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TWO NEW DOLABELLANE DERIVATIVES FROM THE BROWN ALGA *DICTYOTA PARDARLIS*

Anthony D. Wright, Gabriele M. König and Otto Sticher

Department of Pharmacy, Swiss Federal Institute of Technology
(ETH) Zurich, CH-8092 Zürich, Switzerland

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ABSTRACT-The secondary metabolite chemistry of two species of brown algae from the family Dictyotaceae was investigated. From *Dictyota pardarlis f. pseudohamata* Cribb two novel diterpenes **1**, **2** and the previously reported diterpene **3** were isolated. Analysis of the results of NOE experiments performed on **1** enabled the relative stereochemistry at chiral centres C7 and C8 to be proposed as *R** and *R** respectively for the previously reported compound **4**, (1*R**,3*R**,4*S**,11R*)-3,4-7,8-bisepoxy-dolabella-12(18)-ene. From *Spatoglossum macrodontum* J.Agardh the three sterols cholesterol, 24-methylene-cholesterol and fucosterol were the major isolates.

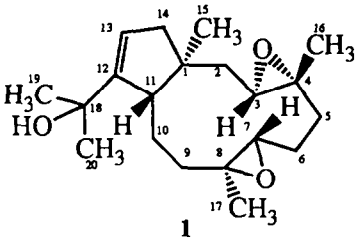
INTRODUCTION

Brown algae are known to be a rich source of secondary metabolites [1,2,3]. For the family Dictyotaceae this is particularly true[1,2,3]. This family of algae has been demonstrated to contain sesqui- and diterpenes of many structural types including diterpenes possessing the dolabellane skeleton[4,5]. Chemical investigations of brown algae from the Great Barrier Reef (Australia) are however few[6] and in this respect the current study provides more information as to the secondary metabolites to be found in algae from this region. In this paper we report the structural elucidation of two new dolabellanes (**1** and **2**) and the complete NMR characterisation of **3**, a metabolite first reported by Bheemasankara *et al.*[7].

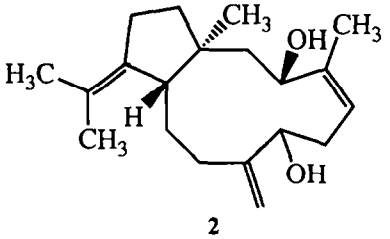
DISCUSSION

Compound **1** is a white crystalline material of the molecular formula $C_{20}H_{32}O_3$. The presence of two signals for sp^2 carbons in the ^{13}C NMR spectrum for a single carbon-carbon double bond, dictated that the molecule be tetracyclic. IR spectroscopy indicated the prescence of an OH function (3300 cm^{-1}) as did the ^{13}C NMR data (82.7(s) ppm). Four further oxygenated carbons

were present by ^{13}C NMR spectroscopy (61.5 (s), 61.7(s), 63.0(d), 64.9(d) ppm) and, because of their shielded nature, the presence of two epoxide functions was proposed. The main skeleton of this diterpene was thus bicyclic. The results from two dimensional long and short range ^{13}C-^{1}H NMR correlation experiments (Table 1) permitted the molecular skeleton of **1** to be deduced as that of a dolabellane with a tertiary hydroxy and two epoxide functions. Comparison of the ^{13}C NMR data for **1** with those of[6] **4** (Table 1) clearly indicated that these two molecules were extremely similar. The major differences between the two sets of ^{13}C NMR data being accounted for by the positioning of the OH function at C18 and migrating the C12-C18 double bond in **4** to C12-C13 in **1**.



1



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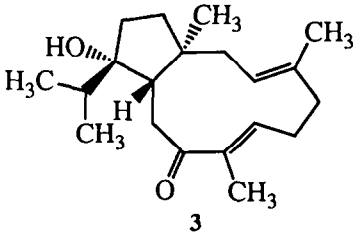

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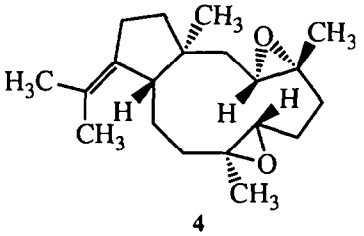


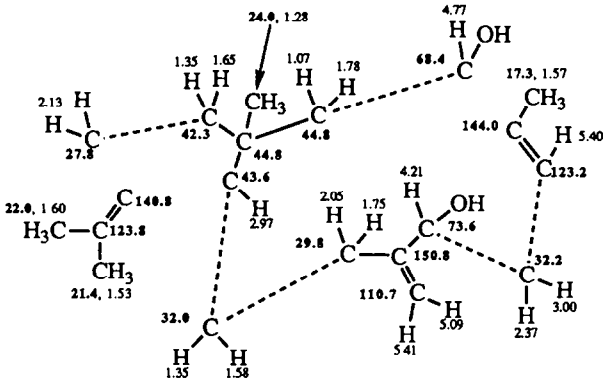
4

The structure for **1** contains six chiral centres. All of these centres are proposed to have the same relative stereochemistry as those of **4**, since the proton and carbon chemical shifts values for these centres in both compounds show remarkable similarity. The relative stereochemistry at C7 and C8 was not proposed by the authors[6] of **4** however, here the stereochemistry is proposed as 7*R** and 8*R** on the basis of NOE experiments performed on **1** (see Experimental). The results of the NOE experiments clearly illustrated that both of the methine protons associated with the epoxy functions must be on the same face of the molecule. It is also clear from models that sterically the C17-methyl group must be down relative to the C7 methine proton (i.e. the two groups have a *trans* relationship). **1** and **4** are thus *(1R*,3R*,4S*,7R*,8R*,11R*,12E)-3,4-7,8-bisepoxy-dolabella-12-en-18-ol* and (*1R*,3R*,4S*,7R*,8R*,11R**)-3,4-7,8-bisepoxy-dolabella-12(18)-ene respectively.

Table 1 ^{13}C (75 MHz, $CDCl_3$) data for **1** and **4**, and ^1H (300MHz, $CDCl_3$) NMR data for **1**.

Carbon	^{13}C ppm		δ ^1H	Long range correlation
no.	**4**	**1**	**1**	^{13}C-^1H (J 10Hz)
1	44.5(s)	43.7(s)		
2	38.6(t)	40.7(t)	1.52(m), 1.70(dd, J 2.8, 11.1Hz)	
3	63.8(d)	63.0(d)	3.03(dd, J 2.6, 11.1Hz)	63.0, 40.7
4	60.7(s)	61.5(s)		
5	37.3(t)	37.4(t)	1.32(m), 2.22(ddd, J 3.3, 3.3, 13.8Hz)	64.9, 63.0, 61.5, 37.4
6	23.4(t)	23.5(t)	1.69(m), 2.00(m)	
7	63.6(d)	64.9(d)	2.80(d,J 8.7 Hz).	64.9, 37.4, 23.5
8	60.7(s)	61.7(s)		
9	36.6(t)	36.0(t)	2.01(m), 1.45(m)	61.7, 16.8
10	27.6(t)	25.4(t)	1.50(m), 2.05(m)	
11	43.2(t)	46.9(d)	2.45(brd, J 11.8 Hz)	150.2, 125.7, 46.9, 43.7, 40.7, 36.0, 25.4
12	141.3(s)	150.2(s)		
13	27.6(t)	125.7(d)	5.57(s)	82.7, 48.1, 46.9, 43.7
14	40.4(t)	48.1(t)	2.00(m), 2.37(d, J 16.8 Hz)	150.2, 125.7, 48.1, 40.7, 22.5
15	23.8(q)	22.5(q)	1.29(s)	48.1, 46.9, 43.7, 40.7
16	15.3(q)	16.5(q)	1.29(s)	63.0, 61.5, 37.4, 16.5
17	17.5(q)	16.8(q)	1.48(s)	
18	123.6(s)	82.7(s)	8.22(s,OH)	
19	21.7(q)	26.8(q)	1.38(s)	150.2, 82.7, 26.8
20	22.0(q)	26.7(q)	1.48(s)	150.2, 82.7, 26.7

Compound **2**, a diterpene of the molecular formula $C_{20}H_{32}O_2$, contains three carbon carbon double bonds (110.7, 123.2, 123.8, 140.8, 144.0, 150.8 ppm) and two secondary hydroxy functions (68.4, 73.6 ppm, 3320 cm^{-1}), apparent from ^{13}C NMR and IR spectroscopy: It is thus a bicyclic molecule. Examination of the ^1H-^1H cosy spectrum of **2** (Fig. 1) reveals the presence of three spin systems; -CH-CH_2-CH_2-, -CH_2-CHOH- and -C(CH_3)=CH-CH_2-CHOH-C(=CH_2)-CH_2-CH-, as delineated in Scheme 1. When this information is combined with the short and long range ^{13}C-^1H NMR correlation data the 11-membered ring system of a basic dolabellane is evident (Table 2, and Scheme 1).



Scheme 1 - - - Indicates connectivities established from ^{1}H-^{1}H COSY, Fig. 1.
—— Indicates connectivities established from ^{13}C-^{1}H COSY, Table 2.

The connectivities between C12 and its two neighbours, C 11 and C13, were deduced from comparisons of the ^{13}C NMR data for **2** with those of **4**. The C3-C4 bond then follows as a consequence. The proposed molecule has four chiral centres and one double bond which required stereochemical assignment. The C4-C5 double bond was assigned as *Z* since the C16 methyl group had a carbon shift δ>17. From ^{13}C NMR data comparisons between **2** and **4** it is evident that the relative stereochemistry at C1 is *R** as it is at C11. NOE experiments performed on **2** (see Experimental), enabled the stereochemistry at C3 to be proposed as *R**. This latter deduction was made on the basis of the NOE observed between the C1 methyl group (δ1.28) and the irradiated proton (H3) whose resonance is at δ4.77. This implies that both groups must be down. The stereochemistry at C7 could not be proposed unambiguously from these experiments. **2** is thus *(1R*,3R*,4Z,11R*)-dolabella-4, 8(17),12(18)-trien-3,7-diol.*

The third diterpene to be isolated from this alga was **3** which had been previously reported from an Indian species of *Dictyota dichotoma* [7].

Compounds **1**, **2** and **3** were subjected to antibacterial and antifungal testing. In TLC-bioautographic tests none of the compounds exhibited activity towards *E.coli*, *M.luteus* and *P.oxalicum* at the 10μg level[8].

The second alga, from the family Dictyotaceae, investigated was *Spatoglossum macrodontum*. This plant sample appeared to be devoid of sesqui- and diterpenes, its major lipophyllic constituents being fats, pigments and the sterols cholesterol, 24-methylene cholesterol and fucosterol.

Table 2 ^1H (300 MHz, CD_3OD) and ^{13}C (75 MHz, CD_3OD) NMR data for **2**

Carbon no.	^{13}C ppm	δ ^1H	Long range correlations ^{13}C-^1H (J 10Hz)
1	44.8(s)		
2	44.8(t)	1.07(dd,J 2.1, 13.3 Hz), 1.78(m)	24.0, 44.8
3	68.4(d)	4.77(dd, J 2.0, 11.2 Hz)	
4	144.0(s)		
5	123.2(d)	5.40(m)	17.3, 123.2
6	32.2(t)	3.00(ddd, J 3.3, 3.4, 13.3 Hz), 2.37(brd, J 15.5 Hz)	
7	73.6(d)	4.21(brs)	
8	150.8(s)		
9	29.8(t)	1.75(m), 2.05(m)	
10	32.0(t)	1.35(m), 1.58(m)	
11	43.6(d)	2.97(m)	
12	140.8(s)		
13	27.8(t)	2.13(m)	
14	42.3(t)	1.35(m), 1.65(m)	
15	24.0(q)	1.28(s)	24.0, 42.3, 43.6, 44.8
16	17.3(q)	1.57(s)	123.2, 144.0
17	110.7(t)	5.41(s), 5.09(s)	29.8, 73.6, 110.7
18	123.8(s)		
19	22.0(q)	1.60(s)	21.4, 22.0, 123.8,140.8
20	21.4(q)	1.53(s)	22.0, 123.8, 140.8

Table 3 ^1H (300 MHz, $CDCl_3$) and ^{13}C (75 MHz, $CDCl_3$) NMR data for **3**.

Carbon No.	^{13}C ppm	δ ^1H
1	45.4(s)	
2	42.8(t)	1.78(m), 2.14(dd, J12.0, 13.0Hz)
3	125.1(d)	5.35 (dd, J 2.2, 12.1 Hz)
4	133.8(s)	
5	39.4(t)	2.35(m)
6	24.1(t)	2.31(m), 2.50(m)
7	144.7(d)	6.35(dd, J 1.0, 10.0 Hz)
8	135.5(s)	
9	208.7(s)	
10	36.9(t)	2.40(dd, J 10.8, 12.8 Hz), 2.94 (dd, J 1.0, 12.8 Hz)
11	47.8(d)	1.70(m)
12	86.7(s)	
13	39.9(t)	1.46(m), 1.71(m)
14	29.9(t)	1.45(m), 1.64(m)
15	23.8(q)	1.07(s)
16	15.0(q)	1.49(s)
17	11.8(q)	1.73(s)
18	34.2(d)	1.60(dq, J 7.0, 7.0 Hz)
19	17.7(q)	0.72 (d, J 7.0 Hz)
20	18.1(q)	0.88 (d, J 7.0 Hz)

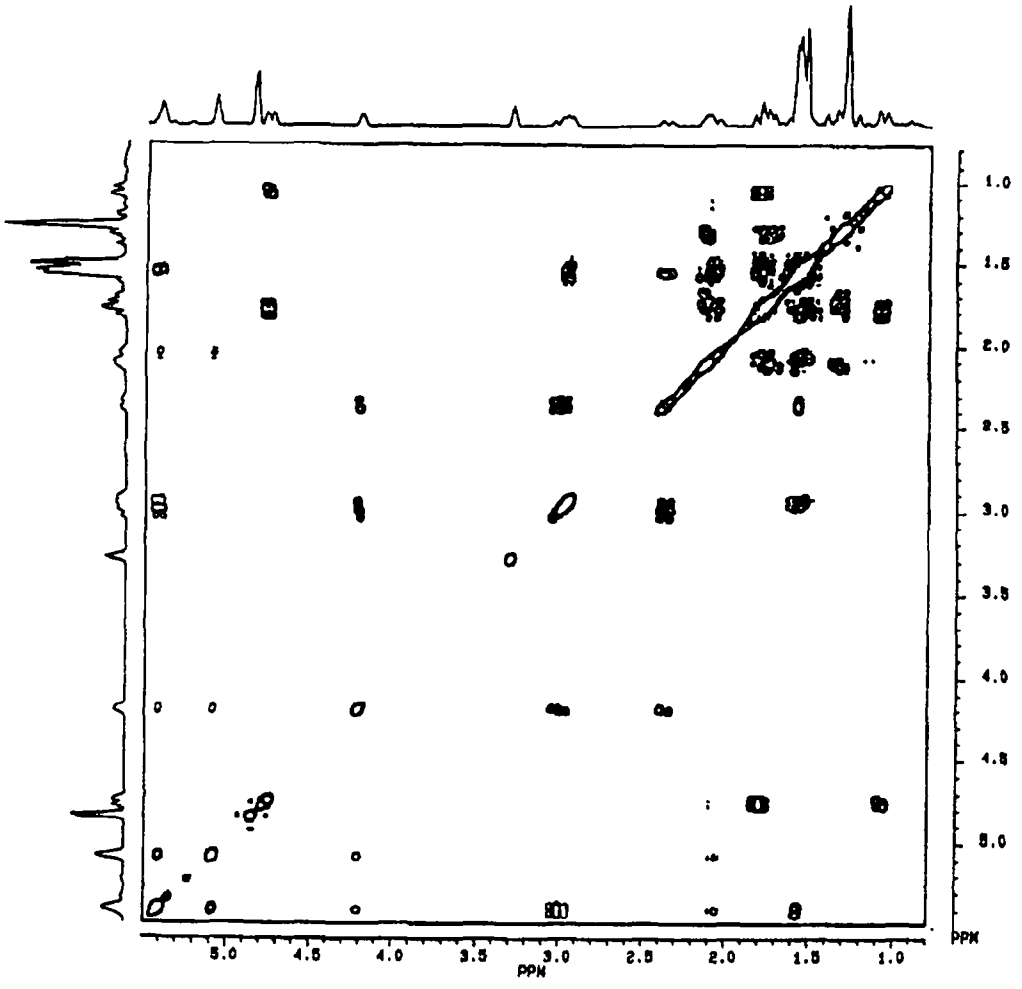


Figure 1. Symmetrized contour plot of a 300MHz absolute value COSY45 spectrum of a solution of **2** in CD_3OD, T=298K. A 1Kx512W data matrixc has been transformed with sine-bell filters in both domains using 1989 DISNMR software. 16 scans were made for each of 256 individual transients.

EXPERIMENTAL

GENERAL PROCEDURES. Melting points were measured on a stereomicroscope (Wild Ltd., Heerbrugg, Switzerland) fitted with a Mettler FP52 hot-stage apparatus and are uncorrected. Mass spectra were recorded on either a Hitachi-Perkin-Elmer RMU-6M (EIMS) or a ZAB2-SEQ (FABMS) mass spectrometer. All ^1H NMR and ^{13}C NMR were recorded using a Bruker AC300 NMR spectrometer. Unless otherwise stated the NMR solvent is $CDCl_3$ containing a trace of

$CHCl_3$ which is used as the internal reference for ^1H NMR measurements (δ7.26). IR spectra were recorded on a Perkin-Elmer 781 infrared spectrometer as either liquid films or Nujol mulls and UV spectra were obtained, in ethanol, on a Perkin-Elmer Lambda 3 UV/VIS spetrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter using $CHCl_3$ as solvent.
Silica gel (type 60, Merck) was used for column chromatography, and aluminium backed plates coated with silica gel 60 F_{254} 0.2mm thick (Merck) were used for TLC. HPLC was carried out with a Waters 6000A solvent delivery system connected to a Rheodyne HPLC injector and a Knauer differential refractometer. HPLC columns were from Knauer (250mm x 8mm, filled with LiChromosorb Si60 5μm). All solvents were HPLC grade.

PLANT MATERIAL.All plant materials were collected by divers using self contained underwater breathing apparatus (SCUBA). After collection plant materials were deep frozen until they were freeze dried. Voucher specimens were deposited with the James Cook University's Botany Department Herbarium (JCT).

ISOLATION FROM *Dictyota pardarlis f. pseudohamata* **(A7749)**. A sample of the alga was collected from Florence Bay, Magnetic Island and on return to the laboratory deep frozen and subsequently freeze dried. The dry tissue (108g) was then exhaustively extracted with DCM (3l) to yield 5.5g (5.1%) of DCM soluble material. Vacuum liquid chromatography (VLC)[9] of this material over silica gel, using hexane with increasing proportions of EtOAc as an eluent system , afforded 12 fractions, each of 100ml. VLC of fraction 4, employing eluent elution as described above, yielded 9x60ml fractions.

(1R,3R*,4S*,7R*,8R*,11R*,12E)-3,4-7,8-bisepoxy-dolabella-12-en-18-ol*, **1**:(73mg, 1.33%); Fraction 5, after HPLC separation with hexane/EtOAc (95/5) as the eluent, afforded a white crystalline solid mp 118.5-119.5°; $[\alpha]_D^{25}$ +57.6° (c=0.024, $CHCl_3$); mass measurement observed 320.235, $C_{20}H_{32}O_3$ requires m/e 320.229; FABMS (% rel. int.) 320 (M^+, 4) 319(16), 303(32), 285(9) , 257(9), 191(8), 177(6) 161(11), 155(25), 137(68), 133(45), 77(55); IR (film) 3300, 2910, 1450, 1390 and 785cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1; NOE experiments, irradiation at δ3.03 caused enhancement of the resonance at δ2.80 (1%) and δ1.69 (1.2%). Irradiation at δ2.80 caused enhancement of the resonance at δ3.03 (1.5%).

(1R, 3R*,4Z, 11R*)-dolabella-4,8(17),12(18)-trien-3,7-diol*; **2**:(15mg, 0.3%); HPLC separation, using hexane/EtOAc (50/50) as eluent , of combined fractions 10 and 11 from the VLC of the DCM soluble plant extract, afforded a diterpene, which was a white crystalline solid mp 206-207°; $[\alpha]_D^{25}$ + 66.0° (c=0.005, $CHCl_3$); mass measurement observed 304.235, $C_{20}H_{32}O_2$ requires m/e 304.240; FABMS (% rel.int.) 304 (M^+, 10) 303(6), 288(20), 287(96), 273(11), 269(15), 205(20), 187(11), 175(11), 166(11), 149(30), 137(94), 119(14); IR (film) 3320, 2930, 1450, 1380, 1005 cm^{-1} ; ^1H NMR (300 MHz, $CDCl_3$) δ1.13 (dd, 1H, J 2.3, 13.1 Hz, 2-H), 1.27 (s, 3H, 1-Me), 1.54 (s, 3H, 18-Me), 1.57 (s, 3H, 8-Me), 1.61(s, 3H, 18-Me'), 1.3-1.75(m, 5H, 10 and 14-H_2 and 9-H), 1.82 (dd, 1H, J 11.5, 13.1 Hz, 2-H'), 2.12 (m, 3H, 13-H_2 and 9-H'), 2.45 (brd, J 15.5 Hz, 6-H), 2.85 (d, 1H, 11.2 Hz, 11-H), 2.95 (ddd, 1H, J 2.7, 11.8, 15.5Hz , 6-H'), 4.30 (brs, 1H, 7-H), 4.75 (brd, 1H, J 11.5 Hz, 3-H), 5.12 (s, 1H, 17-H), 5.39 (s, 1H, 17-H'), 5.44 (d, 1H, J 11.5 Hz, 5-H); ^1H NMR (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD) see Table 2; NOE experiments; irradiation at δ4.21 caused enhancement of the resonances at δ3.00 (3.5%), 2.37 (3%) and 1.75 (2%). Irradiation at δ4.77 caused enhancement of signals at δ3.00 (13%), 1.28 (5%) and 1.07 (5%).

3: HPLC separation of fraction 5 from the VLC of the DCM soluble extract, using DCM/EtOAc (95/5) as the eluent, afforded two diterpenes; **1** and the previously described metabolite **3**; (14mg, 0.3%); a white crystalline solid with identical physical and spectroscopic data to the

compound reported by Bheemasankara, *et al.* [7]. Table 3 contains complete proton and carbon assignments for **3**. These assignments are based on short range ^{13}C-^{1}H NMR correlation experiments optimised for J 125 Hz.

ISOLATION FROM *Spatoglossum macrodontum*.(A7750).A sample of the alga collected from Florence Bay, Magnetic Island, was freeze dried. The plant tissue (214g) was then exhaustively extracted with DCM to afforded 10.7g (0.05%) of extract. VLC of the DCM extract, over silica with hexane and diethyl ether, so the polarity of the eluent increased, afforded 18 fractions, each of 75 ml. Examination of these fraction by TLC and ^{1}H NMR indicated that the major secondary metabolites were, aside from pigments and lipids, steroidal. The major steroids that were identified on the basis of their ^{1}H NMR, ^{13}C NMR, $[\alpha]_D$ and mp's were cholesterol (63mg, 0.006%), 24-methylene cholesterol (39mg, 0.004%) and fucosterol (34mg, 0.003%).

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