Neolemnane-Type Sesquiterpenoids from a Formosan Soft Coral
Paralemnalia thyrsoides

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Six new sesquiterpenoids, paralemnolins D—I (1—6), have been isolated from the EtOAc extract of the soft coral Paralemnalia thyrsoides. The structures of these metabolites were determined by extensive spectroscopic analysis and by comparison of their spectral data with those of related metabolites. The absolute stereochemistry of these metabolites was established by application of the Mosher’s method on 1 and on the basis of the absolute structures of other related compounds previously isolated from the soft corals of the genera Paralemnalia and Lemnalia. Cytotoxicity of these metabolites toward a limited panel of cancer cell lines also is reported.

Key words Paralemnalia thyrsoides; soft coral; sesquiterpenoid; paralemnolin

Soft corals belonging to the genera Paralemnalia1—4) and Lemnalia5—12) have been found to be a rich source of neolemnane and nardosinane sesquiterpenoids. Our previous investigation on the chemical constituents of a soft coral Paralemnalia thyrsoides has resulted in the isolation of three terpenoids, paralemnolins A—C.13) Continuing study on searching new metabolites of this soft coral again afforded six new metabolites paralemnolins D—I (1—6) (neolemnanoids). We describe herein the isolation, structure elucidation and biological activity of these compounds.

Results and Discussion

Paralemnolin D (1) was isolated as a colorless oil, [α]D 25 +67° (c = 1.32, CHCl3). Its HR-ESI-MS exhibited a pseudomolecular ion peak at m/z 301.1781 [M+Na]+ (Calcd for 301.1780), corresponding to the molecular formula of C17H26O3. Thus, five degrees of unsaturation were deduced. The 13C-NMR and DEPT spectra displayed 17 carbon signals, including four methyls, four methylenes, five methines, and four quaternary carbons. The NMR signals [δH 2.10 (3H, s); δC 170.8 (C) and 20.9 (CH3)] and IR absorptions at 3504 and 1730 cm−1, together with the observation of two oxygen-bearing carbon resonances [δ 72.1 (CH) and 76.3 (CH)] in the 13C-NMR spectrum, indicated the presence of an acetyl and a hydroxy group. Furthermore, two trisubstituted double bonds [δ 125.7 (CH), 142.1 (C), 138.3 (CH), 129.6 (C)] were assigned from the 13C-NMR and DEPT spectra of 1. The above functionalities revealed that 1 is a bicyclic compound. The skeleton of 1 was established by extensive 2D NMR analysis (1H–1H COSY, HMOC, and HMBC). To establish the proton sequences in 1, the 1H–1H COSY spectrum was used to reveal the connectivity of H-4/H-5, H-5/H-6, H-6/H-7, H-9/H-10, and H-10/H-11 (Fig. 1). The vinyl methyl [δ 1.57 (3H, d, J = 1.2 Hz)] attached at C-3 was confirmed by the HMBC correlations from H3-14 to C-2, C-3 and C-4. The methyl group attached at C-1 and the connectivities from C-7 to C-8 and C-8 to C-9 were deduced from the following HMBC correlations: H3-13/C-1, C-2, C-8, C-12 and H2-7/C-8, C-9. Moreover, a methyl [δ 0.87 (3H, d, J = 6.6 Hz)] attached at C-12 was confirmed by the HMBC correlations from H3-15 to C-1, C-11, and C-12. The placement of the acetyl group at C-4 was confirmed by the HMBC correlation of H-4 (δ 6.49) with C-3 and the carbonyl group of ester. Thus, the hydroxyl group should be positioned at C-5, and the planar structure of 1 was established. The relative stereochemistry and detailed 1H-NMR data assignment of 1 were elucidated from the NOE correlations observed in a NOESY experiment (Fig. 2). The Z geometry of the 2,3-double bond was established by an NOE interaction between H-2 and H3-14. Also, H3-13 was found to show NOE interactions

Fig. 1. Key 1H–1H COSY and HMBC Correlations of 1—6

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with H-2, H1-15 and one proton (δ 2.31, m) of H1-7, but not with H1-12. H1-14 also showed an NOE correlation with H-5 instead of H-4, while no NOE correlation could be observed between H-4 and H-5, suggesting that H2-15, H1-13, and H-5 should be positioned on the same face and were arbitrarily assigned as β-oriented protons. Moreover, the NOE interaction between H-4 and H-12 suggested that both H-4 and H-12 should be positioned on the α face. Thus, the structure of 1 was established unambiguously.

Paralamenolin E (2) was obtained as a white powder, [α]D 25° +68° (c=0.68, CHCl3). The molecular formula of 2, C19H28O4, was established by HR-ESI-MS, requiring six degrees of unsaturation. The IR spectrum of 2 showed the presence of ester groups (νmax 1739 cm⁻¹). The 13C-NMR and DEPT spectra of 2 showed signals of 19 carbons (Table 2), including two ester carbonyls (δ 170.3 (C), 170.8 (C)), and two trisubstituted double bonds [δ 126.4 (CH), 141.0 (C), 138.8 (CH), 129.0 (C)]. The above functionalities revealed that 2 is a bicyclic compound. The NMR spectral data of 2 were analogous to those of 1 with the exception that the resonance of hydroxyl methine in 1 was replaced by that of an acetyl methine in 2, which was further confirmed by the assistance of the HMBC and 1H-1H COSY spectra as shown in Fig. 1. The relative stereochemistry of 2 was found to be the same as that of 1, on the basis of the observed NOE correlations (Fig. 2).

Paralamenolin F (3) was obtained as a colorless oil, [α]D 25° −6° (c=1.28, CHCl3). According to the HR-ESI-MS (m/z 359.1835, [M+Na]⁺) and 13C-NMR spectral data, its molecular formula was established as C17H24O4, implying six degrees of unsaturation. The IR spectrum of 3 showed the presence of ester functionality (νmax 1745 cm⁻¹) and the absence of hydroxyl group. The 13C- and 1H-NMR spectra revealed the presence of two acetyl groups [δα 2.09 (3H, s), 2.07 (3H, s); δβ 170.0 (C), 169.9 (C), 21.1 (CH3), 20.9 (CH3)], one double bond [δα 127.8 (CH), 140.4 (C)]. A trisubstituted epoxide was confirmed from the resonances of two oxygen-bearing carbons at δα 58.7 (C) and 71.8 (CH), and δβ 2.62 (s) in the HMBC spectrum. Comparison of the NMR spectral data of compounds 1—3 revealed that the C-2-C-3 double bond of 1 and 2, was oxidized as an epoxide in compound 3. The location of the epoxide was confirmed by the HMBC correlations from H2-14 to C-2, C-3, and C-4 (Fig. 1). The same as that for compound 2, the locations of two acetyl groups at C-4 and C-5 were revealed by the relevant HMBC correlations, as shown in Fig. 1. Thus, the planar structure of 3 was established. The relative stereochemistry of 3 was deduced by the key NOE interactions (Fig. 3). From the NOESY spectrum of 3, H1-13 was found to show NOE interactions with H-4, H-5, H2-15 and one proton (δ 1.97, m) of H1-6, but not with H-2 and H-12, revealing the β-orientation of these protons. Furthermore, H2-2 (δ 2.62, s) exhibited NOE interactions with H1-14, H1-15 and H-12, suggesting the β-orientation of H-2 and H1-14, as revealed by the molecular model in Fig. 3. Thus, the structure of 3 was established unambiguously. Compound 4 was obtained as a colorless oil. The HR-ESI-MS of 1 exhibited a pseudomolecular ion [M+Na]⁺ peak at m/z 315.1573 and established a molecular formula of C17H24O4, implying six degrees of unsaturation. The 13C-NMR and DEPT spectra of 4 displayed 17 carbon signals: four methyls, four methylenes, four methines, and five quaternary carbons. From the 1H- and 13C-NMR spectra of 4, the resonances at δ 170.3 (C), 20.6 (CH3) and δ 2.17 (s) disclosed the presence of an acetyl group. Furthermore, one ketone (δ 201.4), one trisubstituted epoxide [δα 59.6 (CH) 66.1 (C)], and one trisubstituted double bond [δα 142.0 (CH) 126.6 (C)] were also found in the 13C-NMR spectrum. The above functionalities accounted for four of the six degrees of unsaturation, suggesting a bicyclic structure for 4. Comparison of the NMR spectral data of 4 with those of 1—3 disclosed that they should possess the same carbon skeleton. The gross structure of 4 was established by the careful inspection of the 2D NMR (1H-1H COSY and HMBC) correlations as illustrated in Fig. 1. The presence of NOE interactions between H-2 and H1-14 suggested the Z geometry of the 2,3-double bond. The β-oriented H1-13 was found to show NOE correlations with H1-15, H-2 and one proton of H1-7 (δ 2.40, m), but not with H1-12, suggesting the β-orientation of this C-7 attached proton, H1-13, and H1-15. Moreover, H-4 was found to show NOE interactions with H-12 and the α-oriented proton at C-10 (δ 1.94, m), and H-9 also showed an NOE interaction with the α-oriented proton of H1-7 (δ 1.57, m), revealing the α-positions of H-4, H-9 and H-12. Thus, the structure of 4 was established unambiguously. Compound 5 was found to possess the same molecular formula as that of 2 (C19H28O4), as revealed from HR-ESI-MS. The above result, together with the comparison of the 2D NMR spectral data of 5 with those of 2, disclosed that both compounds had similar structures. However, due to the overlap of proton signals in the 1H-NMR spectrum of 5, observed in CDCl3 solution, the NOESY spectrum was recorded in pyridine-d5. In this spectrum H1-13 was found to show NOE correlations with H1-15 and H1-14, but not with H-12 and H-2. Moreover, NOE correlations were observed.
between H-14 and H-4, and H-14 and H-5. Thus, H-13, H-15, H-4, and H-5 were assigned as β-protons. The absence of NOE correlation between H-14 and H-2 suggested the E geometry of 2,3-double bond. By analysis of these NOE interactions and a molecular model as shown in Fig. 5, the relative structure as 5 was confirmed.

Paralemnolin I (6) possesses a molecular formula of C_{19}H_{28}O_{5} as revealed by its HR-ESI-MS and NMR spectral data (Tables 1, 2). Comparison of the 13C-NMR spectral data of 6 with those of 1—5 (Table 2) indicated that these compounds possess similar carbon skeleton. Inspection of the 13C-NMR spectral data of 6 allowed the assignment of a 2,3-double bond [δ 130.6 (C) and 136.7 (CH)] and 8,9-epoxide [δ 68.3 (C), 60.7 (CH)]. Two acetyl groups attached at C-4 and C-5 were confirmed by the HMBC correlations from H-3-14 to C-2, C-3 and C-4, and the COSY correlation between H-4 (δ 5.25) and H-5 (δ 4.84) (Fig. 1). The relative stereochemistry was deduced by the assistance of the NOE correlations observed in a NOESY experiment (Fig. 6). The E geometry was determined due to the absence of the NOE correlation between H3-14 and H-2. In the NOESY spectrum of 6, the β-oriented H-13 was found to show NOE correlations with H-15, H-14 and the β-oriented proton of H-2-7 (δ 1.91, m), but not with H-12. Moreover, NOE correlations were observed between H-14 and H-4, H-14 and H-5, and H-4 and H-5. Thus, the β-orientation of H-15, H-4 and H-5 was suggested. Furthermore, H-2 was found to show an NOE correlation with H-12, while the α-oriented proton of H-2-7 (δ
On the basis of the above results and biogeniceral nardosinanoids isolated from the soft corals of the gen-
served by application of the Mosher’s method, which therefore, the absolute structure of 1 was determined as shown in formula 1. The absolute stereochemistries of sev-
eral nardosinanoids were considered to be identical as illustrated in formula 1—6. Thus, the absolute structures of 1—6 were established.

All metabolites have been submitted for cytotoxicity evaluation toward cancer cell lines, including HepaS9T/VGH (human liver carcinoma), KB (human oral epidermoid carci-
noma), Hela (human cervical epithelial carcinoma), and Daoy (human medulloblastoma). The result showed that compound 2 exhibited moderate cytotoxicity toward Daoy cancer cell line with the ED50 values of 17.5 μM. Other com-

other compounds did not show inhibitory activity against the growth of the above four cancer cell lines.

### Experimental

**General Experimental Procedures** Melting points were determined using a Fisher–Johns melting point apparatus and were uncorrected. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were recorded on a Jasco FT-5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Mercury-Plus 300 FT-NMR at 300 MHz for 1H and 75 MHz for 13C in CDCl3 using TMS as internal standard. LR-ESI-MS and HR-ESI-MS were obtained on a Burker APEX II Mass spectrometer. Precoated silica gel plates (Merek, Kieselgel 60 F-254, 0.2 mm) were used for analytical TLC. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC-10AT apparatus equipped with the Merck Hibar Si-60 column (250×21 mm, 7 μm) or Merek Hibar RP-18e column (250×10 mm, 5 μm).

**Animal Material** The soft coral *Paralemmalia thyrsoides* was collected by hand using scuba at Green Island, which is located off the southeast coast of Taiwan, in July 2004, at a depth of 15 m, and was stored in a freezer until extraction. A voucher specimen was deposited in the Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. GIPT-401).

**Extraction and Isolation** The soft coral (1.8 kg fresh wt) was collected and freeze-dried. The freeze-dried organism was minced and extracted exhaus-

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