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Article

Update of Spectroscopic Data for 4-Hydroxydictyolactone and Dictyol E Isolated from a *Halimeda stuposa* — *Dictyota* sp. Assemblage †

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† Dedication: We dedicate this paper to the late Peter Thomas Murphy (Townsville, Australia) for his passionate contributions to Australian natural products chemistry and marine biodiversity research.

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Abstract: The methanol extract of an assemblage of *Halimeda stuposa* and a *Dictyota* sp., yielded three natural products characteristic of *Dictyota* sp., and one of *Halimeda* sp. These included the xenicane diterpene 4-hydroxydictyolactone (**1**), and the diterpenes dictyol E (**2**), 8α,11-dihydroxypachydictyol A (**3**) and indole-3-carboxaldehyde (**4**). A minor revision of **1** and new spectroscopic data for **1** and **2** are provided, along with associated anti-cancer activities of compounds **1**–**4**.

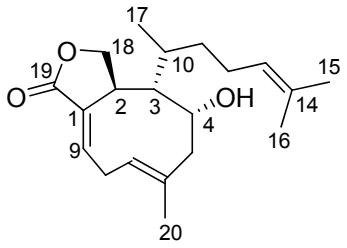
Keywords: *Dictyota*; Dictyotaceae; *Halimeda stuposa*; anti-cancer activity; xenicanes; diterpenes; 4-hydroxydictyolactone; dictyol E; 8α,11-dihydroxypachydictyol A; indole-3-carboxaldehyde

1. Introduction

There have been many reports involving chemical investigations of algae from the genera *Halimeda* [1–4] and *Dictyota* [5–10], with *Dictyota* species in particular being a prolific source of novel terpenoids. Considering the well documented history of terpenoid chemistry having significant biological activity [11–13], this genus of alga is an attractive target for the discovery of novel bioactive metabolites.

While investigating marine derived extracts for their anti-cancer activity, the ethanol (EtOH) extract of the green-brown alga assemblage of *Halimeda stuposa* and *Dictyota* sp. was found to have significant activity and an unusual profile in the NCI 60 cell line COMPARE analysis [14] The methanol (MeOH) extract of a large scale recollection was subjected to bioassay-guided fractionation, using C_{18} flash vacuum liquid chromatography and preparative C_{18} HPLC, to yield the xenicane lactone 4-hydroxydictyolactone (**1**) [15], as well as the known diterpenes dictyol E (**2**) [16], 8α,11-dihydroxypachydictyol A (**3**) [17], and indole-3-carboxaldehyde (**4**) [18] (Figure 1). Described below are a minor revision of **1**, as well as CD data and molecular modelling studies, in accordance with the absolute configuration previously reported [19], and NMR evidence confirming the presence of the minor *cis* conformer of **1** [20]. Also presented are the complete ^{1}H-NMR data for **2**, as well as the biological activities of **1**–**4** against a panel of human tumour and normal mammalian cell lines.

Figure 1. Structures of the xenicane lactone 4-hydroxydictyolactone (**1**), the diterpenes dictyol E (**2**) and 8α,11-dihydroxypachydictyol A (**3**), and indole-3-carboxaldehyde (**4**).



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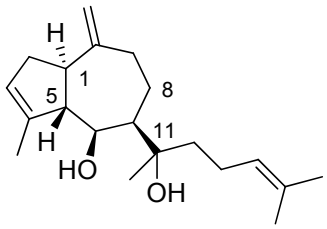

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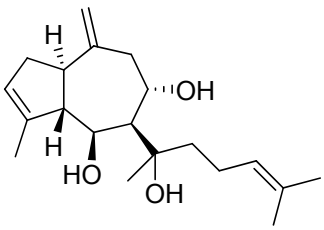

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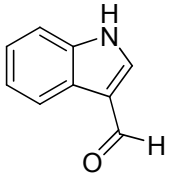

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2. Results and Discussion

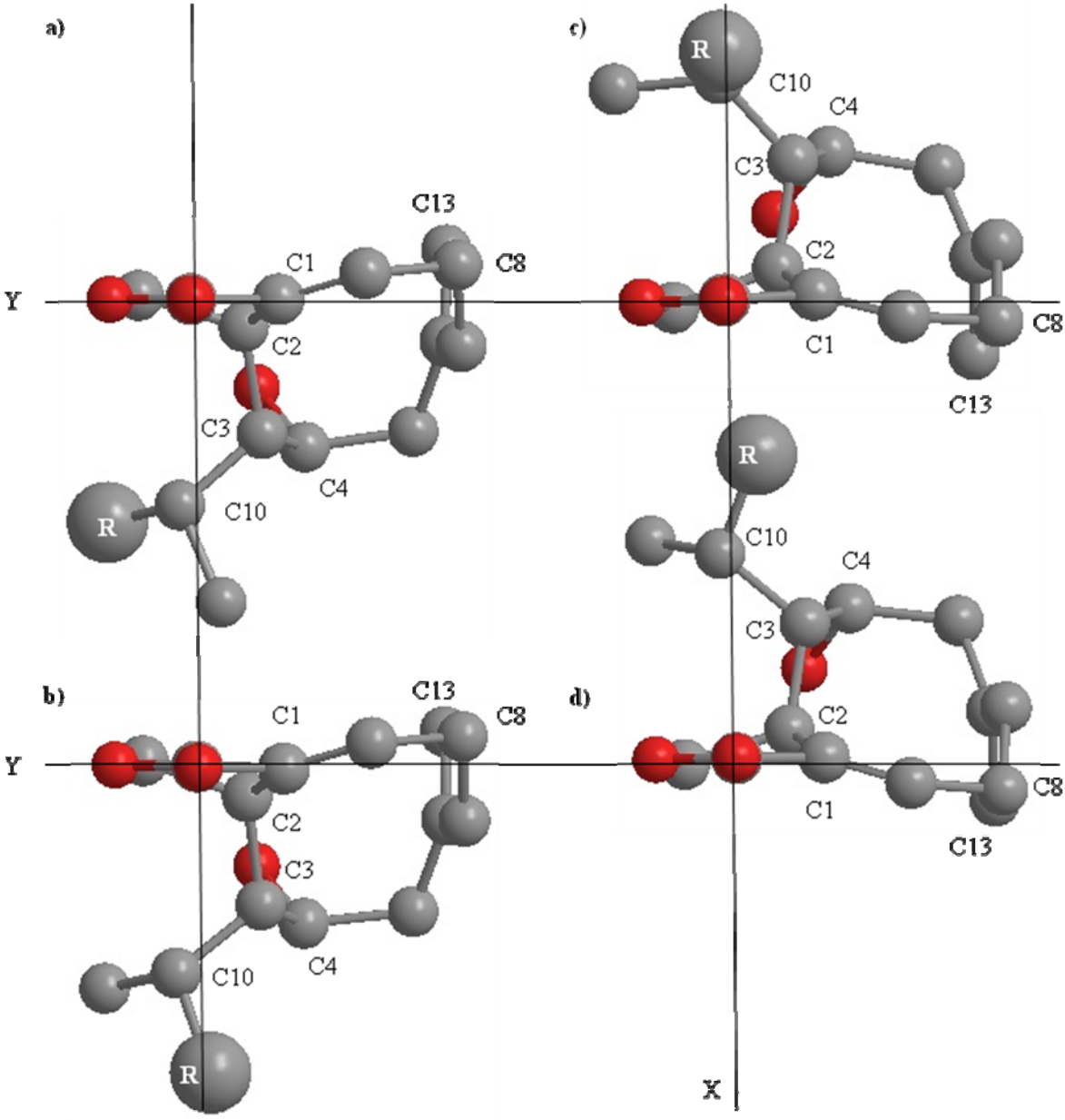
4-Hydroxydictyolactone (**1**) was isolated from the MeOH extract with a HRESIMS molecular weight indicative of the molecular formula $C_{20}H_{30}O_3$ and corresponding to six double bond equivalents. ^{1}H- and ^{13}C-NMR resonances (Supporting information Table S1) were identical to those first reported for the naturally occurring [15] and the synthetic 4-hydroxy-dictyolactone (**1**) [19], except for the C-7 and C-13 resonances. HSQC correlations (Supporting information Figure S4) were observed from δ_H 5.32 (H-7) to δ_C 125.3 and from δ_H 5.02 (H-13) to δ_C 123.9, indicating that the original assignments of these carbons were reversed. The C-1–C-9 double bond was assigned an *E*-configuration owing to the large coupling constants exhibited due to the axial-axial orientation of H-1 (δ 5.32, dd, 11.4, 4.2 Hz) and H_a-2 (δ 3.20, dddd, 17.5, 11.4, 2.2, 2.2) [21] whilst ^{13}C-NMR data for C-20 (δ_C 20.0) confirmed the *E* geometry of C-6–C-7 [22]. All other spectroscopic data matched that reported [15], however, as previously noted by Williams *et al.* [19], a differing optical rotation for the naturally occurring **1** {$[\alpha]^{21}_D$ −87° (*c* 0.25, $CHCl_3$)}was observed. Guella *et al.* [20] showed that **1** undergoes a slow conformation medium-ring flipping between the predominant *trans*- (C-20 *trans* to H-3) and the minor *cis*-conformer (C-20 *cis* to H-3). Further inspection of the ^{1}H and COSY NMR data confirmed the presence of the minor *cis*-conformer (Supporting Information Table S1), the ratio of which may influence the optical rotation. Closer inspection of the ^{1}H-NMR of the 50% MeOH flash column fraction revealed the presence of both conformers, however, only the *trans*-conformer was detected in the MeOH extract due to overlapping signals and concentration. Molecular modeling studies, where the geometry of both the double bonds (C-2–C-9 and C-6–C-7) in the carbocycle was constrained to *E*, were performed to determine which of the 16 possible stereoisomers (taking into consideration both *trans*- and *cis*-carbocycle conformations giving a total of 32 possibilities) matched the coupling constants observed in the ^{1}H-NMR data. As expected four possible structures matched the ^{1}H-NMR data, *trans*-2*R*,3*R*,4*S*,10*R*, *trans*-2*R*,3*R*,4*S*,10*S*, *trans*-2*S*,3*S*,4*R*,10*S* and *trans*-2*S*,3*S*,4*R*,10*R*.

The naturally occurring [15, 20] and synthetic studies [19] report measurement of optical rotation, but no CD data. The absolute configuration at C-2 of **1** was corroborated by CD measurement. The CD spectrum of **1** showed a large negative Cotton effect at 226 nm ($\Delta\varepsilon$ = −38.44, $\pi\rightarrow\pi^*$), and a small positive Cotton effect at 258 nm ($\Delta\varepsilon$ = 5.42, $n\rightarrow\pi^*$). Applying the quadrant rule [23]; viewing the ring along the O-C-19-C-1 axis, resulted in C-3 extending into the negative upper right quadrant. This finding is reconcilable with an *S* configuration at C-2 (Figure 2c,d) and in agreement with the naturally occurring [15] and the synthetic 4-hydroxydictyolactone (**1**) [19], *trans*-2*S*,3*S*,4*R*,10*R*, where 10*R* has previously been determined by x-ray crystallography [24] and synthetic studies [20].

Dictyol E (**2**) was also isolated from the MeOH extract with a HRESIMS molecular weight indicative of the molecular formula $C_{20}H_{32}O_2$ and corresponding to five double bond equivalents. Initial comparison of experimental ^{1}H- and ^{13}C-NMR resonances (Table 1) with those reported for the naturally occurring dictyol E (**2**) [16], indicated that the literature ^{1}H-NMR data was incomplete and that a full assignment of the structure was required. NMR resonances (Table 1) confirmed the presence of two trisubstituted double bonds (δ_C 141.0, 132.0, 124.2, 124.2; δ_H 5.34, 1H, br s; 5.16, 1H, br t, *J* = 6.9 Hz) and one disubstituted double bond (δ_C 152.0, 107.4; δ_H 4.78, 1H, s; δ_H 4.76, 1H, s) as well as three olefinic methyls (δ_C: 25.7, 15.9, 17.5; δ_H 1.82, 3H, s; 1.69, 3H, s; 1.62, 3H, s), a tertiary methyl

(δ_C: 25.3; δ_H 1.26, 3H, s) and an oxy-methine (δ_C: 74.4; δ_H 4.20, 1H, dd, J = 7.8, 2.0 Hz), consistent with reported values. Five additional methylenes and three methines were also observed.

Figure 2. The possible *trans*-conformers, based on ^{1}H-NMR coupling constants, viewed along the C=O bond towards C-18, of 4-hydroxydictyolactone (**1**) as obtained from MM2 calculations [25]; (**a**) *trans*-2*R*,3*R*,4*S*,10*R*; (**b**) *trans*-2*R*,3*R*,4*S*,10*S*; (**c**) *trans*-2*S*,3*S*,4*R*,10*S* and (**d**) *trans*-2*S*,3*S*,4*R*,10*R*. R=$(CH_2)_2CHC(CH_3)_2$.



Analysis of the COSY NMR data for **2** (Table 1) showed an extended ^{1}H-^{1}H spin system from H-3 (δ_H 5.34, 1H, br s) to H_2-9 (δ_H 2.69, 1H, ddd,14.8, 4.6, 2.4 Hz) via H-1 (δ_H 2.60, 1H, q, J = 9.1 Hz) and H-5 (δ_H 2.37, 1H, m), as well as long-range ^4J COSY NMR correlations from H-3 to H_3-17 and H-5, from H-5 to H_3-17 and from H_3-18 to H-1 and H_2-9. In addition, gHMBC correlations from δ_H 2.60 (H-1) to δ_C 33.7 (C-2), 60.4 (C-5), 74.4 (C-6) and 152.0 (C-10) and from δ_H 2.37 (H-5) to 124.2

(C-3), 141.0 (C-4), 74.4 (C-6) and 152.0 (C-10) confirmed H-1 and H-5 as the bridgehead protons and readily identified the perhydroazulene skeleton.

Table 1. ^{1}H- and ^{13}C-NMR data (300 MHz and 75 MHz, $CDCl_3$) for dictyol E (**2**).

No.	^{13}C δ (m)	^{1}H δ (m, *J* Hz)	COSY	gHMBC
1	46.0 (d)	2.60 (1H, q, 9.1)	H_2-2, H-5, H_2-18	C-2, C-5, C-6, C-10, C-18
2	33.7 (t)	2.51 (1H, m)	H-1, H_b-2	C-1, C-3, C-4, C-5
		2.22 (1H, dd, 14.8, 7.8)	H-1, H_a-2, H-3	C-1, C-3, C-4, C-5
3	124.2 (d)	5.34(1H, br s)	H_b-2, H_3-17, H-5	C-1, C-2, C-4, C-5, C-17
4	141.0 (s)			
5	60.4 (d)	2.37 (1H, m)	H-1, H_3-17, H-3, H-6	C-1, C-3, C-4, C-6, C-10
6	74.7 (d)	4.20 (1H, dd, 7.8, 2.0)	H-5, H-7	C-4, C-5, C-7, C-8,
7	48.7 (d)	1.67 (1H, m)	H-6	C-9, C-11, C-12
8	21.6 (t)	1.81 (1H, m)	H_b-8, H_a-9	C-6, C-10, C-19
		1.73 (1H, m,)	H_a-8	C-7, C-11
9	40.6 (t)	2.69 (1H, ddd, 14.8, 4.6, 2.4)	H_b-9, H_2-8	C-1, C-7, C-8, C-10, C-18
		2.13 (1H, m)	H_a-9, H_2-18	C-8, C-10, C-18
10	152.0 (s)			
11	76.3 (s)			
12	40.9 (t)	1.74 (2H, t, 8.6)	H_2-13	C-7, C-11, C-13, C-14, C-19
13	23.2 (t)	2.12 (1H, dd, 14.8, 8.6)	H_2-12, H_b-13, H-14	C-11, C-12, C-14, C-15
		2.02 (1H, dq, 14.8, 6.9)	H_2-12, H_b-13, H-14	C-11, C-12, C-14, C-15
14	124.2 (d)	5.16 (1H, br t, 6.9)	H_2-13, H_3-20,	C-12, C-13, C-16, C-20
15	132.0 (s)			
16	25.7 (q)	1.69 (3H, s)	H-14	C-14, C-15, C-20
17	15.9 (q)	1.82 (3H, br s)	H-3, H-5	C-3, C-4, C-5
18	107.4 (t)	4.78 (1H, br s)	H-1	C-1, C-5, C-9, C-10
		4.76 (1H, br s)	H_b-9	C-1, C-5, C-9, C-10
19	25.3 (q)	1.24 (3H, s)		C-7, C-11, C-12
20	17.5 (q)	1.62 (3H, br s)		C-14, C-15, C-16

Analysis of gHMBC correlations for **2** (Table 1) between δ_H 1.74 (H-12) and δ_C 76.3 (C-11), δ_C 23.2 (C-13), δ_C 124.2 (C-14) and δ_C 25.3 (C-19), and between δ_H 2.12/2.02 (H-13$_{a/b}$) and δ_C 76.3 (C-11), δ_C 40.9 (C-12), δ_C 124.2 (C-14) and δ_C 25.3 (C-19), confirmed the presence of a 6-methylhept-5-en-2-ol side chain. Furthermore, gHMBC correlations from δ_H 1.26 (H_3-19) to δ_C 48.7 (C-7) and from δ_H 1.67 (H-7) to δ_C 76.3 (C-11) and δ_C 40.9 (C-12) allowed the 6-methylhept-5-en-2-ol side chain to be positioned at C-7. Based on these observations, the planar structure of **2** was confirmed as reported [16].

The configuration of the C-3–C-4 double bond must be *Z* in order to form the five-membered ring [26]. The relative stereochemical assignment was confirmed as 1*R*,5*S*,6*R*,7*S* by the positive optical rotation ($[\alpha]^{21}_D$ +21° $CHCl_3$; *c* 0.11) [27] and comparison with literature values [16].

A further two compounds were also isolated from the assemblage, 8α,11-dihydroxypachydictyol A (**3**) and indole-3-carboxaldehyde (**4**). Their spectroscopic data matched those reported in the literature [17,18].

Outlined in Table 2 are the cytotoxic activities of **1**–**4** against a panel of human and mammalian cell lines. From these data there appears to be no obvious SAR, with **1**–**3** having approximately the same

activities against the three human tumour cell lines SF-268, MCF-7 and H460. However, the response of compounds **1**–**3** against HT-29, a human colon tumour cell line, and CHO-K1, a Chinese hamster ovary non-tumour cell line, were between two and four-fold less active as compared to those for the three cancer cell lines mentioned above, suggesting some selectivity. Indole-3-carboxaldehyde (**4**) was not active against any of the cell lines.

Table 2. Cytotoxicity data [GI_{50} (μM)] for compounds **1**–**4** against the human tumour cell lines SF-268, MCF-7, H460, HT-29, the normal human cell line WI38, and the mammalian cell line CHO-K.

Compound	SF-268 [a]	MCF-7 [b]	H460 [c]	HT-29 [d]	CHO-K1 [e]
1	25	27	20	61	72
2	16	22	17	46	48
3	20	38	20	88	103
4	NA[f]	NA	NA	NA	NA

[a] SF-268 Central nervous system-glioblastoma cells; [b] MCF-7 Breast-pleural effusion adenocarcinoma cells; [c] H460 Lung-large cell carcinoma cells; [d] HT-29 Colon-recto-sigmoid colon adenocarcinoma cells; [e] CHO-K1 Sub-clone of Chinese hamster ovary cells; [f] NA = not active.

3. Experimental

3.1. General Procedures

General experimental procedures are as described previously [28]. CD spectra were collected on a JASCO J-715 spectropolarimeter with a 0.1 dm cell.

3.2. Plant Material

The green/brown algal assemblage of *Halimeda stuposa* (Udoteaceae, Caulerpales) and *Dictyota* sp., (Dictyotaceae, Dictyotales) was collected from the passage between Shaw and Maher Islands, Queensland, at a depth of 7 m, in October 1987. Collection of this material was conducted under the Queensland Fish or Marine Products Permit no. 1780 and the GBRMPA Permit no. 87/293. A voucher specimen (Accession number AQ642006) has been lodged with the Queensland Herbarium.

3.3. Bioassay

Cellular bioassays were undertaken as described previously [28].

3.4. Extraction and Isolation

Freeze dried plant material was extracted with dichloromethane (CH_2Cl_2) (3 × 400 mL) followed by MeOH (3 × 400 mL). The MeOH extract (2.7 g) was then subjected to reversed phase C_{18} flash vacuum chromatography (RP-C18, 0%, 20%, 50%, 70%, 90% and 100% MeOH in H_2O and 1:1 MeOH:CH_2Cl_2). The 50% MeOH fraction was further purified by semi-preparative C_{18} HPLC (4 mL/min, gradient elution from 10% CH_3CN:H_2O to 73% CH_3CN:H_2O over 14 min through a 250 × 10 mm, 5 μm Phenomenex Luna C_{18} column) to yield the known compound indole-3-

carboxyaldehyde (**4**, 0.8 mg, 0.03% dry wt of extract), which had identical physical and spectroscopic properties to those previously published [18].

The active fractions, 90% and 100% MeOH, were each pre-adsorbed onto C_{18}, packed into a cartridge, then subjected to C_{18} preparative HPLC (9.5 mL/min, gradient elution from 50% H_2O:CH_3CN:0.1% HCO_2H to 100% CH_3CN:0.1% HCO_2H over 40 min, followed by 20 min with 100% CH_3CN:0.1% HCO_2H through 250 × 21 mm, 5 μm Phenomenex Luna C_{18} column). The 90% MeOH fraction yielded, 4-hydroxydictyolactone (**1**, 4.9 mg, 0.18% dry wt of extract) as well as 8α,11-dihydroxypachydictyol A (**3**, 10.4 mg, 0.39% dry wt of extract), and the 100% MeOH fraction yielded dictyol E (**2**, 5.5 mg, 0.20% dry wt of extract). The known compounds had identical physical and spectroscopic properties to those previously published [15–17].

3.4.1. 4-Hydroxydictyolactone (**1**)

Pale yellow oil. $[\alpha]^{21}_{D}$ −87° ($CHCl_3$; *c* 0.25); IR ν_{max}^{film} cm^{-1}: 3436, 2931, 1739, 1455; UV (PDA) $\lambda_{max}^{CH_3CN/H_2O}$ nm: 220; CD λ_{max} (Δε) (MeOH; 1.9×10^{-4} M) 226 (−38.44), 258 (5.42) nm; ^{1}H- (300 MHz, $CDCl_3$) and ^{13}C- (75 MHz, $CDCl_3$) NMR data see Table S1; HRESIMS *m/z* $[M+Na]^+$ 341.2103 (calcd for $C_{20}H_{30}O_3Na$ 341.2087) [15].

3.4.2. Dictyol E (**2**)

Pale yellow oil. $[\alpha]^{21}_{D}$ +21° ($CHCl_3$; *c* 0.11); ^{1}H- (300 MHz, $CDCl_3$) and ^{13}C- (75 MHz, $CDCl_3$) NMR data (Table 1) were consistent with published values [16].

3.4.3. 8α,11-Dihydroxypachydictyol A (**3**)

Pale yellow oil. ^{1}H-NMR and ^{13}C-NMR spectral data were consistent with published values [17].

3.4.4. Indole-3-carboxaldehyde (**4**)

Pale yellow solid. ^{1}H-NMR and ^{13}C-NMR spectral data were consistent with published values [18].

4. Conclusions

Four compounds, the xenicane diterpene 4-hydroxydictyolactone (**1**), and the diterpenes dictyol E (**2**), 8α,11-dihydroxypachydictyol A (**3**) and indole-3-carboxaldehyde (**4**), were isolated from an assemblage of *Halimeda stuposa* and a *Dictyota* sp. Although there are many reports on the isolation of xenicane diterpenes from algae of the genera *Dictyota* sp. [12,29–34], *Pachydictyon* sp. [35–37], *Glossophora* sp. [38] and *Dilophus* sp. [15,16,39], with the latter three genera now recognized as *Dictyota* species [40], and of pachydictyane diterpenes from algae of the genera *Dictyota* sp. [41], *Sargassum* sp. [42], *Glossophora* sp. [26] and *Cystoseira* sp. [43], there are very few that discuss their cytotoxic properties (xenicanes: [12,21,29]; pachydictyanes: [21,42–44]). The bioactivity results and the updated spectroscopic data presented in the current work clearly show that more detailed and concerted investigations of these two classes of diterpenes are warranted.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/17/3/2929/s1.

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