THE CONSTITUENTS.—Air-dried and ground alga (300 g) was extracted with $CHCl_3$ (3×800 ml) under continuous stirring. Evaporation afforded 11.8 g residue which was taken up in hexane-Et_2O (1:4). The solution was passed through a column of Florisil and the eluate evaporated. The residue (8 g) was subjected to plc on Si gel using 25% Et_2O in hexane as eluent. Fractions of 50 ml were collected, and those exhibiting similar tlc profile were combined. Compound **6** was eluted immediately after **4** and subjected to repeated careful plc (Lichroprep Si 60) with the following solvent systems: 3% Me_2CO in hexane; 15% diisopropyl ether in hexane. Compound **5** was collected in a more polar fraction and further purified by plc (Lichroprep Si 60, 5% Me_2Co in hexane).

3(S)-HYDROXY-16-ACETOXY-1(R),11(S),12(R)-DOLABELL-4(E),8(E),18-TRIENE (**5**).—Natural diterpenoid **5** was a colorless oil (240 mg), $[\alpha]^{25}D = +43.9°$ (c=1 in EtOH); ir ν max ($CHCl_3$) 3600, 3480, 1725, 1459, 1370, 1245, 1020, and 890 cm^{-1}; ms *m/z* 346 (M^+, $C_{22}H_{34}O_3$), 331 (M^+-Me), 328 (M^+-H_2O), 286 (M^+-AcOH), 268 (M^+-H_2O-AcOH), 255, 241, 221, 201, 187, 159, 147, 131, 121, 107. For pmr and cmr see Tables 1 and 2, respectively. **5** was identical in all respect to the previously reported (8) compound, whose $[\alpha]^{25}D$ was +42.7°.

DETERMINATION OF THE ABSOLUTE STEREOCHEMISTRY OF **5**.—*Preparation of the* p-*bromobenzoate* **7**: To a solution of **5** (30 mg) in CH_2Cl_2 (3 ml) and pyridine (0.5 ml), an excess (30 mg) of *p*-bromobenzoylchloride was added, and the mixture was refluxed for 48 h. After partitioning with H_2O the organic phase was concentrated *in vacuo* and chromatographed on a Si gel column (10% Me_2CO in hexane). The *p*-bromobenzoate **7** was obtained as a white crystalline solid (20 mg), mp 158-159°; ms *m/z* 528 (M^+, $C_{29}H_{37}O_4Br$); uv (hexane) 244 nm (ε=18,000); pmr ($CDCl_3$, 250 MHz, δ scale) 7.87 and 7.55, 4H, AA'XX' system (BrC_6H_4COO-); 5.69, 1H, dd, (-C*H*OCOC$_6$H$_4$Br); 5.64 and 5.29, 1H each, dd, (-C=C-); 4.95 and 4.70, 1H each, bs, (-C=CH_2); 4.73 and 4.48, 2H, AB system (J=12 Hz), (-CH_2OAc); 1.80, 3H, s, (CH_3COO-); 1.72 and 1.55, 3H each, s, (CH_3C=); 1.27, 3H, s, (CH_3C-).

Application of Nakanishi's method: A solution of **7** (10^{-4} M) in hexane was used to record the cd spectrum from 300 to 210 nm (1 cm cell). In the region of the uv maximum of **7**, the spectrum shows at 240 nm a negative cd ellipticity (Δε=−23.1), indicating an *S*-configuaration of the chiral centre at C-3.

3(S)-ACETOXY-1(R),11(S),12(R)-DOLABELL-4(E),8(E),18-TRIEN-16-ALE (**6**).—On recrystallization from EtOH, **6** was obtained as white needles (750 mg), mp 72-73°, $[\alpha]^{25}D = -13.9°$ (c=1 in EtOH); ir ν max ($CHCl_3$) 1725, 1690, 1445, 1370, 1245, 1020, 960, 895 cm^{-1}; uv λ max (EtOH) 224 nm (ε=10,100); high resolution ms 344.2360 obsd ($C_{22}H_{32}O_3$ requires 344.2351) *m/z* 344, 300 (M^+-CHO-Me), 284 (M^+-AcOH), 269 (M^+-AcOH-Me), 255, 241, 216, 201, 187, 145, 135, 121, 109. For pmr and cmr see tables 1 and 2, respectively. The following estimated internuclear distances (Ångstrom) based on the results of NOEDS experiments were obtained with Dreiding models; C-1 Me/H-3 (2.0); C-1 Me/H-9 (2.5); H-3/H-6 (2.2); H-5/H-16 (2.5).

PHOTOISOMERIZATION OF **6** TO GIVE **4**.—A solution of **6** (30 mg) in EtOH (3 ml) in a quartz cell (1 cm path) was deoxygenated with pure N_2 and then irradiated at 280 nm with a Hanau Q 400 mercury

vapor lamp by means of interference filters (Zeiss Jena). The reaction was followed by uv spectroscopy (isosbestic point at 233 nm) and analytical hplc (Whatman Partisil PXS 10/25 Si gel, 10% Et_2O in hexane, 1.5 ml/min, room temperature). The reaction was stopped when more polar by-products began to appear. The constituents of the reaction mixture were separated by preparative hplc (Whatman Partisil M 9, 10/25 Si gel, 10% Et_2O in hexane, 3 ml/min, room temperature) to give 5 mg of **4** and 20 mg of starting material. The physical properties of the semisynthetic products were identical with those of the natural metabolite.

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