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EXTENSIVE 1D AND 2D NMR AND X-RAY STUDIES OF DITERPENES ISOLATED FROM THE MARINE ALGA *DICTYOTA PARDALIS* f. *PSEUDOHAMATA*

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ABSTRACT.—From the lipophilic extract of the marine alga *Dictyota pardalis* f. *pseudohamata*, four new diterpenes [**1–4**] of the dolastane and dolabellane classes have been isolated and characterized by spectroscopic (nmr, ir, ms, and X-ray) methods. Together with the new natural products, the previously reported compounds **5–8** were also isolated. Single-crystal X-ray analysis of compounds **1**, **9**, and **11** has permitted their absolute configurations to be assigned. For compounds **5** and **6**, complete stereochemical assignments are reported. Unambiguous ^{1}H- and ^{13}C-nmr assignments are made for compounds **1–8**. The results of the current investigations are compared with the results of a former study of the same algal species.

Algae of the genus *Dictyota* have previously been shown to be rich sources of terpenes possessing various carbon skeletons (1,2). On two separate collecting trips to the Great Barrier Reef, Australia, we were able to collect *Dictyota pardalis* f. *pseudohamata* Cribb (Dictyotaceae). The aim of these collections was to compare the results of the current investigation with those of an earlier study (3,4) of the same species from almost the same location.

RESULTS AND DISCUSSION

The CH_2Cl_2 solubles obtained from the freeze-dried alga *D. pardalis* f. *pseudohamata* were fractionated by vlc using Si gel. Fractions which contained terpenes, as judged by ^{1}H-nmr spectroscopy, were further purified by normal-phase hplc on Si gel to yield eighteen terpenoid metabolites, ten of which have been reported previously (5). The current report describes the isolation and characterization of the other eight compounds [**1–8**].

Compound **1** had the molecular formula $C_{20}H_{30}O_2$ as deduced by mass spectrometry. Its ir spectrum indicated the presence of carbonyl and hydroxyl functionalities (1715 and 3480 cm^{-1}). The presence of five resonances in the ^{13}C-nmr spectrum of **1** for two carbon-carbon double bonds [117.4 (d), 123.1 (d), 126.7 (d), 139.8 (s) ppm] and one carbon-oxygen double bond [214.1 (s) ppm], and the absence of any further resonances for sp or sp^2 hybridized carbon atoms, indicated **1** to be a tricyclic molecule.

The ^{1}H-^{1}H COSY spectrum of **1** allowed five proton spin-systems to be elaborated: a conjugated diene moiety (δ 5.58 br m, 5.86 dd, 5.94 br d); coupling of methylene protons to a methine proton occurred twice [(δ 1.37 m, 2.19 ddd and 2.31 ddd, 2.66 dd, 1.45 dd)]; two intercoupling methylene protons (δ 1.70 m, 1.95 m, 1.60 m, 1.10 m); and resonances typical of an isopropyl moiety (δ 1.75 m, 0.88 d, 0.96 d). At this point it became obvious that compound **1** was closely related to the previously reported compound **7** (6). Further analysis of spectroscopic data (Tables 1 and 2) suggested the two to have identical planar structures, thus the differences between them must be

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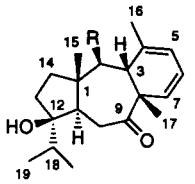

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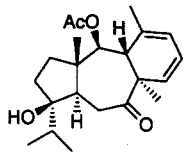
2 R=OAc



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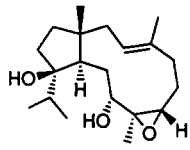

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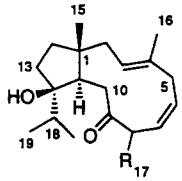
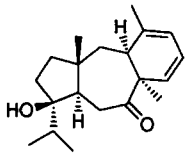


5 R=β-CH_3
6 R=α-CH_3



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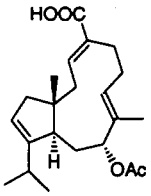

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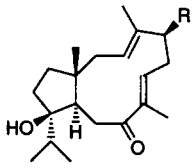
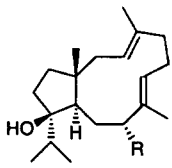
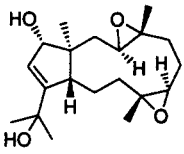


9 R=OAc
12 R=H



10 R=OH
11 R=*p*-bromobenzoate



13

stereochemical. NOESY data revealed an nOe interaction between H-3 (δ 2.19 ddd) and H_3-17 (δ 1.12 s) indicating the six- and seven-membered rings to be cis fused, as in **7**. Compound **1** therefore had to differ from **7** in the relative stereochemistry at centers C-1 and/or C-11 and/or C-12 and/or C-3 and C-8. The presence of an nOe interaction between H_3-15 (δ 1.17 br s) and H-3 (δ 2.19 ddd) indicated them to be on the same side of the molecule in contrast to **7**. To prove this contention an X-ray study of **1** was undertaken (Figure 1). The results of this analysis not only unambiguously showed the difference in relative stereochemistry between **1** and **7** to be as proposed from the nmr data, but also allowed the absolute configuration of **1** to be established. Compound **1** is (1*R*,3*S*,4*Z*,6*Z*,8*R*,11*R*,12*R*)-12-hydroxydolasta-4,6-dien-9-one.

The resultant X-ray structure of **1** is best described in terms of the conformations of the three rings. The seven-membered ring approximates a chair conformation with C-1 lying on the local mirror. Five atoms of the ring conform well to the approximate mirror symmetry, but C-8 and C-9 are twisted out of mirror-related positions such that torsion angles about the C-3, C-8 and C-9, C-10 bonds differ in magnitude by 28°, and the torsion angle about the C-8, C-9 bond differs from zero by 20.5°. The five-membered ring has the envelope conformation with C-1 at the flap position, with the other four atoms forming a torsion angle of −2.2°. The 1,3-cyclohexadiene ring has a twist conformation with the local twofold axis bisecting C-5, C-6 and C-3, C-8. The diene unit is not planar. Torsion angles about the two double bonds are −2.6° for $\Delta^{6,7}$ and −5.7° for $\Delta^{4,5}$, and the torsion angle about the central bond of the diene (C-5, C-6) is −13.1°. Molecules are linked in the crystal by weak hydrogen bonds involving the hydroxyl

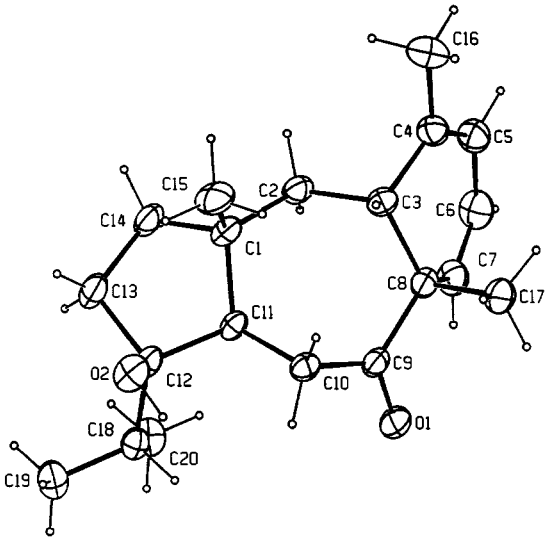


FIGURE 1. ORTEP drawing of **1**.

substituent OH-2 and carbonyl oxygen O-1. The O...O distance is 2.937(3) Å, and the angle about H is 160(2)°.

Compound **2** analyzed for $C_{22}H_{32}O_4$ by mass spectrometry. Of the seven degrees of unsaturation implied by the molecular formula, four were accounted for by multiple bonds; two carbon-carbon double bonds (δ 137.0 s, 119.7 d, 124.5 d, 127.5 d), a keto group (δ 213.3 s), and an acetate function (δ 169.7 s, 21.0 q), indicating **2** to be tricyclic. Comparison of all the spectroscopic data of **2** with those for **1**, and the results of a 2D ^{1}H-^{1}H COSY experiment, revealed that the two molecules were identical, except for the presence of an additional acetoxyl function in **2**. On the basis of ^{1}H-^{1}H couplings observed in the ^{1}H-nmr spectrum of **2** it was apparent that this functionality had to reside at either C-2 or C-10. The proton-detected 2D long-range (J=10 Hz) ^{1}H-^{13}C COSY (HMBC) spectrum of **2** showed heteronuclear couplings between C-2 (δ 80.0 d) and H-3 (δ 2.35 dd), as well as from C-12 (δ 85.3 s) to H_2-10 (δ 2.36 dd, 2.72 dd), which allowed the acetate group to be unambiguously positioned at C-2. The relative stereochemistry of **2** at C-1, C-3, C-8, C-11, and C-12 was identical to that of **1** on the basis of comparable nOe interactions and inter-proton coupling patterns. For C-2 it was concluded that the acetate function had to be β, based on a 9.8 Hz coupling between H-2 (δ 4.99 d) and H-3 (δ 2.35 dd), as well as nOe effects observed between H-2 and H-11 (δ 1.80 m). Compound **2** is (1*R**, 2*R**, 3*S**, 4*Z*, 6*Z*, 8*S**, 11*S**, 12*S**)-2-acetoxy-12-hydroxydolasta-4,6-dien-9-one.

Compound **3**, a further dolastane derivative, had the same molecular formula, $C_{22}H_{32}O_4$, as **2**. Interpretation of spectroscopic data for **3** indicated that it was a stereoisomer of **2**. Comparison of the ^{13}C-nmr data for the two compounds revealed that the major differences between them lay in the vicinity of the C-3, C-8 ring junction (see Table 2). From a NOESY spectrum of **3**, significant nOe effects could be observed between H_3-17 (δ 1.36 s) and H-7 (δ 6.00 d), H-11 (δ 2.27 dd), and H-2 (δ 5.19 d), and between H-3 (δ 3.09 br d) and H_3-15 (δ 0.99 s), and between the isopropyl protons (δ 0.93 d, 0.95 d) and H-11. These data clearly indicated the only relative difference between **3** and **2** to be at C-8; the C-3, C-8 ring junction is trans fused in **3**. Compound **3** is (1*R**,2*R**,3*S**,4*Z*,6*Z*,8*R**,11*S**,12*S**)-2-acetoxy-12-hydroxydolasta-4,6-dien-9-one.

Compound **4** had a molecular formula of $C_{20}H_{34}O_3$. Of the four degrees of unsaturation implied by the molecular formula, only one could be accounted for by the presence of a multiple bond; a carbon-carbon double bond as deduced from ^{1}H- and ^{13}C-nmr data (δ 5.40 br d, 123.7 d, 133.7 s), indicating **4** to be tricyclic. Further analysis of the nmr data of **4** and comparison with earlier published data (3,4) for *Dictyota* metabolites indicated **4** to be a dolabellane derivative. The ^{13}C-nmr spectrum showed four resonances for oxygenated carbons [δ 84.4 s, 77.8 d, 64.7 s, 63.2 d] being for one tertiary and one secondary hydroxyl function, and an epoxide. The secondary hydroxyl group was positioned at C-9 on the basis of a chain of proton-proton couplings from H-11 (δ 1.83 dd) to H_2-10 (δ 2.01 m, 1.63 m) to H-9 (δ 3.40 br d). In the same fashion, it was possible to assign the single carbon-carbon double bond as being $\Delta^{3,4}$, since H_2-2 (δ 2.25 m, 1.86 m) coupled to H-3 (δ 5.40 d), which in turn was long-range coupled to H_3-16 (δ 1.65 br s). A third proton spin-system was traced from H_2-5 (δ 2.27 m) through H_2-6 (δ 1.99 m, 1.60 m) to H-7 (δ 2.65 dd), which was part of the epoxy function. The latter function must thus reside between C-7 and C-8. The final functionality, the tertiary hydroxyl, was positioned at C-12, as in other dolabellanes and dolastanes of this algal sample (see Table 2). With the basic skeleton of **4** established, all that remained was the determination of stereochemistry. The $\Delta^{3,4}$ double bond was assigned with the *E* configuration based on the ^{13}C-nmr chemical shift of CH_3-16 (15.9 q), which is in the same range as those of compounds **5**, **6**, and **9**. The relative stereochemistries of the six chiral centers within **4** were assigned from the results of a single NOESY measurement. Diagnostic nOe interactions were observed between H-7 (δ 2.65 dd), H-3 (δ 5.40 br d), H-9 (δ 3.40 br d), and H_3-15 (δ 1.05 s), indicating them to be on the one side and also fixing the epoxide as trans. The nOe as well as the ^{13}C-nmr data were all consistent with centers C-1, C-11, and C-12 being the same in a relative stereochemical sense, as the equivalent centers in **1–3**, **9**, and **11**. Compound **4** is (1*R**,3*E*,7*S**,8*R**,9*R**,11*R**,12*R**)-7,8-epoxy-9,12-dihydroxydolabella-3-ene.

Compounds **5** and **6** were reported as C-8 isomers isolated from an Indian Ocean collection of *Dictyota dichotoma* (6). The nature of the configuration at C-8 was, however, never resolved. After assigning all carbon and proton resonances of **5** and **6** via 2D nmr methodologies, extensive nOe measurements, in the form of NOESY spectra, were made. From these measurements the relative position of H-8 in **5** and **6** was assigned. The observation of cross-peaks in the NOESY spectrum of **5**, between H_3-15 (δ 1.07 s) and H-10 (δ 2.55 dd), and between H_3-19 (δ 0.90 d), H-11 (δ 2.03 m), H-10 (δ 2.24 dd) and H-8 (δ 3.33 m), and between H-3 (δ 5.24 br d) and H-6 (δ 5.84 dddd) clearly indicated H_3-17 to be β. Compound **5** is (1*R**,3*E*,6*Z*,8*R**,11*R**,12*R**)-12-hydroxydolabella-3,6-dien-9-one.

In compound **6**, H_3-19 (δ 0.95 d) and H_3-20 (0.93 d) as well as H-18 (δ 1.70 m) showed an nOe with H-10α (δ 2.25 br d), which in turn had a nOe interaction with H_3-17 (δ 1.21 d). All these substituents were thus on one side (α) of the molecule. H_3-15 (δ 1.16 s) demonstrated a nOe with H-10 (δ 2.60 dd), indicating the two to be β oriented. Compound **6** is (1*R**,3*E*,6*Z*,8*S**,11*R**,12*R**)-12-hydroxydolabella-3,6-dien-9-one.

Compounds **7** and **8** were shown to be identical with compounds previously reported (6). Extensive 2D nmr measurements made on these two compounds permitted their ^{1}H-nmr (Table 1) and ^{13}C-nmr (Table 2) data to be unambiguously assigned for the first time.

The previously reported compounds **9** and **10**, also from *Dictyota pardalis* f. *pseudohamata* (5), were recently crystallized. As the absolute configurations of these molecules were not known and our crystals appeared to be of suitable quality, single-crystal X-ray analyses were undertaken. The results of these analyses secured the absolute configuration for compound **9** (Figure 2) and indicated the conformation of the

TABLE 1. ^{1}H-Nmr Data (300 MHz, $CDCl_3$) for Compounds **1–8**.[a]

Proton	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2	1.37 (m)	4.99 (d, J=9.8)	5.19 (d, J=10.8)	2.25 (m), 1.86 (m)	2.05 (m), 1.87 (m)	1.94 (m)	1.35 (m), 1.51 (m)	3.19 (br dd, J=12.8, 12.9), 1.80 (dd, J=4.2, 12.9)
3	2.19 (ddd, J=1.2, 4.1, 8.8)	2.35 (dd, J=1.1, 9.8)	3.09 (br d, J=10.8)	5.40 (br d, J=12.2)	5.24 (br d, J=11.4)	5.29 (m)	2.41 (dd, J=3.6, 11.2)	6.03 (dd, J=4.2, 12.8)
5	5.58 (br m)	5.71 (br m)	5.78 (m)	2.27 (m)	2.58 (br dd, J=6.8, 16.6), 2.71 (br dd, J=7.3, 16.6)	2.73 (ddd, J=2.2, 5.8, 14.5), 2.60 (m)	5.60 (br d, J=5.7)	2.17 (m), 2.79 (m)
6	5.86 (dd, J=5.0, 9.5)	5.93 (br d, J=9.4)	5.84 (dd, J=5.4, 9.7)	1.99 (m), 1.60 (m)	5.84 (dddd, J=1.5, 6.8, 7.3, 10.6)	5.77 (dddd, J=1.0, 5.8, 8.4, 10.6)	5.80 (dd, J=5.7, 9.8)	2.65 (m), 2.11 (m)
7	5.94 (br d, J=9.5)	5.99 (dd, J=4.7, 9.4)	6.00 (d, J=9.7)	2.65 (dd, J=2.1, 10.9)	5.52 (ddd, J=1.8, 8.0, 10.6)	5.54 (ddd, J=2.2, 8.5, 10.6)	5.96 (d, J=9.8)	5.23 (br d, J=11.3)
8					3.33 (m)	3.47 (br dq, J=7.3, 7.5)		
9				3.40 (br d, J=4.5)				5.40 (dd, J=6.5, 10.5)
10	2.31 (ddd, J=2.1, 12.5), 2.66 (dd, J=11.9, 12.5)	2.36 (dd, J=3.0, 13.5), 2.72 (dd, J=12.1, 13.5)	2.50 (dd, J=7.8, 15.8), 2.89 (dd, J=11.9, 15.8)	2.01 (m), 1.63 (m)	2.55 (dd, J=6.2, 16.1), 2.24 (dd, J=4.6, 16.1)	2.60 (dd, J=7.5, 16.7), 2.25 (br d, J=16.7)	2.62 (d, J=10.1) 2.70 (d, J=9.3)	2.00 (m), 1.61 (m)
11	1.45 (dd, J=2.1, 11.9)	1.80 (m)	2.27 (dd, J=7.8, 11.9)	1.83 (dd, J=8.5, 2.0)	2.03 (m)	2.04 (dd, J=1.8, 7.5)	2.26 (dd, J=9.3, 10.1)	2.00 (m)
13	1.70 (m), 1.95 (m)	1.91 (m), 1.60 (m)	2.00 (m), 1.57 (m)	1.32 (m), 1.58 (m)	1.39 (m), 1.67 (m)	1.30 (m), 1.60 (m)	1.30 (m), 1.50 (m)	5.19 (br s)
14	1.60 (m), 1.10 (m)	1.45 (m), 1.30 (m)	1.75 (m), 1.34 (m)	1.42 (m), 1.90 (m)	1.55 (m), 1.87 (m)	1.58 (m), 1.94 (m)	1.97 (m), 1.60 (m)	2.00 (m), 2.32 (m)
15	1.17 (br s)	1.18 (s)	0.99 (s)	1.05 (s)	1.07 (s)	1.16 (s)	0.96 (s)	1.28 (br s)
16	1.78 (s)	1.86 (br s)	1.81 (s)	1.65 (br s)	1.62 (br s)	1.44 (br s)	1.78 (br s)	
17	1.12 (s)	1.14 (s)	1.36 (s)	1.30 (s)	1.16 (d, J=7.1)	1.21 (d, J=7.3)	1.17 (s)	1.54 (br s)
18	1.75 (m)	1.76 (m)	1.66 (m)	2.15 (m)	1.67 (m)	1.70 (m)	1.67 (m)	2.15 (m)
19	0.88 (d, J=6.7)	0.88 (d, J=6.7)	0.93 (d, J=6.5)	0.93 (d, J=6.9)	0.90 (d, J=6.8)	0.95 (d, J=6.8)	0.95 (d, J=6.7)	0.89 (d, J=6.6)
20	0.96 (d, J=6.7)	0.96 (d, J=6.7)	0.95 (d, J=6.5)	0.98 (d, J=6.9)	0.92 (d, J=6.8)	0.93 (d, J=6.8)	0.97 (d, J=6.7)	1.06 (d, J=6.6)
OAc		2.00 (s)						2.00 (s)

[a]Coupling constants are in Hz.

TABLE 2. ^{13}C-Nmr Data (75.5 MHz, $CDCl_3$) for Compounds **1–8**.

Carbon	Compound							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	43.5 s[c]	48.5 s	51.8 s[a]	44.9 s	45.5 s	45.9 s	43.3 s	44.8 s
2	41.8 t	80.0 d	80.9 d	43.0 t	42.5 t	42.3 t	40.6 t	41.9 t
3	43.1 d	46.9 d[a]	47.3 d	123.7 d	123.0 d	122.8 d	44.3 d	147.2 d
4	139.8 s	137.0 s	132.7 s	133.7 s	135.2 s	136.7 s	136.4 s	129.8 s
5	117.4 d	119.7 d	123.5 d	37.6 t	36.2 t	38.4 t	117.8 d	35.3 t
6	123.1 d	124.5 d	122.0 d	24.2 t[a]	130.1 d	134.9 d	121.6 d	25.8 t
7	126.7 d	127.5 d	135.1 d	63.2 d	132.7 d	127.2 d	126.8 d	132.2 d
8	52.6 s	51.7 s	53.7 s[a]	64.7 s	45.7 d	47.3 d	53.1 s	133.5 s
9	214.1 s	213.3 s	216.6 s	77.8 d	213.2 s	212.9 s	213.1 s	79.9 d
10	37.3 t	37.6 t	39.6 t	31.1 t[a]	35.6 t	36.8 t	38.2 t	31.7 t
11	50.4 d	47.1 d[a]	44.6 d	46.5 d	46.7 d	48.8 d	45.7 d	47.9 d
12	85.0 s	85.3 s	84.8 s	84.4 s	86.0 s	86.0 s	83.3 s	152.6 s
13	34.7 t[a]	33.8 t	35.3 t[b]	43.0 t[b]	40.4 t[b]	40.8 t[a]	41.4 t[a]	118.9 d
14	39.8 t[a]	36.9 t	39.0 t[b]	32.1 t[b]	32.9 t[b]	33.9 t[a]	35.8 t[a]	48.6 t
15	19.3 q	14.6 q	14.2 q	21.5 q	22.6 q	20.9 q	23.3 q	22.4 q
16	22.2 q	25.4 q	22.5 q	15.9 q	18.0 q	15.8 q	22.1 q	173.3 s
17	22.9 q	21.8 q	13.8 q	14.6 q	17.8 q	17.6 q	25.0 q	11.0 q
18	36.6 d	36.4 d	37.7 d	35.9 d	35.6 d	36.2 d	37.5 d	26.9 d
19	18.2 q	18.2 q	18.0 q	18.8 q	18.1 q	17.9 q	18.5 q	22.5 q
20	17.3 q	17.2 q	17.2 q	17.4 q	17.3 q	16.9 q	17.5 q	21.8 q
Acetate		169.7 s	171.1 s					170.4 s
		21.0 q	22.1 q					21.4 q

[a-b]Assignments with the same superscripts may be interchangeable.
[c]Multiplicities determined by DEPT.

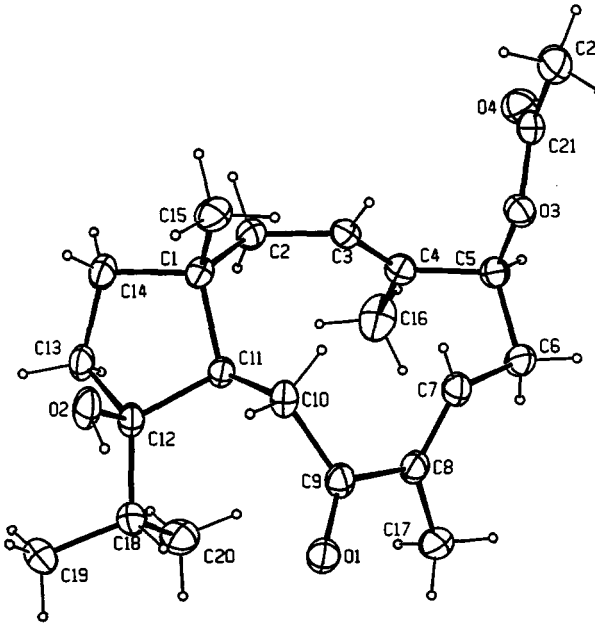


FIGURE 2. ORTEP drawing of **9**.

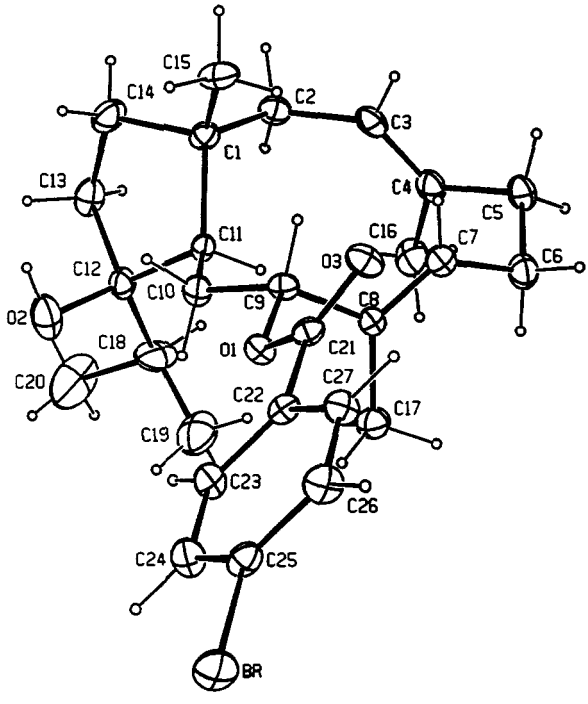


FIGURE 3. ORTEP drawing of **11**.

cycloundecadiene ring to be such that methyl groups C-16 and C-17 are both α-oriented. Their directions vary from being parallel by 36.3°. Both double bonds are *E*, and both deviate substantially from planarity, forming CC=CC torsion angles of −172.2° about C=C $\Delta^{3,4}$ and −171.3° about C=C $\Delta^{7,8}$. The ketone oxygen atom is *S*-trans to the $\Delta^{7,8}$ double bond, but not quite coplanar with it, forming a C=C, C=O torsion angle of −152.7°. The five-membered ring is in a half-chair conformation, with C-1 lying on the local twofold twist axis. Molecules are linked in the crystal by weak intermolecular hydrogen bonds involving the OH group and acetate carbonyl oxygen. The O...O distance is 3.004(4) Å, and the angle about H is 151°. Compound **9** is thus (1*R*,3*E*,5*S*,7*E*,11*R*,12*R*)-5-acetoxy-12-hydroxydolabella-3,7-dien-9-one.

The crystals of compound **10**, although of a high quality, were all unfortunately twinned, and as such were not suitable for analysis. As a knowledge of the absolute configuration of this molecule was considered useful information, the *p*-bromobenzoate derivative was produced [**11**]. X-ray analysis of this compound (Figure 3) enabled its absolute configuration to be deduced. Compound **10** is (1*R*,3*E*,7*E*,9*R*,11*R*,12*R*)-9-hydroxydolabella-3,7-dien-12-ol.

The X-ray structure of **11** indicated that the conformation of the 11-membered ring is similar to that of **9**, except for the position of C-10. The seven endocyclic torsion angles not involving C-10 differ by a mean value of only 7.0°, but the C-9, C-10 bond is puckered in the opposite sense in the two molecules. The C-8, C-9, C-10, C-11 torsion angle is +75.1° for the acetate and −50.5° for the *p*-bromobenzoate. This 11-membered ring conformation is evidently more strained, as the two *E* double bonds deviate more from planarity, with torsion angles of −170.6° about C=C $\Delta^{3,4}$ and −166.4° about C=C $\Delta^{7,8}$. The methyl groups at C-16 and C-17 are slightly closer to being parallel, forming an angle of 30.8°. The conformational change in the 11-membered ring is accompanied by a change to the envelope conformation for the 5-membered ring, with

C-14 at the flap position. The OH group does not engage in hydrogen bonding, which may account for the apparent conformational disorder in the vicinity of C-12.

The results of this and the earlier investigation of *Dictyota pardalis* f. *pseudohamata* (3,4) clearly show that this algal species is capable of producing a far greater variety of secondary metabolites with greater variations in functionalities than previously thought. The first study of *D. pardalis* f. *pseudohamata* yielded exclusively dolabellanes, with **12** being the only metabolite common to both samples (5). The current investigation has yielded both dolabellanes, with acetate functionalities, as well as further cyclized diterpenes and dolastanes. All isolates, with the exception of **8**, have an identical substitution pattern in the five-membered ring. Noteworthy is the obvious occurrence of many isomeric compounds in this second investigation, which may be providing a glimpse of the vast biosynthetic potential of this algal species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—X-ray data were collected on an Enraf-Nonius CAD4 diffractometer using graphite-monochromated CuK_α or MoK_α radiation. For all three compounds the refinement of the mirror-image structure was carried out. The reported vs. alternate models gave the following R_w values for the three compounds: **1**: 0.0545 vs. 0.0546; **9**: 0.0591 vs. 0.0593; **11**: 0.040 vs. 0.081. The difference is highly significant for the bromo compound **11**, and the absolute configuration thus determined agrees with the better fitting configuration of the other two compounds. Remaining data as per König *et al.* (7).

PLANT MATERIAL.—All algal materials were collected from Magnetic Island, Queensland, Australia. The plants were all obtained from a depth of 0–3 m during July 1987, and then deep frozen. A voucher specimen is deposited with the Department of Botany and Tropical Agriculture, James Cook University of North Queensland, Australia; voucher number JCT A8084.

EXTRACTION AND ISOLATION.—Deep-frozen algal tissue was freeze dried. Dry tissue (105.0 g) was extracted with CH_2Cl_2 (2.5 liters) and then with MeOH (2 liters). From both extracts the CH_2Cl_2 solubles (10.2 g) were taken, combined, and chromatographed over Si gel with petroleum ether containing increasing proportions of EtOAc as eluent; 15 fractions, each of approximately 90 ml, were obtained.

Hplc separation of vlc fraction 6 [LiChrosorb Si60, 5 μm, hexane-*t*-butylmethylether (7:1.5)] yielded compounds **5** and **6**.

(1R,3E,6Z,8R*,11R*,12R*)-12-Hydroxydolabella-3,6-dien-9-one* [**5**].—10 mg, 0.009%; oil; $[\alpha]^{25}D$ −17.8° (c=0.37, $CHCl_3$); ^1H nmr (300 MHz, $CDCl_3$), see Table 1; ^{13}C nmr (75.5 MHz, $CDCl_3$), see Table 2; eims *m/z* (rel. int.) 304 (M^+, 5), 275 (3), 261 (4), 243 (6), 205 (7), 191 (5), 181 (90), 163 (14), 121 (100), 107 (42).

(1R,3E,6Z,8S*,11R*,12R*)-12-Hydroxydolabella-3,6-dien-9-one* [**6**].—8 mg, 0.008%; oil; $[\alpha]^{25}D$ +17.9° (c=0.79, $CHCl_3$); ^1H nmr (300 MHz, $CDCl_3$), see Table 1; ^{13}C nmr (75.5 MHz, $CDCl_3$), see Table 2; eims *m/z* (rel. int.) 304 (M^+, 6), 275 (4), 261 (5), 243 (7), 191 (10), 181 (87), 163 (15), 149 (20), 121 (91).

Hplc separation of vlc fraction 7 (LiChrosorb Si60, 5 μm, hexane- *t*-butylmethylether (8:2)) yielded compounds **7** (183 mg, 0.17%) and **8** (180 mg, 0.17%) with identical spectroscopic data to those published (6). Assignment of ^1H nmr (300 MHz, $CDCl_3$), see Table 1; ^{13}C nmr (75.5 MHz, $CDCl_3$), see Table 2.

Hplc separation of vlc fraction 8 [LiChrosorb Si60, 5 μm, hexane- *t*-butylmethylether (7:3)] yielded compound **1**.

(1R,3S,4Z,6Z,8R,11R,12R)-12-Hydroxydolasta-4,6-dien-9-one [**1**].—25 mg, 0.02%; crystalline, mp 121–124°, $[\alpha]^{25}D$ −87.0° (c=0.85, $CHCl_3$); ir ν max 3450, 2930, 2915, 1685 cm^{-1}; uv λ max (EtOH) (ε) 260 nm (1100); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* (rel. int.) 302 (M^+, 4), 284 (38), 269 (7), 181 (91), 121 (100), 107 (89); hreims, observed, *m/z* 302.2224, $C_{20}H_{30}O_2$ requires 302.2246.

Hplc separation of vlc fraction 10 [LiChrosorb Si60, 5 μm, hexane-Me_2CO (8:2)] yielded compounds **2** and **3**.

(1R,2R*,3S*,4Z,6Z,8S*,11S*,12S*)-2-Acetoxy-12-hydroxydolasta-4,6-dien-9-one* [**2**].—10 mg, 0.009%; clear oil; $[\alpha]^{25}D$ −107.0° (c=0.31, $CHCl_3$); ir ν max 3520, 2970, 2880, 1790, 1700 cm^{-1}; uv λ max (EtOH) (ε) 260 (3300); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* (rel. int.) 360 (M^+, 0.1), 342 (0.2), 300 (4), 282 (9), 257 (12), 239 (8), 211 (7), 193 (15), 181 (34), 119 (44), 43 (100); hreims, observed, *m/z* 360.2298, $C_{22}H_{32}O_4$ requires 360.2302.

(1R,2R*,3S*,4Z,6Z,8R*,11S*,12S*)-2-Acetoxy-12-hydroxydolasta-4,6-dien-9-one* [**3**].—2.1 mg, 0.002%; clear oil; $[\alpha]^{25}D$ +40.0° (*c*=0.21, $CHCl_3$); ir ν max 3400, 2960, 2920, 1740, 1690 cm^{-1}; uv λ max (EtOH) (ε) 263 nm (3227); ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* (rel. int.) 360 (M^+, <1), 300 (3), 282 (2), 267 (2), 257 (15), 197 (19), 181 (43), 159 (71), 135 (57), 121 (60), 43 (100); hreims, observed, *m/z* 300.2056, $C_{20}H_{30}O_2$ requires 300.2046.

Hplc separation of vlc fraction 11 {LiChrosorb Si60, 5 μm, hexane-Me_2CO (8:2)] yielded compound **4**.

(1R,3E,7S*,8R*,9R*,11R*,12R*)-7,8-Epoxy-9,12-dihydroxydolabella-3-ene* [**4**].—9.0 mg, 0.009%; clear oil; $[\alpha]^{25}D$ +51.5° (*c*=0.26, $CHCl_3$); ir ν max 3400, 2960, 2860, 1385 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* (rel. int.) 322 (M^+, 1), 304 (10), 286 (25), 261 (28), 243 (28), 193 (35), 137 (38), 121 (100), 107 (41), 93 (45); hreims, observed, *m/z* 322.2520, $C_{20}H_{34}O_3$ requires 322.2509.

(1R,3E,5S,7E,11R,12R)-5-Acetoxy-12-hydroxydolabella-3,7-dien-9-one [**9**].—Spectroscopic data as reported (5), mp 80.0° (dec).

Preparation of p-*bromobenzoate derivative of compound* **10**.—18 mg of crystalline **10** (mp 69.0–71.0°) were dissolved in 3 ml anhydrous CH_2Cl_2, and 32 mg of *p*-bromobenzoyl chloride were added. After 5 h the reaction mixture was quenched with H_2O and the organic-soluble material separated from the aqueous phase. Hplc separation on LiChrosorb Si60, 5 μm, yielded 10 mg of **11**.

Compound **11**.—Mp 137.6–138.6°; $[\alpha]^{25}D$ −17.1° (*c*=0.14, $CHCl_3$); uv λ max (EtOH) (ε) 244 nm (27 785); ^1H nmr (300 MHz, $CDCl_3$) δ 0.95 (3H, d, *J*=6.8 Hz, H_3-20), 1.00 (1H, d, *J*=8.6 Hz, H-19), 1.26 (3H, s, H_3-15), 1.41 (m), 1.55 (3H, br s, H_3-16), 1.59 (3H, br s, H_3-17), 1.71 (m), 1.79 (m), 1.84 (m), 1.99 (m), 2.10 (m), 2.26 (m), 2.30 (m), 5.08 (1H, ddd, *J*=1.2, 4.0, and 11.8 Hz, H-3), 5.32 (1H, br d, *J*=11.4 Hz, H-7), 5.69 (1H, dd, *J*=4.6 and 10.7 Hz, H-9), 7.54 (m), 7.87 (m); ^{13}C nmr (75.5 MHz, $CDCl_3$) δ 11.0

TABLE 3. Crystal Structure Data[2] for Compounds **1**, **9**, and **11**.

	Compound		
	1	**9**	**11**
Crystal dimensions	0.32×0.28×0.20 mm	0.53×0.33×0.15 mm	0.40×0.28×0.25 mm
Crystal color	Colorless	Colorless	Colorless
Molecular formula	$C_{20}H_{30}O_2$	$C_{22}H_{34}O_4$	$C_{27}H_{34}O_3Br$
Mol wt	302.5	362.5	486.5
a	6.7733(4) Å	9.2548(7) Å	8.0318(4) Å
b	13.6248(13) Å	12.3989(8) Å	7.7260(3) Å
c	19.8294(12) Å	9.3391(8) Å	19.9968(11) Å
α	90°	90°	90°
β	90°	98.382(7)°	92.915(5)°
γ	90°	90°	90°
V	1830.0(4) $Å^3$	1060.2(3) $Å^3$	1239.3(2) $Å^3$
F_{000}	664	396	510
μ (CuK_α)	5.0 cm^{-1}	5.8 cm^{-1}	16.6 cm^{-1}
λ (CuK_α)	1.54184 Å	1.54184 Å	0.71073 Å
Z	4	2	2
D_{calc}	1.097 gcm^{-3}	1.136 gcm^{-3}	1.304 gcm^{-3}
θ_{range}	2–75°	2–75°	1–25° hemisph. 25–30° quadrant.
Refined variables	204	235	280
Unique data	3473	2236	5848
Observed data	2611	2087	3877
R	0.047	0.047	0.041
R_w	0.054	0.059	0.040
Space group	$P2_12_12_1$	$P2_1$	$P2_1$
Temperature	23°	23°	20°
Min. rel. transm.	84.55%	77.09%	90.97%
Max. rel. transm.	99.62%	99.33%	99.86%
Av. rel. transm.	92.37%	87.79%	95.51%
Intensity decay	4.7%	5.1%	0%
Min. residual	−0.24 $eÅ^{-3}$	−0.17 $eÅ^{-3}$	−0.16 $eÅ^{-3}$
Max. residual	0.24 $eÅ^{-3}$	0.22 $eÅ^{-3}$	0.98 $eÅ^{-3}$

[2]Atomic coordinates for all X-ray structures have been deposited with the Cambridge Crystallographic Data Center and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

(q, C-17), 16.3 (q, C-16), 17.7 (q, C-19), 18.7 (q, C-20), 22.9 (q, C-15), 24.4 (t, C-6), 30.2 (t, C-10), 32.4 (t, C-13)[a], 34.9 (d, C-18), 39.3 (t, C-5), 41.4 (t, C-2)[a], 41.6 (t, C-14)[a], 45.5 (d, C-11), 45.6 (s, C-1), 81.7 (d, C-9), 88.3 (s, C-12), 124.7 (d, C-3), 127.6 (s, C-1')[b], 130.0 (s, C-4')[b], 130.7 (s, C-8)[b], 131.1 (d, C-2')[c], 131.5 (d, C-3')[c], 131.9 (d, C-5')[c], 132.4 (d, C-6')[c], 133.4 (s, C-4)[b], 135.0 (d, C-7), 165.1 (s, C=O). ([a,b,c]Resonances with the same superscript may have assignments interchanged).

SINGLE CRYSTAL X-RAY ANALYSIS OF **1**, **9**, and **11**.—See Table 3 for general data. ORTEP representations of **1** (Figure 1), **9** (Figure 2), and **11** (Figure 3) show the absolute configuration for each of these molecules. All calculations were carried out using the MolEN programs (8).

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