Paralemnolins J—P, New Sesquiterpenoids from the Soft Coral *Paralemnalia thyrsoides*

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Six new sesquiterpenoids, paralemnolins J—O (1—6), along with one novel norsesquiterpenoid, paralemnolin P (7), have been isolated from the soft coral *Paralemnalia thyrsoides*. The structures of metabolites 1—7 were established on the basis of extensive NMR study and chemical methods. The structure of 5 was further confirmed by a single-crystal X-ray analysis. Cytotoxicity of these metabolites toward a limited panel of cancer cell lines also is described.

**Key words** sesquiterpenoid; paralemnolin; *Paralemnalia thyrsoides*; cytotoxicity

Soft coral of *Paralemnalia thyrsoides* (Acyonaceae) has been found to be a rich source of a variety of sesquiterpenes and norsesquiterpenes.1—8) Our previous chemical investigation of the Formosan soft coral *P. thyrsoides* resulted in the isolation of twelve sesquiterpenoids, paralemnolins A—I, 2,3) paralemnanone,4) isoparalemnanone,4) and paralemnanol. 4) Our continuing search for bioactive compounds from this organism has further resulted in the isolation of seven paralemnolins J—P (1—7). Herein, we report the structural elucidation of these metabolites 1—7, and the structure of 5 was unambiguously established by a single-crystal X-ray analysis.

In the cytotoxicity testing, we observed that paralemnolins M, N (4, 5) exhibited moderated cytotoxicity toward a human medulloblastoma cell line.

**Results and Discussion**

Paralemnolin J (1) was isolated as a white powder with a molecular formula of C_{17}H_{26}O_{3} requiring five degrees of unsaturation, as established by HR-electrospray ionization (ESI)-MS. The 13C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of 1 showed signals of 17 carbons, including one ketone (δ_C 212.9 qC), one trisubstituted carbon–carbon double bond (δ_C 137.7 qC and 123.9 CH), one oxygenated methane (δ_C 66.2 CH_2; δ_H 3.71, 4.56), and one acetoxy group (δ_C 170.8 qC, 20.8 CH_3; δ_H 2.05, s). The above functionalities account for three of the five degrees of unsaturation, suggesting a bicyclic skeleton for 1. The gross structure of 1 was determined by 2D NMR spectroscopic analysis. The 1H–1H correlation spectroscopy (COSY) spectrum revealed three spin systems as shown in Fig. 1. Careful inspection of the heteronuclear multiple bond correlation (HMBC) spectrum led to the establishment of the planar structure of 1 (Fig. 1). The H-6, H_{14}, and H_{15} positioned on the β face were established by the following nuclear Overhauser enhancement (NOE) cross peaks: H_3-15/H_{14} and H_{13}-H_{6}. The 1H-NMR spectral data and the physical properties of 1 were found to be in full agreement with those of a semisynthetic product, 9,10) of which the absolute stereochemistry was shown as in formula 1. This compound has been isolated recently from an Indonesian soft coral *Nephthea* sp., however, the stereochemistry at C-11 was not established.11)

Paralemnolin K (2) was obtained as a colorless oil. The molecular formula of 2, deduced from HR-ESI-MS, was found to be the same as that of 1. The 1H- and 13C-NMR data of 2 are very similar to those of 1 except for the chemical.

![Fig. 1. Key 1H–1H COSY and HMBC Correlations 1—7](image-url)
shifts and splitting patterns of H₇-12. Analyses of ¹H-¹H COSY and HMBC correlations led to the establishment of the same planar structure for both compounds (Fig. 1). By comparison of the NOESY interactions of 1 and 2, it was found that the relative configurations at C-4, C-5, and C-6 of both compounds were identical. Thus, 2 was found to be an 11-epimer of 1.

Paralemnolin L (3) was assigned a molecular formula of C₁₉H₂₈O₄ by HR-ESI-MS and ¹³C-NMR spectral data. Thus, six degrees of unsaturation was deduced. The IR absorption (1743 cm⁻¹) and NMR spectral data (δH 2.09, 3H, s and 1.93, 3H, s; δC 170.5 qC, 168.3 qC, 21.3 CH₃, and 20.7 CH₃) revealed the presence of two acetates. Carbon resonances at δ 122.5 (CH), 139.8 (qC), 134.2 (CH), and 120.3 (qC) were deduced as two trisubstituted double bonds. Above data suggested 3 to be bicyclic. Moreover, an acetoxyl-bearing carbon (δC 71.3; δH 5.30) was also found in the HMOC spectrum.

Two spin systems (a and b) of 3 were established by ¹H-¹H COSY spectrum (Fig. 1). The connectivity between C-11 to C-6, C-11, and C-12. The HMBC correlations from H-7 to the carbon signal at δ 170.5 and from H-12 to the carbon resonance at δ 168.3 revealed the locations of two acetoxy groups at C-7 and C-12, respectively. The above data and the other HMBC correlations illustrated in Fig. 1 established the planar structure of 3. The relative configuration of 3 was elucidated from a 2D NMR experiment, which showed that H₃-14, H₃-15, H-6, and H-7 are located on the β face of the molecule. The Z geometry of 11,12-double bond was determined by an NOE correlation between H₃-13 and H-12.

Compound 4 was found to possess the same molecular formula as that of 3. Some signals in ¹H- and ¹³C-NMR spectrum of 4 measured in CDCl₃ at room temperature or low temperature even at −50 °C gave mostly broad signals, suggesting the existence of slowly interconverting conformations in CDCl₃ solution (Tables 1 and 2). But still, the 2D NMR spectra were well resolved for the assignment of the gross structure. Inspection of the ¹H-¹H COSY and HMBC spectral data of 4 led to the establishment of the same planar structure as that of 3 (Fig. 1). By comparison of the NOESY spectra of 3 and 4, it was found that both compounds have the same relative configurations for all chiral centers. The absence of NOE correlation between H₃-13 and H-12 and the appearance of C-13 signal of 4 at upper field (δ 13.2) relative to that of 3 (δ 17.8), suggested the E geometry of 11,12-double bond of 4. Also, NOE correlations between H₃-15 with H₃-12, H-6, and H-7 suggested the β-orientation of these protons.

The same molecular formula C₁₉H₂₈O₄ as those of 3 and 4, was deduced for 5 from HR-ESI-MS. The ¹H- and ¹³C-NMR spectral data of 5 were found to exhibit broader signals than those of 4. The carbon resonances at δ 168.0 (qC) and 171.0 (qC), coupled with two methyl protons resonating at δ 2.15 and 2.03, suggested the presence of two acetoxy groups. By comparison of the spectral data of 5 with those of 3 and 4, it was found that 5 should be closely related to 3 and 4. The partial 2D NMR spectral data were shown in Fig. 1, which did not provide enough information for the complete elucida-

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**Table 1. ¹H-NMR Spectral Data of Compounds 1—7**

<table>
<thead>
<tr>
<th>H #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>1</td>
<td>5.60 ddd (6.0, 6.0, 3.0)</td>
<td>5.56 ddd (5.7, 3.0, 2.7)</td>
<td>5.41 t (2.7)</td>
<td>5.46d</td>
<td>2.08 m; 2.12 m</td>
<td>5.61 ddd (3.0, 2.7, 2.4)</td>
<td>5.51 ddd (5.1, 2.7, 2.4)</td>
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<tr>
<td>2</td>
<td>1.98 m; 2.15 m</td>
<td>2.01 m; 2.10 m</td>
<td>1.96 m</td>
<td>1.94 m; 2.05 m</td>
<td>1.27 m; 1.66 m</td>
<td>2.05 m</td>
<td>1.86 m; 1.98 m</td>
</tr>
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<td>1.49 m</td>
<td>1.47 m</td>
<td>1.37 m</td>
<td>1.38 m</td>
<td>1.35 m; 1.45 m</td>
<td>1.40 m</td>
<td>1.37 m</td>
</tr>
<tr>
<td>4</td>
<td>2.08 m</td>
<td>1.94 m</td>
<td>1.64 m</td>
<td>1.61 m</td>
<td>2.59 brs</td>
<td>1.48 m</td>
<td>1.52 m</td>
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<tr>
<td>5</td>
<td>2.37 m</td>
<td>2.46 m</td>
<td>3.54 d (6.3)</td>
<td>2.67 d (5.4)</td>
<td>2.59 brs</td>
<td>2.52 d (4.9)</td>
<td>3.35 d (5.7)</td>
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<tr>
<td>6</td>
<td>5.30 ddd (12.0, 6.0, 6.3)</td>
<td>5.26d</td>
<td>5.33d</td>
<td>5.51 dt (13.5, 3.9)</td>
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<td>2.44 m</td>
<td>1.82 m</td>
<td>1.82 m</td>
<td>2.30 m</td>
<td>1.85 m; 2.19 m</td>
<td>1.76 m; 1.96 m</td>
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<td>2.44 m</td>
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<td>2.24 m</td>
<td>5.22 brs</td>
<td>2.33 ddd (15.3, 4.8, 1.8)</td>
<td>2.24 ddd (14.7, 5.1, 2.1)</td>
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<td>2.51 m</td>
<td>2.51 m</td>
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<td>2.18 m</td>
<td>2.25 m</td>
<td>3.86 d (7.5)</td>
<td>6.92 d (1.5)</td>
<td>6.76d</td>
<td>6.83d</td>
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<td>4.56 ddd (10.5, 3.0)</td>
<td>1.03 d (7.2)</td>
<td>1.73 d (1.5)</td>
<td>1.76 brs</td>
<td>1.86 s</td>
<td>1.33 s</td>
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<tr>
<td>12</td>
<td>0.97 d (6.9)</td>
<td>0.83 d (6.6)</td>
<td>0.75 d (6.6)</td>
<td>0.79 d (6.9)</td>
<td>0.75 d (5.7)</td>
<td>0.74 d (6.0)</td>
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<td>1.31 s</td>
<td>1.11 s</td>
<td>1.17 s</td>
<td>1.13 s</td>
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<td>14</td>
<td>11-OH</td>
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<td>2.14 s</td>
<td>2.15 s</td>
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<tr>
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<td>2.05 s</td>
<td>2.04 s</td>
<td>1.93 s</td>
<td>2.01 s</td>
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<td>2.04 s</td>
<td>1.93 s</td>
<td>2.01 s</td>
<td>2.03 s</td>
<td></td>
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</tbody>
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* Spectra recorded at 300 MHz in CDCl₃ at 25 °C.  
  b J-values (in Hz) in parentheses.  
  c Broad signals.
tion of the gross structure. Thus, in order to resolve the structure of 5, a single-crystal X-ray crystallographic analysis (Fig. 2) was undertaken which led to the full structure elucidation and the full assignment of 1H- and 13C-NMR spectral data of 5.

Paralemnolin O (6) was obtained as a white powder. The molecular formula of C_{16}H_{24}O_{3}, was established by HR-ESI-MS, requiring five degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3497 cm\(^{-1}\) and 1734 cm\(^{-1}\)), one olefinic double bond (\(\delta_{\text{C}}\) 140.3 cQ and 124.9 CH), two oxygenated carbons (\(\delta_{\text{C}}\) 71.9 CH; 80.5 qC), one acetoxyl group (\(\delta_{\text{C}}\) 170.0 HC, 21.5 CH\(_3\); 20.8 2,04 s), and one keto group. By comparison of the NMR spectral data of 6 with H\(_3\)-13, H-6, and H-7 revealed that these protons were attached at C-11 were confirmed by the HMBC correlations. The hydroxy group. The hydroxy and aldehyde groups at C-7, C-11, and C-12. The planar structure of 6 was established by the inspection of the 2D NMR spectroscopic data as illustrated in Fig. 1. The position of H-6, H-7, H-14, and H-15 on the same face (\(\beta\) face) was established by the following NOE cross peaks: H\(_{\text{b}}\)-15/H\(_{\text{a}}\)-14, H\(_{\text{b}}\)-15/H-6, and H\(_{\text{b}}\)-15/H-7. The relative configuration of C-11 was unable to be determined due to the insufficient NOE correlations (Fig. 3).

Paralemnolin P (7) was isolated as a colorless oil. HR-ESI-MS, 1H- and 13C-NMR, and DEPT spectra established the molecular formula of C\(_{17}\)H\(_{26}\)O\(_{3}\), implying five degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3497 cm\(^{-1}\)), one olefinic double bond (\(\delta_{\text{C}}\) 140.3 cQ and 124.9 CH), two oxygenated carbons (\(\delta_{\text{C}}\) 71.9 CH; 80.5 qC), one acetoxyl group (\(\delta_{\text{C}}\) 170.0 HC, 21.5 CH\(_3\); 20.8 2,04 s), and one keto group. By comparison of the NMR spectral data of 7 with those of a known metabolite (paralemnolin B), it was found that the ketone at C-7 (\(\delta_{\text{C}}\) 207.3) in paralemnolin B was converted to an acetoxy group (C-7, \(\delta_{\text{C}}\) 72.0 CH; \(\delta_{\text{H}}\) 5.30) in 7. NOE correlations of H\(_{\text{b}}\)-14 with H\(_{\text{b}}\)-13, H-6, and H-7 revealed that these protons were located on the same face. Thus, the structure of 7 was established unambiguously.

The cytotoxicity of the compounds 1–7 against Hepa597/VGH (human liver carcinoma), KB (human oral epidermoid carcinoma), Hela (human cervical epitheloid carcinoma), and Daoy (human medulloblastoma) cancer cell lines was measured. The result showed that compounds 4 and 5 exhibited moderated cytotoxicity toward Daoy cancer cell line with the ED\(_{50}\) values of 9.4, and 8.6 µg/ml, respectively. Other compounds did not show inhibitory activity against the growth of the above four cancer cell lines.
parameters were 4450/0/209; goodness-of-fit on 2,5-diphenyltetrazolium bromide colorimetric method.\(^{13,14}\)

Paralemnolin M (4): Colorless oil; [\(\alpha\)\(D\)]\(^{20}\) \(= -66\) (c = 1.24, CHCl\(_3\)); IR (neat) \(v_{\text{max}}\) 2957, 2924, 1734, and 1647 cm\(^{-1}\); \(1\)H- and \(13\)C-NMR data, see Tables 1 and 2; ESI-MS \(m/z\) 343 [M + Na\(^+\)]; HR-ESI-MS \(m/z\) 343.1884 [M + Na\(^+\)] \((\text{Calcd for C}_{17}\text{H}_{26}\text{O}_{4}\text{Na}, 317.1730)\).

Paralemnolin N (5): White powder; mp 85–87 °C; \([\alpha]_{D}^{25} = 94\) (c = 1.16, CHCl\(_3\)); IR (neat) \(v_{\text{max}}\) 3497, 2966, 1734, and 1236 cm\(^{-1}\); \(1\)H- and \(13\)C-NMR data, see Tables 1 and 2; ESI-MS \(m/z\) 287 [M + Na\(^+\)]; HR-ESI-MS \(m/z\) 287.1625 [M + Na\(^+\)] \((\text{Calcd for C}_{19}\text{H}_{28}\text{O}_{4}\text{Na}, 343.1885)\).

Paralemnolin O (6): White powder; mp 77–79 °C; \([\alpha]_{D}^{25} = -15\) (c = 1.72, CHCl\(_3\)); IR (neat) \(v_{\text{max}}\) 3497, 2966, 1734, and 1236 cm\(^{-1}\); \(1\)H- and \(13\)C-NMR data, see Tables 1 and 2; ESI-MS \(m/z\) 287 [M + Na\(^+\)]; HR-ESI-MS \(m/z\) 287.1625 [M + Na\(^+\)] \((\text{Calcd for C}_{19}\text{H}_{28}\text{O}_{4}\text{Na}, 343.1885)\).

Paralemnolin P (7): Colorless oil; [\(\alpha\)\(D\)]\(^{25}\) \(= 184\) (c = 1.52, CHCl\(_3\)); IR (neat) \(v_{\text{max}}\) 3497, 2966, 1734, and 1236 cm\(^{-1}\); \(1\)H- and \(13\)C-NMR data, see Tables 1 and 2; ESI-MS \(m/z\) 287 [M + Na\(^+\)]; HR-ESI-MS \(m/z\) 287.1625 [M + Na\(^+\)] \((\text{Calcd for C}_{19}\text{H}_{28}\text{O}_{4}\text{Na}, 343.1885)\).

X-Ray Diffraction Analysis of Paralemnolin N (5)\(^{12}\) A suitable colorless crystal (0.53×0.45×0.32 mm\(^3\)) of 5 was grown by slow evaporation of the EtOAc solution. Diffraction intensity data were acquired with a Rigaku AFC7s single-crystal X-ray diffractometer with graphite-monochromated MoK\(_{\alpha}\) radiation (\(\lambda = 0.71073\) Å). Crystal data for 5: \(\text{C}_{19}\text{H}_{28}\text{O}_{4}\) (formula weight 320.41), approximate crystal size, 0.53×0.45×0.32 mm\(^3\), orthorhombic, space group, \(P2_{1}2_{1}2_{1}\) (\#4), \(a = 298(2)\) Å, \(b = 12.321(7)\) Å, \(c = 16.8426(615)\) Å, \(V = 1820.2(3)\) Å\(^3\), \(D_{\text{c}} = 1.169\) mg/m\(^3\), \(Z = 4\), \(F(000) = 696\), \(\mu_{\text{cyc}} = 0.08\) mm\(^{-1}\). A total of 21811 reflections were collected in the range 2.05°<\(\theta<28.9°\), with 4450 independent reflections, completeness to \(\theta_{\text{max}} = 99.5\%\); psi-scan absorption correction applied; full-matrix least-squares refinement on \(F^2\), the number of data/restraints/parameters were 4450/0/209; goodness-of-fit on \(F^2 = 1.013\); final \(R\) indices \([I>2\sigma(I)]\), \(R_{I} = 0.0533\), \(wR_{I} = 0.1190\); \(R\) indices (all data), \(R_{I} = 0.0940\), \(wR_{I} = 0.1364\); largest difference peak and hole, 0.175 and −0.144 e/Å\(^3\).

Cytotoxicity Testing Cancer cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicity assays of the test compounds 1—7 were performed using the MITT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.\(^{13,14}\)

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References and Notes
12) Crystallography data of 5 have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC600210. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).