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New Diterpenoids from the Octocoral *Pachyclavularia violacea* Collected in Papua New Guinea

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The authors wish to dedicate this paper to Professor Paul Scheuer on the 50th anniversary of his outstanding academic career at the University of Hawaii

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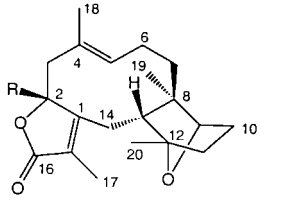
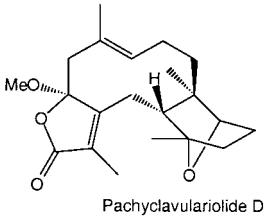
Abstract—Six new diterpenoids, pachyclavulariolides A (**1**) to F (**6**), with cembrane and briarane carbon skeletons have been isolated from specimens of *Pachyclavularia violacea* collected in Papua New Guinea. The structures of the new diterpenoids were elucidated by analysis of spectroscopic data. A single crystal X-ray diffraction analysis of pachyclavulariolide B (**2**) confirmed its structure. Pachyclavulariolide F (**6**) showed in vitro cytotoxicity. © 2000 Elsevier Science Ltd. All rights reserved.

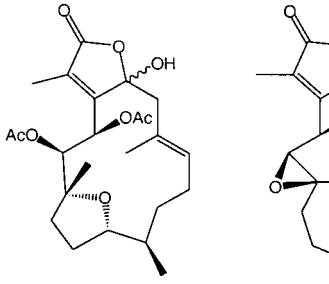
Marine octocorals are an extremely rich source of novel diterpenoids[1] that often have potent biological activities relevant to drug development. The tubulin-stabilizing antimitotic diterpene glycoside eleutherobin, isolated from an *Eleutherobia* sp. collected in Western Australia,[2] and the antiinflammatory diterpene glycoside pseudopterosin A, isolated from *Pseudopterogorgia elisabethae* collected in the Caribbean,[3] represent two examples of octocoral diterpenoids with exciting drug potential. As part of our ongoing screen of marine invertebrates for the presence of biologically active secondary metabolites,[4] it was found that extracts of the octocoral *Pachyclavularia violacea* collected in Papua New Guinea showed moderate activity in assays for in vitro cytotoxicity and G2 cell cycle checkpoint inhibitors.[5] Assay guided fractionation of the *P. violacea* extract resulted in the isolation of six new diterpenoids, pachyclavulariolides A (**1**) to F (**6**), whose structures are described below.



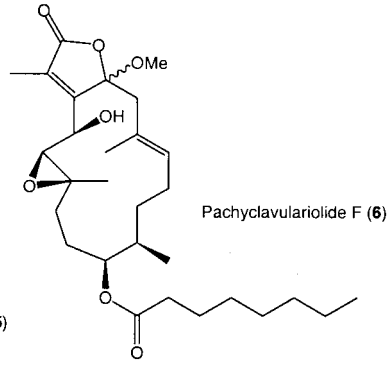
R=H Pachyclavulariolide A (**1**)
R=OH Pachyclavulariolide B (**2**)
R=OMe Pachyclavulariolide C (**3**)



Pachyclavulariolide D (**4**)



Pachyclavulariolide E (**5**)



Pachyclavulariolide F (**6**)

Specimens of *P. violacea* were collected by hand using SCUBA on shallow reefs near Motupore Island, Papua New Guinea. Freshly collected animals were deep frozen on site and transported back to Vancouver over dry ice. Frozen specimens were thawed and extracted exhaustively with MeOH. Concentration of the combined MeOH extracts in vacuo gave a deep green gum that showed G2 checkpoint inhibition at 1 μg/mL and in vitro cytotoxicity against murine leukemia P388 at 10 μg/mL. The crude gum was suspended in water and sequentially partitioned against hexanes, $CHCl_3$, and EtOAc. Only the hexanes soluble material showed G2 checkpoint inhibition and cytotoxicity. Fractionation of the hexanes soluble material by sequential application of Sephadex LH20 chromatography (eluent: MeOH), gradient silica-gel flash chromatography (eluent:

Keywords: *Pachyclavularia violacea*; diterpenoids; octocoral.
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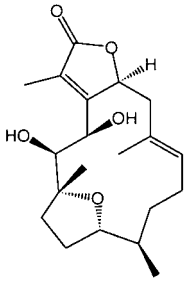
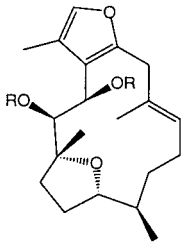
hexane/EtOAc 9:1 to hexane/EtOAc 1:1), and reversed phase HPLC (eluent: MeOH/H_2O 85:15) gave pure samples of pachyclavulariolides A (**1**) to F (**6**).

Pachyclavulariolide A (**1**) was isolated as a white amorphous solid that gave an intense $[M+H]^+$ ion at *m/z* 317.21150 in the positive ion HRFABMS appropriate for a molecular formula of $C_{20}H_{28}O_3$ (ΔM, −0.53 ppm) requiring seven sites of unsaturation. The ^{1}H NMR spectrum of pachyclavulariolide A (**1**) acquired at room temperature in CD_2Cl_2 contained many very broad peaks that indicated the molecule existed in at least two slowly interconverting conformations. When the ^{1}H NMR data for **1** were recorded in CD_2Cl_2 at −20°C, many of the broad peaks separated into two distinct sets of sharp resonances. Recording the ^{1}H NMR data for **1** in C_6D_6 at elevated temperature (55°C) resulted in a spectrum that contained only a single set of sharp resonances consistent with rapid conformational interconversion. Therefore, all of the ^{1}H and ^{13}C NMR data used for the structure elucidation of **1** were recorded in C_6D_6 at 55°C.

The ^{1}H NMR spectrum of pachyclavulariolide A (**1**) contained four methyl singlets (δ 0.76 (C-19), 1.05 (C-20), 1.35 (C-18), 1.65 (C-17)), which in conjunction with the 20 carbons present in the molecular formula, suggested a diterpenoid structure. Resonances at δ 123.2 (C), 129.1 (CH), 129.6 (C), 163.6 (C), and 173.0 ppm (C) in the ^{13}C NMR spectrum were assigned to a trisubstituted olefin, a tetrasubstitued olefin, and a carbonyl, which accounted for three sites of unsaturation. The absence of ^{13}C NMR evidence for additional unsaturated functional groups required that pachyclavulariolide A (**1**) had to be tetracyclic. HMBC correlations observed between the ^{1}H resonance at δ 1.65, assigned to an olefinic methyl (Me-17), and the olefinic and carbonyl ^{13}C resonances at δ 163.6 (C-1), 123.2 (C-15), and 173.0 (C-16) showed that the carbonyl was conjugated to the tetrasubstituted olefin and that the olefinic methyl was attached to the α carbon of this fragment. A pair of geminal allylic methylene proton resonances at δ 1.92 (H-14′) and 2.22 (H-14) both showed HMBC correlations to the olefinic carbon resonances at δ 163.6 (C-1) and 123.2 (C-15), and the downfield proton resonance (δ 2.22) showed an additional HMBC correlation to a carbinol methine carbon resonance at δ 78.6 (C-2) that was correlated in the HMQC spectrum to a proton resonance at δ 4.65 (H-2). A COSY correlation attributed to homoallylic coupling was observed between the H-2 (δ 4.65) and Me-17 (δ 1.65) resonances. Taken together, the above NMR evidence indicated the presence of a butenolide substructure in **1** that was identical to that found in the known metabolite pachyclavulariolide (**7**) isolated from *P. violacea* specimens collected in Vanuatu (**7**: ^{13}C NMR C_6D_6 δ 166.8 (C-1), 80.7 (C-2), 124.4 (C-15), 175.6 (C-16)).[6]



Pachyclavulariolide (**7**)



Pachyclavulariadiol (**8**) R=H
Pachyclavulariadioldiacetate (**9**) R= Ac

COSY, HMQC, and HMBC data routinely identified the branched six-carbon alkene appendage extending from C-3 to C-7. The H-2 resonance (δ 4.65) showed COSY correlations to a pair of geminal methylene proton resonances at δ 2.75 (H-3) and 1.52 (H-3′) that both showed HMQC correlations to a carbon resonance at δ 42.5 (C-3). HMBC correlations were observed between the Me-18 resonance (δ 1.35) and C-3 (δ 42.5), C-4 (δ 129.1), and C-5 (δ 129.6), and between H-5 (δ 5.04) and Me-18 (δ 18.5), C-6 (δ 24.3), and C-3 (δ 42.5). COSY correlations were observed between H-5 (δ 5.04) and H-6 (δ 1.92), H-6′ (δ 1.89), and Me-18 (δ 1.35), and between H-6/H-6′ and H-7 (δ 1.22) and H-7′ (δ 1.05). The H-7 and H-7′ resonances both showed HMQC correlations to a carbon resonance at δ 37.7 (C-7).

Evidence for the remaining fragment of **1** started with the observation of COSY correlations between both of the H14 (δ 2.22) and H-14′ (δ 1.92) resonances and a methine resonance at δ 1.18 (H-13), that was correlated in the HMQC spectrum to a carbon resonance at δ 54.6 (C-13). A methyl singlet at δ 1.05 (Me-20) showed HMBC correlations to the C-13 methine carbon resonance at δ 54.6 and also to a quaternary carbon at δ 86.2 (C-12) and to a methylene carbon at δ 38.8 (C-11), demonstrating that C-13 was attached to the oxygenated quaternary carbon C-12. HMBC correlations observed between a second methyl singlet at δ 0.76 (Me-19) and the C-13 methine (δ 54.6) and C-7 methylene (δ 37.7) resonances showed that the quaternary carbon (C-8) bearing this methyl was attached to both C-13 and C-7 to form a ten membered ring encompassing C-1 to C-8 and C-13/C-14. The Me-19 singlet (δ 0.76) showed additional HMBC correlations to C-8 (δ 49.4) and an oxygenated methine carbon resonance at δ 87.0 (C-9), that was correlated in the HMQC spectrum to a proton resonance at δ 3.52 (H-9). The H-9 (δ 3.52) resonance showed a single COSY correlation to a resonance at δ 1.50 (H-10), one of a pair of geminal methyelene proton resonances (δ 1.68, H-10′), that showed HMQC correlations to a carbon resonance at δ 26.1 (C-10). Additional HMBC correlations observed between H-9 (δ 3.52) and C-12 (δ 86.2) and C-11 (δ 38.8) established the presence of both an ether linkage and a two carbon bridge between C-9 and C-12, completing the constitution of pachyclavulariolide A (**1**).

The configuration of the $\Delta^{4,5}$ olefin and the relative stereochemistries of the chiral centers in **1** were determined via a series of difference NOE experiments. Irradiation of the Me-18 resonance at δ 1.35 induced a NOE in the H-6′ resonance at δ 1.89 demonstrating that the $\Delta^{4,5}$ olefin had the E configuration. When the Me-19 resonance at δ 0.76 was irradiated, strong NOEs were observed in the H-14′ (δ 1.92) and H-9 (δ 3.52) resonances, but no NOEs were observed in the H-10 or 10′ resonances (δ 1.50/1.68). These observations were consistent with Me-19 and C-14 being exo substituents on the oxanorbornane fragment. Finally, irradiation of the H-2 resonance at δ 4.65 induced a strong NOE in the H-13 resonance at δ 1.18 indicating that

Table 1. [1]H NMR data (ppm) (assignments are based on COSY, HMQC, and HMBC data) for pachyclavulariolides A (**1**) to F (**6**) recorded in benzene-d_6 at 500 MHz

Carbon no.	A (**1**)[a,b]	B (**2**)[a,b]	C (**3**)[a,b]	D (**4**)[b]	E (**5**)[b]	F (**6**)[b]
2	4.65 (dd, 11.0, 2.1)	–	–	–	–	–
2-OH	–	2.82	–	–	5.38	–
3	2.75 (dd, 11.0, 1.8)	2.79	3.08 (d, 15.3)	2.90 (d, 13.5)	3.09	2.81 (d, 14.4)
3′	1.52	1.95	1.75	2.10 (d,13.5)	3.09	2.58 (d, 14.4)
5	5.04	5.47	5.63	4.52	6.30	4.86
6	1.92	2.03	1.98	1.69	2.15	2.00
6′	1.89	1.96	2.01	2.00	1.92	1.70
7	1.22	1.40	1.35	1.11	2.12	1.20
7′	1.05	1.12	1.15	1.11	1.05	0.96
8	–	–	–	–	1.26	1.50
9	3.52 (d, 5.1)	3.57 (d, 5.0)	3.54 (d, 5.3)	3.46 (d, 4.7)	3.50	4.96
10	1.50	1.55	1.53	1.55	1.27	1.42
10′	1.68	1.75	1.75	1.65	1.63	1.28
11	1.32	1.40	1.35	1.36	1.50	1.82
11′	1.12	1.40	1.32	1.36	1.32	1.12
13	1.18	2.24	1.98	1.25	5.02 (d, 1.53)	3.35 (d, 6.17)
14	2.22 (dd, 9.9, 15.8)	2.28	2.26 (dd, 9.8, 16.1)	2.05	6.52 (d, 1.5)	4.14 (dd, 3.9, 6.7)
14′	1.92	2.06	2.02	1.87	–	–
14-OH	–	–	–	–	–	2.14 (d,3.9)
15	–	–	–	–	–	–
17	1.65 (s)	1.59 (s)	1.63 (s)	1.70 (s)	1.75 (s)	2.28 (s)
18	1.35	1.51	1.50	1.70	1.90	1.65
19	0.76 (s)	0.82 (s)	0.82 (s)	0.82 (s)	0.65 (d, 6.4)	0.72 (d, 6.9)
20	1.05 (s)	1.18 (s)	1.18 (s)	1.35 (s)	1.45	1.10
21	–	–	2.91 (s)	2.81 (s)	–	–
22	–	–	–	–	1.40 (s)	2.09 (t, 7.2)
23	–	–	–	–	–	1.50
24	–	–	–	–	1.59 (s)	1.19
25	–	–	–	–	–	1.19
26	–	–	–	–	–	1.19
27	–	–	–	–	–	1.18
28	–	–	–	–	–	0.90 (t, 7.1)
29	–	–	–	–	–	3.18 (s)

[a] Recorded in 328 K.
[b] Coupling constants are in Hz.

H-2 and H-13 were on the same face of the 10 membered ring, completing the relative stereochemical assignment of pachyclavulariolide A as shown in **1**.

Pachyclavulariolide B (**2**) was obtained as a white solid that gave a $[M+H]^+$ ion at *m/z* 333.20633 in the HRFABMS appropriate for a molecular formula of $C_{20}H_{28}O4$ (ΔM +0.15 ppm), differing from the molecular formula of **1** simply by the addition of an oxygen atom. The room temperature NMR data collected for **2** also showed broad ^{1}H peaks and many doubled ^{13}C peaks indicating slow conformational interconversion as was observed with pachyclavulariolide A (**1**). Therefore, all the NMR data used to solve the structure of **2** (Tables 1 and 2) were also collected in C_6D_6 at 55°C. Examination of the NMR data obtained for **2** indicated that it was simply the 2-hydroxy analog of **1**. In the ^{1}H NMR spectrum of **2**, the resonance assigned to H-2 (**1**: δ 4.65) in **1** was missing and in its place was an exchangeable OH resonance at δ 2.82, while in the ^{13}C spectrum of **2** the C-2 resonance had a chemical shift of δ 106.5 appropriate for a hemiketal. Pachyclavulariolide B (**2**) gave crystals when the C_6D_6 NMR sample was slowly evaporated. A single crystal diffraction analysis was carried out on one of these crystals in order to confirm the stereochemical assignments of pachyclavulariolides A (**1**) and B (**2**). An ORTEP drawing of the structure of **2** determined by single crystal X-ray diffraction is shown in Fig. 1.

Analysis of the MS and NMR data (Tables 1 and 2) obtained for pachyclavulariolides C (**3**) and D (**4**) showed that they were the two epimers of the 2-methoxy derivative of pachyclavulariolide A (**1**). Irradiation of the methoxy resonance at δ 2.91 in the ^{1}H NMR spectrum of pachyclavulariolide C (**3**) induced a strong NOE in the H-13 resonance (δ 1.98) demonstrating the 2-MeO group was on the same face of the 10 membered ring as H-13, as shown. The corresponding irradiation (*Me*O, δ 2.81) in pachyclavulariolide D (**4**) failed to generate any significant NOEs.

Pachyclavulariolide E (**5**) was obtained as a white amorphous solid that gave a $[M+H]^+$ ion at *m/z* 451.23322 in the HRFABMS appropriate for a molecular formula of $C_{24}H_{34}O_8$ (ΔM 0.06 ppm) requiring eight sites of unsaturation. The ^{13}C NMR spectrum of **5** contained a suite of resonances at δ 168.4 (C-16), 156.6 (C-1), 128.5 (C-15), 106.6 (C-2) and 9.7 (C-17) that could be assigned to a γ-hydroxy- or γ-alkoxybutenolide by comparison with the ^{13}C NMR data for pachyclavulariolides B (**2**) and C (**3**) (Table 2). HMBC correlations observed between an exchangeable proton resonance at δ 5.38 (O*H*-2) and the butenolide ^{13}C resonances at δ 106.6 (C-2) and 156.6 (C-1) confirmed the presence of a γ-hydroxybutenolide in **5**. Additional unsaturated functionality was indicated by ^{13}C NMR resonances at δ 168.4 (C-23), 173.0 (C-21), 132.4 (C-4), and 131.6 (C-5) assigned to two esters and a trisubstituted olefin, respectively. The lack of evidence for

Table 2. ^{13}C NMR data (assignments are based on HMQC and HMBC data) (ppm) for pachyclavulariolides A (**1**) to F (**6**) recorded in benzene-d_6 at 100 MHz

Carbon no.	A (**1**)[a]	B (**2**)[a]	C(**3**)[a]	D (**4**)	E (**5**)	F (**6**)
1	163.6	160.6	158.0	161.7	156.6	154.9
2	78.6	106.5	108.8	112.2	106.6	110.1
3	42.5	46.2	44.0	52.2	46.4	46.1
4	129.1	128.3	127.2	130.0	132.4	130.1
5	129.6	130.1	130.3	131.0	131.6	131.2
6	24.3	24.3	24.6	24.9	25.3	26.8
7	37.7	37.2	38.3	37.8	32.4	31.9
8	49.4	48.9	49.3	50.7	39.5	38.3
9	87.0	86.7	86.4	85.9	85.3	73.7
10	26.1	25.8	25.7	25.4	31.4	23.5
11	38.8	38.1	38.3	39.5	39.7	34.8
12	86.2	86.1	86.1	87.3	82.9	63.2
13	54.6	49.9	49.9	53.7	75.4	64.3
14	26.5	26.8	27.1	23.7	70.7	63.3
15	123.2	124.6	127.0	127.2	128.5	133.4
16	173.0	170.5	169.7	170.8	168.4	170.7
17	9.1	8.1	8.4	10.5	9.7	9.8
18	18.5	19.5	20.0	17.7	19.5	17.8
19	20.5	20.0	19.9	19.7	15.9	16.3
20	18.5	18.1	18.5	20.6	23.5	16.1
21	–	–	49.5	51.1	173.0	172.8
22	–	–	–	–	20.0	34.5
23	–	–	–	–	168.4	25.0
24	–	–	–	–	20.0	31.5
25	–	–	–	–	–	31.5
26	–	–	–	–	–	31.9
27	–	–	–	–	–	22.6
28	–	–	–	–	–	14.0
29	–	–	–	–	–	50.3

[a] Recorded at 328 K.

further unsaturated functional groups showed that pachyclavulariolide E (**5**) was tricyclic.

HMBC correlations observed between the ester carbonyl resonance at δ 168.4 (C-23) and a methine resonance at δ 6.52 (H-14) and a methyl resonance at δ 1.59 (Me-24), and between the second ester carbonyl resonance at δ 173.0 (C-21) and a methine resonance at δ 5.02 (H-13) and a methyl resonance at δ 1.40 (Me-22) identified two secondary acetate substructures. Subtracting the acetate carbons from the molecular formula of **5** confirmed that the molecule had a diterpenoid core. The methine resonance at δ 6.52 (H-14) showed additional HMBC correlations to both of the olefinic carbons (δ 156.6, C-1 and128.5, C-15) and the ketal carbon (δ 106.6, C-2) of the γ-hydroxybutenolide moiety indicating that one of the carbons bearing an acetate was attached to C-1 of the butenolide. Starting with the H-14 methine proton resonance, it was possible to show by detailed analysis of the COSY and HMBC data that pachyclavulariolide E contained a substituted fourteen membered carbocylic ring extending from C-1 to C-2 with an ether bridge between C-10 and C-12, that had a constitution identical to corresponding substructure found in the previously described pachyclavulariadioldiaceate (**9**).[7] The relative configuration of pachyclavulariadioldiaceate (**9**) was established by a single crystal X-ray diffraction analysis on the co-occurring diol analog **8**. Both **8** and **9** were isolated from Australian specimens of *P. violacea*. Therefore, we have assumed that the relative configurations of the chiral centers at C-8 (R^*), C-9 (S^*), C-12 (R^*), C-13 (S^*) and C-14 (R^*) in pachyclavulariolide E (**5**) are the same as the corresponding centers in **8** and **9** as shown. Even though a difference NOE was observed between the OH-2 and H-13 resonances, the conformational mobility of the fourteen membered carbocyclic ring precluded an unambiguous assignment of the relative configuration at C-2 in **5**.

Pachyclavulariolide F (**6**) was isolated as a white amorphous solid that gave a $[M+H]^+$ peak in the HRFABMS at *m/z* 507.33198 corresponding to a molecular formula of $C_{29}H_{46}O_7$ (ΔM -0.40 ppm) requiring seven sites of unsaturation. The ^{13}C NMR spectrum contained resonances at δ

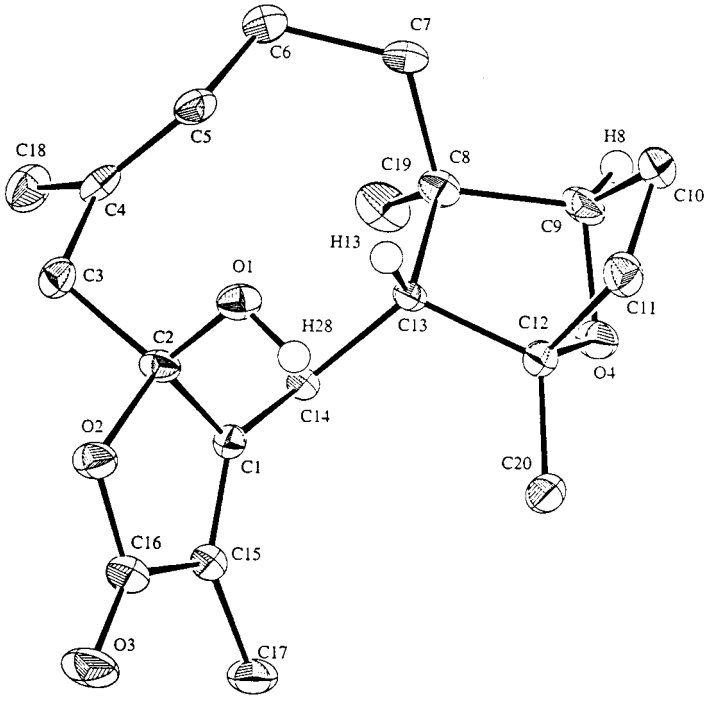
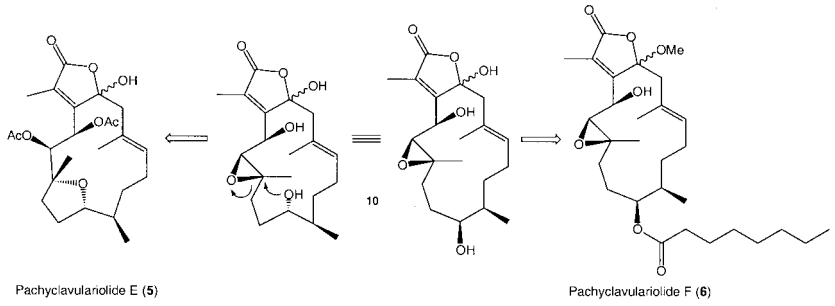


Figure 1. Computer generated ORTEP drawing of pachyclavulariolide B (**2**).



Scheme 1. Proposed biogenetic relationship between pachyclavulariolides E (**5**) and F (**6**).

170.7 (C-16), 154.9 (C-1), 133.4 (C-15), 110.1 (C-2) and 9.8 (C-17) that could be assigned to a γ-hydroxy- or γ-alkoxy butenolide by comparison with the NMR data for pachyclavulariolides B (**2**) to E (**5**) (Table 2). Additional ^{13}C NMR resonances at δ 130.1 (C-4), 131.2 (C-5), and 172.8 (C21) were assigned to a trisubstituted olefin and an ester carbonyl, respectively. The absence of ^{13}C NMR evidence for other unsaturated functionality indicated that pachyclavulariolide F (**6**) contained two rings in addition to the one present in the butenolide. The ^{1}H NMR spectrum of **6** contained a methyl resonance at δ 3.18 (Me-29) that showed a HMBC correlation to the ketal carbon resonance at δ 110.1 (C-2) demonstrating that the butenolide had a methoxy substituent at C-2. A methyl triplet at δ 0.90 (J=7.1 Hz: C-28) and a methylene triplet at δ 2.09 (J=7.3 Hz: C-22) in the ^{1}H NMR spectrum, in conjunction with the observation of a fragment ion at m/z 363 (M+H−144) in the FABMS attributed to loss of $C_8H_{16}O_2$, indicated the presence of a octanoate fragment in **6**. Subtracting the nine carbons present in the methoxy and octanoate fragments from the total of twenty nine carbons present in the molecular formula confirmed that pachyclavulariolide F (**6**) had a diterpenoid core.

Analysis of the COSY, difference NOE, HMQC, and HMBC data established the nature of the C-2 substituent extending from C-3 to C-11 as shown in **6**. The H-9 methine proton resonance had a chemical shift of 4.96 ppm indicating that the octanoate residue was attached to C-9. A deshielded methine proton resonance at δ 4.14 (H-14) showed HMBC correlations to the butenolide carbon resonances at δ 154.9 (C-1), 133.4 (C-15), and 110.1 (C-2) demonstrating that the oxymethine carbon (C-14) was attached to C-1. A COSY correlation observed between the H-14 resonance (δ 4.14) and an exchangeable proton resonance at δ 2.14 (O*H*-14) demonstrated that there was an alcohol attached to C-14. The H-14 resonance (δ 4.14) showed an additional COSY correlation to a methine resonance at δ 3.35 (H-13), that was correlated in the HMQC spectrum to a carbon resonance at δ 64.3 (C-13), providing evidence for a bond between the two oxymethine carbons C-13 and C-14. A methyl singlet at δ 1.10 (Me-20) showed HMBC correlations into both the C-13 (δ 64.3) and C-11 (δ 34.8) resonances and also to a non-protonated carbon resonance at δ 63.2 (C-12). This HMBC evidence showed that the non-protonated carbon (C-12) bearing the methyl group had to be bonded to C-13 and C-11, completing the fourteen membered carbocylic ring extending from C-1 to C-2. Since there was only one oxygen atom not yet accounted for and there was a requirement for one additional ring in the structure, it was apparent that there was an epoxide functionality at C-12/C-13. In addition, since all six of the methyl resonances identified in the ^{13}C/HMQC data could be assigned to the C-2 methoxy, the four methyl branches in the diterpenoid core, and the terminal carbon of the octanoate fragment, it was apparent that the octanoate skeleton had to be linear.

The stereochemical assignment for pachyclavulariolide F (**6**) started with the assumption that the relative configurations at C-8 (R^*) and C-14 (R^*) were identical to the corresponding centers in pachyclavulariolide E (**5**). Difference NOE experiments showed that the $\Delta^{4,5}$ bond had the E configuration and that C-14 and C-11 were oriented *trans* on the C-12/C-13 epoxide. Thus, irradiation of the H-5 olefinic resonance at δ 4.86 induced a NOE in the H-3′ resonance at δ 2.58, and irradiation of the H-14 methine resonance at δ 4.14 induced a NOE in the Me-20 resonance at δ 1.10. Assignments of the relative configurations at C-12 and C-9 were based on a putative biogenetic relationship between pachyclavulariolide F (**6**) and pachyclavulariolide E (**5**). One reasonable biogenetic scenario (Scheme 1) would have the C-12/C-13 expoxide in a common precursor **10** undergoing nucleophilic opening by a C-9 alcohol to generate the C-9/C-12 ether and C-13 alcohol found in **5**. This proposal would predict that the C-9 (S^*) and C-13 (S^*) configurations in **6** should be identical to the C-9 and C-13 configurations in **5**, and assuming that there would be backside attack of the C-9 alcohol on C-12 of the epoxide, it would predict that the C-12 (S^*) configuration in **6** should be the opposite of the C-12 configuration in **5**. An alternate scenario where a C-13 alcohol displaces the C-12 ether bond to give the C-12/C13 epoxide and a C-9 alcohol in **6** results in the same prediction for the relative stereochemistries at C-9, C-12, and C-13 in **6**. A difference NOE was observed between the Me-29 and H-14 resonances, but the conformational flexibility of the fourteen membered ring in **6** precludes a completely unambiguous C-2 assignment on the basis of this NOE data alone. Since *P. violacea* was extracted with methanol in the current work, the methyl ketals found in pachyclavulariolides C (**3**), D (**4**), and F (**6**) may be artifacts of the isolation procedure.

Two previous studies of *P. violacea* resulted in the isolation of the cembranoids pachyclavulariadiol (**8**) and its natually occuring mono- and diacetylated (**9**) derivatives from specimens collected in Australia,[7] and pachyclavulariolide (**7**)

from specimens collected in Vanuatu.[6] The *P. violocea* specimens examined in the current study were collected at a site on the southwestern coast of Papua New Guinea that borders on the Coral Sea, not far from either the Australian Barrier Reef or Vanuatu locations that border on the same water mass. The PNG specimens contained two new cembranolides, pachyclavulariolides E (**5**) and F (**6**), that are closely related to the cembranoids **7**, **8**, and **9** found in the Australian and Vanuatu specimens. In addition, the PNG specimens contained the new diterpenoids pachyclavulariolides A (**1**) to D (**4**) that have briarane carbon skeletons. The 9,12 ether bridge that forms an oxanorbornane substructure in pachyclavulariolides A (**1**) to D (**4**) is without precedent among known briarane diterpenoids. Taken together, the two earlier studies and the current study of *P. violacea* indicate that there is a significant geographic variation in diterpenoid content in this octocoral. Pachyclavulariolide F (**6**) was the only one of the new diterpenoids reported herein that had significant biological activity. It showed in vitro cytotoxicity against murine leukemia P388 with an IC_{50}=1.0 μg/mL. The compound(s) responsible for the G2 checkpoint inhibition activity exhibited by the crude extract are still under investigation.

Experimental

Isolation of pachyclavulariolides A to F

Specimens of *P. violacea* were collected by hand using SCUBA on reefs at depths of −15 and −20 m near Sek point off Madang, Papua New Guinea. Freshly collected soft coral was frozen on site and transported to Vancouver over dry ice. A voucher sample of *P. violacea* has been deposited at the National Museum of the Netherlands.

Frozen specimens of *Pachyclavularia violacea* (100 g, wet wt.) were thawed and extracted exhaustively with MeOH (500 ml×3, 24 h between extractions). The MeOH extract was filtered and concentrated in vacuo to give a deep green crude gum (5 g). The crude extract was diluted with water up to 500 ml and partitioned sequentially against hexane (200 ml×3), $CHCl_3$ (200 ml×3) and EtOAc (200 ml×3). Only the hexanes soluble material showed G2 checkpoint inhibition and cytotoxicity.

Purification of the hexane extract (573 mg) was accomplished by repeated fractionation on Sephadex LH-20, silica gel flash chromatography, and reversed phase HPLC. Sephadex LH-20 size-exclusion chromatography (eluent: MeOH) afforded one bioactive fraction (100 mg). This active fraction was chromatographed on silica gel using gradient elution (hexane/EtOAc 1:9 to 1:1) to give one major complex mixture of diterpenoid compounds. This major fraction (20 mg) was chromatographed on reversed phase HPLC with MeOH/H_2O (85:15) to give pure samples of one bioactive diterpenoid, pachyclavulariolide F (**6**) (5.2 mg), and six inactive diterpenoids, pachyclavulariolide A (**1**) (6.2 mg), B (**2**) (3.1 mg), and C (**3**) (0.8 mg), D (**4**) (0.9 mg), and E (**5**).

Pachyclavulariolide A (1). White amorphous solid; $[\alpha]_D$=+17.1° (*c*=2.01 g/100 mL, MeOH); HRFABMS: [M+H] *m/z* 317.21150 ($C_{20}H_{29}O_3$, ΔM −0.53ppm); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Pachyclavulariolide B (2). Crystals from C_6D_6, mp 152.3–154.6°C; $[\alpha]_D$=+23.1° (*c*=0.28 g/100 mL, MeOH); HRFABMS: [M+H] *m/z* 333.20663 ($C_{20}H_{29}O_4$, ΔM 0.15 ppm); LRFABMS, *m/z* [formula, relative intensity %]: 315 ($C_{20}H_{27}O_3$,50); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Pachyclavulariolide C (3). White amorphous solid; $[\alpha]_D$=+11.1° (*c*=0.42 g/100mL, MeOH); HRFABMS: [M+H] *m/z* 347.22162 ($C_{21}H_{31}O_4$, ΔM −1.77 ppm)); LRFABMS, *m/z* [formula, relative intensity %]: 315 ($C_{20}H_{27}O_3$, 19); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Pachyclavulariolide D (4). White amorphous solid; $[\alpha]_D$=+12.1° (*c*=0.32 g/100 mL, MeOH); HRFABMS: [M+H] *m/z* 347.22079 ($C_{21}H_{31}O_4$, ΔM −4.17 ppm); LRFABMS, *m/z* [formula, relative intensity %]: 315 ($C_{20}H_{27}O_3$, 19); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Pachyclavulariolide E (5). White amorphous solid; $[\alpha]_D$=−15.1° (*c*=0.44 g/100 mL, MeOH); HRFABMS: [M+H] *m/z* 451.23322 ($C_{24}H_{35}O_8$, ΔM 0.06 ppm); LRFABMS, *m/z*[formula, relative intensity %]: 433 ($C_{24}H_{33}O_7$, 24); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Pachyclavulariolide F (6). White amorphous solid; $[\alpha]_D$=+32.1° (*c*=1.22 g/100 mL, MeOH); HRFABMS: [M+H] *m/z* 507.33198 ($C_{29}H_{47}O_7$, ΔM −0.40 ppm); LRFABMS, *m/z* [formula, relative intensity %]: 475 ($C_{28}H_{43}O_6$, 70), 363 ($C_{21}H_{31}O_5$, 18), 331 ($C_{20}H_{27}O_4$, 45), 313 ($C_{20}H_{25}O_3$, 33); ^{1}H NMR (C_6D_6, 500 MHz) and ^{13}C NMR (C_6D_6, 125 MHz) data are listed in Tables 1 and 2.

Single crystal X-ray diffraction analysis of pachyclavulariolide B (2)

A crystal of pachyclavulariolide B (**2**) with dimensions 0.45×0.20×0.12 mm was mounted on a glass fibre. Data were collected at −100°C on a Rigaku/ADSC CCD area detector in two sets of scans (ϕ=0.0 to 190.0°, χ=−90°; and ω=−18.0 to 23.0°, χ=−90°) using 0.50° oscillations with 58.0 second exposures. The crystal-to-detector distance was 40.41 mm with a detector swing angle of −5.53°. The material crystallized in space group $P2_12_12_1$ with *a*=7.390(4), *b*=9.465(1), and *c*=25.138(3) Å. Of the 3509 unique reflections measured (Mo-Kα radiation, $2\theta_{max}$=55.8°, R_{int}=0.080, Friedels not merged), 2311 were considered observed ($I>3\sigma(I)$). The final refinement residuals were *R*=0.033 (on F, $I>3\sigma(I)$) and wR_2=0.094 (on F^2, all data). The data was processed using the d*TREK program[8] and corrected for Lorentz and polarization effects. The structure was solved by direct methods[9] and all non-hydrogen atoms were refined anisotropically, while all methyl and hydroxyl hydrogen atoms were refined isotropically. All other hydrogens were included in calculated positions. This enantiomorph was chosen based on the

known configurations of the various stereocentres. All calculations were performed using the teXsan[10] crystallographic software package of Molecular Structure Corporation.

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References

1. Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7–55, and previous articles in this series.
2. Lindel, T.; Jensen, P. R.; Fenical, W.; Long, B. H.; Casazza, A. M.; Carboni, J.; Fairchild, C. R. *J. Am. Chem. Soc.* **1997**, *119*, 8744–8745.
3. (a) Roussis, V.; Wu, Z.; Fenical W.; Strobel S. A.; van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1990**, *55*, 4916–4922. (b) Mayer, A. M. S.; Jacobsen, P. B.; Fenical, W.; Jacobs, R. S.; Glaser, K. B. *Life Sci.* **1998**, *62*, 401–407.
4. (a) Berlinck, R. G. S.; Britton, R.; Piers, E.; Lim, L.; Roberge, M.; Rocha, R. M.; Andersen, R. J. *J. Org. Chem.* **1998**, *63*, 9850–9856. (b) Cinel, B.; Roberge, M.; Behrisch, H.; van Ofwegen, L.; Castro, C. B.; Andersen, R. J. *Org. Lett.* **2000**, *2*, 257–260.
5. Roberge, M.; Berlinck, R. G. S.; Xu, L.; Anderson, H.; Lim, L. Y.; Curman, D.; Stringer, C. M.; Friend, S. H.; Davies, P.; Vincent, I.; Haggarty, S. J.; Kelly, M. T.; Britton, R.; Piers, E.; Andersen, R. J. *Cancer Res.* **1998**, *58*, 5701–5706.
6. Inman, W.; Crews, P. *J. Org. Chem.* **1989**, *54*, 2526–2529.
7. Bowden, B. F.; Coll, J. C.; Mitchell, S. J.; Raston, C. L.; Stokie, G. J.; White, A. H. *Aust. J. Chem.* **1979**, *32*, 2265–2274.
8. d*TREK: Area Detector Software. Version 4.13. Molecular Structure Corporation (1996–1998).
9. SIR97: Altomare, A.; Burla, M. C.; Cammalli, G.; Cascarano, M.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, A. *J. Appl. Cryst.* **1999**, *32*, 115–119.
10. teXsan: Crystal Structure Analysis Package, Molecular Structure Corporation (1985 & 1992).