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# Pachyclavulariaenones A–C, three novel diterpenoids from the soft coral *Pachyclavularia violacea*

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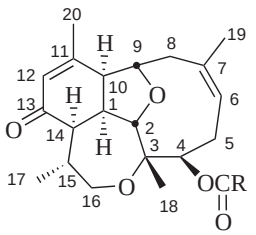
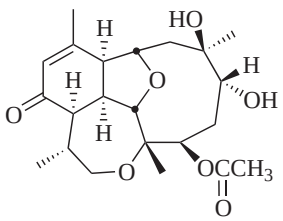
**Abstract**—Three novel diterpenoids named pachyclavulariaenones **A**–**C** (**1**–**3**) have been isolated from the soft coral *Pachyclavularia violacea*. Their structures have been established by spectroscopic methods. The structure of **3** was further confirmed by a single crystal X-ray analysis. © 2001 Elsevier Science Ltd. All rights reserved.

We have previously isolated several cytotoxic briarane diterpenoids, named excavatolides[1–3] and briaexcavatolides,[4] from a Formosan gorgonian *Briareum excavatum*. In our continuing survey of Formosan marine invertebrates with promising cytotoxicity against a variety of cancer cell lines, we encountered the soft coral *Pachyclavularia violacea* which has similar colonial morphology to *Briereum* spp., along the coast of Kenting, located in the southernmost tip of Taiwan, in September 1995. The chemical constituents of *P. violacea* has now become the subject of intense investigation in our laboratory due to the significant cytotoxicity of its organic extract toward P-388 tumour cells ($ED_{50}=0.3$ μg/mL). This present study has led to the isolation of three novel briarellin-type diterpenoids, pachyclavulariaenones **A**–**C** (**1**–**3**).



**1 :** R = $CH_2CH_2CH_3$
**2 :** R = $CH_3$



**3**

Pachyclavulariaenone **A** (**1**) was isolated as colourless oil, $[\alpha]_D^{28}$ −2.7° (*c* 0.6, $CHCl_3$). The molecular formula of $C_{24}H_{34}O_5$ (found *m*/*z* 402.2406, calcd 402.2407) was deduced from HREIMS. Thus, eight degrees of unsaturation was determined for **1**. Inspection of the NMR spectral data (Table 1) for **1** by the assistance of DEPT spectrum revealed the presence of five methyls, five methylenes, seven $sp^3$-hybridized methines, one quaternary $sp^3$-carbons, and two trisubstituted double bonds. The $^1H$ NMR spectrum (Table 1) also showed the presence of five methyl groups including a methyl attached to methine carbon (δ 0.97, 3H, d, $J=7.2$ Hz), a methyl attached to an oxygen-bearing carbon (δ 1.36, 3H, s), two olefinic methyl groups (δ 1.91, 3H, s and δ 1.92, 3H, s), and an *n*-butyryloxy methyl group (δ 0.95, 3H, t, $J=7.5$ Hz). Two methylenes in the *n*-butyryloxyl group showed signals at δ 1.68 and 2.34 ppm, respectively. Two protons showed signals at δ 3.68 (1H, d, $J=10.8$ Hz) and δ 4.33 (1H, dd, $J=3.6$, 1.5 Hz) in $^1H$ NMR spectrum were found to be the protons of the two oxymethines in the THF structural unit of **1**. The second ether ring could be identified by the appearance of an oxymethylene group (δ 3.35, 1H, dd, $J=14.1$, 1.8 Hz, and δ 3.64, 1H, dd, $J=14.1$, 9.0 Hz). The signals at δ 5.93 (1H, s) and 5.82 (1H, dd, $J=10.8$, 6.3 Hz) were assigned as one proton attached to α-carbon of the enone moiety and the other proton attached to a normal olefin, respectively. The molecular framework of **1** was established by the $^1H$–$^1H$ COSY analysis (Fig. 1) and by an HMBC experiment which showed the following key correlations: H-12 and $H_3$-20 to C-10, H-1 and H-14 to C-13, H-2 and $H_3$-17 to C-14, H-4 to C-3, C-6, and ester carbonyl carbon, $H_3$-18 to C-2, C-3 and C-4,

*Keywords*: *Pachyclavularia violacea*; pachyclavulariaenone; diterpenoids.

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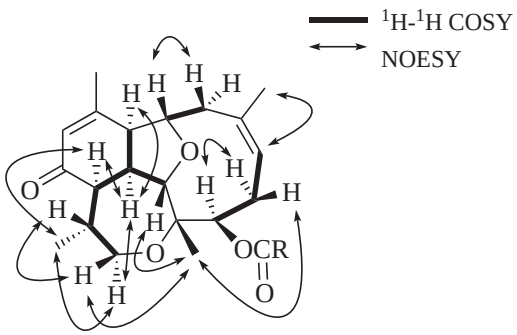
**Table 1.** The $^{1}H$ and $^{13}C$ NMR chemical shifts for **1**–**3**

| Compounds | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| C/H | $^{1}H$[a] | $^{13}C$[b] | $^{1}H$[c] | $^{13}C$[d] | $^{1}H$[e] | $^{13}C$[f] |
| 1 | 3.12 m | 40.41 | 3.12 m | 40.42 | 3.60 m | 38.49 |
| 2 | 3.68 d (10.8)[g] | 87.23 | 3.69 d (10.8) | 87.09 | 3.98 d (9.8) | 85.00 |
| 3 | | 76.99 | | 77.32 | | 76.98 |
| 4 | 5.30 d (7.8) | 71.87 | 5.28 d (7.8) | 72.36 | 5.89 dd (8.5, 3.0) | 71.73 |
| 5α | 3.20 m | 33.06 | 3.20 m | 33.05 | 2.03 m | 38.03 |
| 5β | 1.80 dd (14.1, 5.6) | | 1.79 dd (14.4, 5.7) | | 2.65 m | |
| 6 | 5.82 dd (10.8, 6.3) | 126.87 | 5.79 dd (10.5, 6.3) | 126.73 | 4.69 br d (8.2) | 73.23 |
| 7 | | 131.67 | | 131.64 | | 75.03 |
| 8α | 2.83 br d (5.1) | 38.34 | 2.83 br d (5.1) | 38.36 | 2.34 t (13.7) | 44.81 |
| 8β | 1.94 m | | 1.93 m | | 1.87 br d (2.8) | |
| 9 | 4.33 dd (3.6, 1.5) | 82.43 | 4.33 dd (4.2, 2.1) | 82.42 | 5.00 dd (13.2, 2.0) | 79.30 |
| 10 | 2.87 br s | 47.80 | 2.87 br s | 47.82 | 2.73 d (5.9) | 51.81 |
| 11 | | 156.41 | | 156.31 | | 156.73 |
| 12 | 5.93 s | 126.64 | 5.93 s | 126.52 | 6.02 s | 128.20 |
| 13 | | 198.09 | | 197.90 | | 197.23 |
| 14 | 2.29 t (6.9) | 48.51 | 2.29 t (6.9) | 48.50 | 2.60 br d (4.1) | 49.40 |
| 15 | 2.54 m | 33.06 | 2.54 m | 33.05 | 2.83 m | 31.24 |
| 16α | 3.35 dd (14.1, 1.8) | 65.96 | 3.38 dd (13.8, 1.8) | 65.95 | 3.46 dd (13.3, 1.4) | 64.64 |
| 16β | 3.64 dd (14.1, 9.0) | | 3.64 dd (13.8, 8.7) | | 3.74 d (13.3) | |
| 17 | 0.97 d (7.2) | 19.32 | 0.98 d (6.9) | 19.30 | 1.22 d (7.4) | 17.21 |
| 18 | 1.36 s | 21.02 | 1.36 s | 21.03 | 1.38 s | 18.53 |
| 19 | 1.91 s | 29.09 | 1.91 s | 29.15 | 1.59 s | 26.10 |
| 20 | 1.92 s | 21.87 | 1.92 s | 21.94 | 1.85 s | 21.02 |
| *n*-Butyrate | | | | | | |
| $CH_3$ | 0.95 t (7.5) | 13.49 | | | | |
| $CH_2$ | 1.68 m | 18.49 | | | | |
| $CH_2$ | 2.34 m | 36.51 | | | | |
| CO | | 173.28 | | | | |
| Acetate | | | | | | |
| $CH_3$ | | | 2.10 s | 21.37 | 2.07 s | 21.02 |
| CO | | | | 170.52 | | 170.09 |

[a] Spectra recorded at 400 MHz in $CDCl_3$.
[b] Spectra recorded at 100 MHz in $CDCl_3$.
[c] Spectra recorded at 300 MHz in $CDCl_3$.
[d] Spectra recorded at 75 MHz in $CDCl_3$.
[e] Spectra recorded at 400 MHz in pyridine-$d_5$ at 70°C.
[f] Spectra recorded at 100 MHz in pyridine-$d_5$.
[g] *J* values (in Hz) in parentheses.




**R = $CH_2CH_2CH_3$**

**Figure 1.** Selective $^{1}H$–$^{1}H$ COSY and NOE correlations of pachyclavulariaenone **A** (**1**).

and $H_3$-19 to C-6, C-7, and C-8. Furthermore, the relative stereochemistry of **1** was determined by a NOESY spectrum (Fig. 1). Thus, the structure of compound **1** was unambiguously established.

Pachyclavulariaenone **B** (**2**) was isolated as white powder, mp 52–54°C, $[\alpha]_D^{28}$ −1.9° (*c* 2.2, $CHCl_3$). A molecular formula of $C_{22}H_{30}O_5$ was established for **2** from HREIMS (found *m*/*z* 374.2069, calcd 374.2093). The $^{1}H$ and $^{13}C$ NMR spectra of **2** are very similar to those of **1** (Table 1), except for the signals of the *n*-butyryloxyl group which were replaced by an acetoxyl group. HMBC and NOESY spectra of **2** also gave similar results to those of **1**, suggesting that **2** is simply a structurally similar analogue to **1**. A weak but significant NOE correlation was observed for H-2 and H-9, which has not been found in **1**, revealing that both H-2 and H-9 are positioned in the same face.

Pachyclavulariaenone **C** (**3**) was further obtained as colourless crystals, mp 220–223°C, $[\alpha]_D^{27}$ −82.0° (*c* 0.5, $CHCl_3$). A molecular formula of $C_{22}H_{32}O_7$ (found *m*/*z* 408.2155, calcd 408.2149) was established from HREIMS. The $^{1}H$ NMR spectral data of **3**, measured in $CDCl_3$,[5] also revealed the characteristic signals which

suggested that **3** could be a derivative of **2**. However, the $^{13}C$ NMR spectrum of **3** in $CDCl_3$ gave mostly very weak (or broad) signals. Some of these broadened signals were hardly observed over the noise level. These observations suggested the existence of slowly interconverting conformations for **3** in $CDCl_3$ solution. We found that both $^{1}H$ and $^{13}C$ NMR spectra could be sharpened and well-resolved in pyridine-$d_5$ at 70°C. The $^{13}C$ NMR spectrum of **3** is similar to that of **2**, except that the signals for carbons of the 6,7-double bond disappeared and were replaced by two signals of oxygenated carbons. The assignments of $^{1}H$ and $^{13}C$ NMR spectral data of **3** were assisted by a series of 2D NMR ($^{1}H$–$^{1}H$ COSY, HMQC and HMBC) experiments. The protons of the hydroxy- and acetoxy-bearing methines which show signals at $\delta$ 4.69 (1H, br d, $J=8.2$ Hz) and 5.89 (1H, dd, $J=8.5$, 3.0 Hz) were assigned to H-6 and H-4, respectively. A doublet at $\delta$ 1.22 (3H, d, $J=7.4$ Hz) and four singlets at $\delta$ 1.38, 1.59, 1.85, and 2.07 were attributed to $H_3$-17, $H_3$-18, $H_3$-19, $H_3$-20, and protons of acetoxyl methyl, respectively. Based on the consideration of molecular formula, the second hydroxy group should be placed at C-7.

The relative stereochemistry of compound **3** was also deduced using a NOESY spectrum, which showed that the relative configuration of **3** is similar to that of metabolites **1** and **2**. H-6 was found to show NOE response with H-5β, but not with $H_3$-19. Thus, H-6 and $H_3$-19 should be positioned on the β and α face, respectively. A single-crystal X-ray structure analysis was carried out in order to confirm the molecular structure of **3**. The X-ray structure (Fig. 2) demonstrates the location of the acetoxyl and two hydroxyl groups and unambiguously confirms the relative configuration of **3**.[6] Furthermore, it was found that there are two independent molecules in an asymmetric cell unit. The two molecules are linked by an intermolecular hydrogen bond (O4···O10) and by bridging a water molecule via two hydrogen bonds (O11···O15, and O5···O15).

Although pachyclavulariaenone **A**–**C** possess similar ring structure to that of asbestinins, [7–12] they have a unique methyl substituent at C-11 in comparison with the latter. If the position of Me-11 and H-12 were switched, the carbon skeleton would be identical with asbestinins. Also, pachyclavulariaenones are novel in comparison with eunicellins[13–15] and asbestinins as they contain a conjugated cyclohexenone unit in the molecules. Besides the fact that all diterpenoids of the above two classes reported so far have *trans*-configuration for H-1 and H-14, metabolites **1**–**3** are unprecedented examples as they possess a six-membered carbocyclic ring *cis*-fused to both of the ten-membered carbocyclic and seven-membered ether rings, making three ring-junction protons (H-1, H-10, and H-14) *cis* to each other. The cytotoxicity of compounds **1**–**3** toward P-388, KB, A-549 and HT-29 cancer cell lines was evaluated and the results showed that these compounds are not cytotoxic against the above cells. Further investigations are now being carried out in our group in order to discover the bioactive metabolites from this organism.

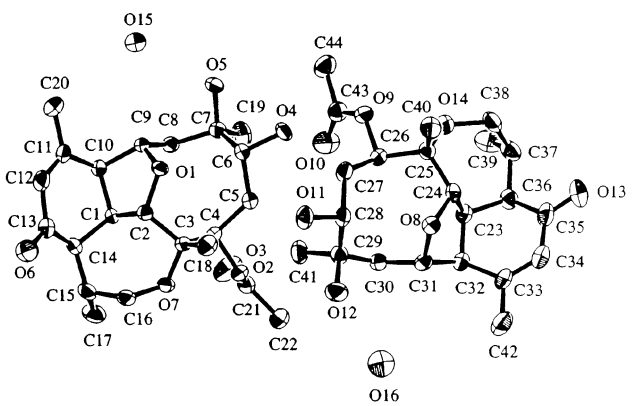



**Figure 2.** A computer-generated ORTEP plot of **3** showing the relative configuration. Hydrogen atoms have been omitted for clarity.


## Acknowledgements

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5. $^{1}H$ NMR ($CDCl_3$, 300 MHz): $\delta$ 5.95 (1H, s, H-12), 5.34 (1H, br s, H-4), 4.70 (1H, dd, $J=12.4$, 3.6 Hz, H-9), 3.78 (1H, d, $J=10.0$ Hz, H-2), 3.50 (1H, d, $J=13.3$ Hz, H-16), 3.36 (1H, dd, $J=13.3$, 1.7 Hz, H-16), 3.34 (1H, m, H-1), 2.74 (1H, d, $J=5.4$ Hz, H-15), 2.61 (1H, dd, $J=6.9$, 3.6 Hz, H-10), 2.50 (1H, d, $J=4.0$ Hz, H-14), 2.30 (1H, br s, H-5), 2.07 (3H, s, acetate methyl), 2.04 (1H, m, H-5), 1.99 (3H, s, $H_3$-20), 1.26 (6H, s, $H_3$-18 and $H_3$-19), 1.03 (3H, d, $J=7.4$ Hz, $H_3$-17). The signals of protons at C-6 and C-8 disappeared.
6. Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.
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