A new briarane-type diterpenoid from the South China Sea Gorgonian 
*Dichotella gemmacea*

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One new briarane-type diterpenoid, dichotellide V (1), along with four known 
analogues, gemmacolide N (2), dichotellide J (3), junceelin A (4) and junceellolide A 
(5), was isolated from the South China Sea gorgonian *Dichotella gemmacea*. All of the 
isolated compounds (1–5) were established by comprehensive analysis of the spectral 
data, especially 1D and 2D NMR (HMQC and HMBC) spectra. The cytotoxic activities 
of these compounds were evaluated.

**Keywords:** *Dichotella gemmacea*; gorgonian; briarane-type diterpenoid; cytotoxicity

1. **Introduction**

Briarane-type diterpenoids are a group of highly oxidised secondary metabolites reported from 
marine organisms, particularly from octocorals (Blunt et al. 2011). These kinds of diterpenoids 
are characterised by a bicycle [8.4.0] ring system fused by a lactone group, with high 
oxidisation and esterification by all kinds of acyls, i.e. acetyl and isovalerate (Grote et al. 2006; 
Su et al. 2007; Sun et al. 2011; Lei et al. 2014). Since the first briarane-type diterpenoid, briarane 
A, was isolated from the West Indian gorgonian *Briareum asbestinum* in 1977 (Burks et al. 1977), more than 500 briarane family members have been discovered (Sung et al. 2002, 2005, 
2008). Until now, these kinds of molecules have been continuously reported in an increasing 
number due to the structural complexity and interesting biological activities, such as cytotoxicity
Hoshino et al. 2005; Ishiyama et al. 2008), anti-inflammatory (Shin et al. 1989), antivirus (Subrahmanyam et al. 1998), insecticide (El Sayed et al. 1997), immunomodulation (Hamann et al. 1996) and antifouling (Qi et al. 2006).

In the course of our ongoing screening for biologically active secondary metabolites from marine sources, we made a collection of the gorgonian Dichotella gemmacea off the coast of Meishan Island, Hainan province of China. Early investigation on this species resulted in the isolation and characterisation of a series of new briarane diterpenoids (Li, La, Li, et al. 2011; Li, La, Sun, 2011, 2012; Sun et al. 2011, 2013), fatty acids (Wang et al. 2011) and steroidal glycosides (Jiang et al. 2013). Our continuing studies of the EtOAc-soluble fraction from the EtOH extract of D. gemmacea have now led to the isolation of one new briarane-type diterpenoid, dichotellide V (1), along with four known analogues, gemmacolide N (2) (Li, La, Li, et al. 2011; Li, La, Sun, 2011), dichotellide J (3) (Sun et al. 2013), junceelin A (4) (Garcia et al. 1999) and junceellolide A (5) (Shin et al. 1989). The structures of these diterpenoids were determined by extensive spectroscopic analysis (1H and 13C NMR, DEPT, HSQC, HMBC, NOESY and HR-ESI-MS), aided by the comparison with reported data of related derivatives. Herein we report the isolation, structure elucidation and biological activities of these compounds.

2. Results and discussion

Dichotellide V (1) was obtained as white, amorphous powder. The molecular formula was established as C41H58O16 on the basis of its NMR spectrum and positive HR-ESI-MS data (m/z, 829.3618 [M + Na]+). Its IR and UV spectra indicated the presence of hydroxyl (3427 cm⁻¹), γ-lactone (1794 cm⁻¹), ester carbonyls (1746, 1732 cm⁻¹) and a conjugated diene system (274 nm). The 1H and 13C NMR (DEPT) spectra (Table S1) presented signals for three acetates and three isovalerate moieties, a tertiary methyl (δH 1.14, s), a secondary methyl (δH 1.13, d, J = 7.0 Hz), a γ-lactone (δC 175.2, s), an exocyclic C-11/C-20 epoxide (δH 3.60, 2.94, each d, J = 2.0 Hz; δC 58.3, s, 49.0, t), a conjugated diene (δC 131.5, d; 128.5, d; 139.5, s; 122.8, d; δH 5.63, t, J = 10.0 Hz; 6.33, d, J = 10.0 Hz; 5.73, d, J = 8.5 Hz), an oxymethylene (δC 62.8, t; δH 5.48, 4.63, each d, J = 15.5 Hz), an oxygenated quaternary carbon and six oxygenated methines, which accounted for nine degrees of unsaturations. Since the total degrees of unsaturations are 13, the remaining 4 degrees of unsaturations led us to conclude that 1 must be tetracyclic. The above NMR data showed that 1 was a briarane-type diterpenoid with a 3, 5 (6)-conjugated diene, γ-lactone ring and an exocyclic C-11/C-20 epoxide.

The 1H and 13C NMR spectra of 1 showed great similarity to those of dichotellide J (3), except that three acetate groups at C-12, C-13 and C-16 in 3 were replaced by three isovalerate groups in 1, respectively. This assignment was confirmed by the HMBC correlations of H-12/H-13/H-16 to the corresponding carbonyl group (δC 172.1, 171.7, 171.9) of the isovalerate group, respectively. Accordingly, the established planar structure of 1 was assigned and further supported by the 1H–1H COSY and HMBC spectra (Figure S1). The relative configuration of 1 at the chiral centres was proved to be the same as that of 3 by a NOESY experiment (Figure S2), showing a β configuration of H-7, H-12, H-13, H-14, Me-15, H-17 and CH2-20, and an α configuration of H-2, H-9, H-10 and Me-18. The geometry of the Δ3 double bond was assigned as (Z) based on the proton coupling constant between H-3 and H-4 (J = 10.0 Hz), while that of Δ5 double bond was determined as (E) due to the NOESY correlation between H-6 and H-2-16 (Figure S2). Thus, the relative configuration of 1 was determined as 1R*, 2R*, 3Z, 5E, 7S*, 8R*, 9S*, 10S*, 11R*, 12R*, 13R*, 14R* and 17R* (Figure 1).

The four known compounds gemmacolide N (2), dichotellide J (3), junceelin A (4) and junceellolide A (5) were also isolated and identified by using their NMR, MS data and comparing their experimental data with those reported in the literature.
All the isolated briarane-type diterpenoids were tested for their cytotoxicity against six human tumour cell lines (H1975, U937, BGC823, MCF-7, A549 and Hela), using the CCK8 method (Wanka et al. 2013). However, none of the compounds exhibited cytotoxic effects on the tested cancer cell lines (IC$_{50} > 100$ μM). Previous studies found that the number and the location of isovaleryl group substitution may increase and decrease the cytotoxicity, respectively (Li, La, Li, et al. 2011; Li, La, Sun, 2011; Li et al. 2013). For example, according to the results of Li et al. (2013), gemmacolide AF which has isovaleryl group at C-12 and acetyl group at C-13 showed cytotoxic activities against A549 and MG63 cells, but gemmacolide AG which has acetyl group at both C-12 and C-13 did not show any cytotoxic activities. They presented that the 12-O-isovalerate may show the positive contribution to the activity. Comparing the results of dichtellide V with these analogues, differentially functionalised group at C-12 and C-13 might be one of the factors to show cytotoxicity. This observation may encourage further investigations on briaranes and the clear structure–activity relationships.

3. Experimental

3.1. General experimental procedures

Optical rotation values were measured with a PerkineElmer 341 polarimeter (Rudolph Research Analytical, Hackettstown, NJ, USA). IR spectra were measured on JASCO FT/IR-480 plus spectrometers (JASCO Ltd, Tokyo, Japan). UV spectra were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Kyoto, Japan). NMR spectra were collected using Bruker 500 MHz NMR spectrometers (Bruker BioSpin, Fällanden, Switzerland) with TMS as an internal standard at room temperature (δ in parts per million, J in Hertz). HR-ESI-MS (including ESI-MS) spectra were recorded on an Applied Biosystems Mariner 5140 spectrometer (Life Technologies Ltd., Grand Island, NY, USA). Column chromatography (CC) was applied on the Buchi Sepacore (C-615/605) system. All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). Silica gel and preparative thin layer chromatography (TLC) plates (20 cm × 20 cm × 0.04 cm) (Qingdao Mar. Chem. Ind. Co. Ltd), Sephadex LH-20 gel...
(Amersham Biosciences, Uppsala, Sweden) and C₁₈ reverse-phased silica gel (150–200 mesh, Merck) were used for chromatography.

3.2. Animal material
The gorgonian *D. gemmacea* were collected from Meishan Island, Hainan province of China in April 2009 (7–10 m depth) and identified by Professor Hui Huang, South China Sea Institute of Oceanology, Chinese Academy of Sciences. A voucher specimen (No. M090405) has been deposited in the Key Laboratory of Marine Bio-resources Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China.

3.3. Extraction and isolation
The fresh gorgonian *D. gemmacea* (ca. 4.0 kg) was exhaustively extracted with 95% EtOH twice and CHCl₃/MeOH (1:1) once at room temperature. After evaporation of the solvent in vacuum, the combined residue was suspended in H₂O and partitioned with EtOAc and n-BuOH to provide the EtOAc extract (18.0 g) and the n-BuOH extract (5.0 g). The EtOAc extract was chromatographed by silica gel CC (300–400 mesh), eluting with a gradient of petroleum ether (PE)/Me₂CO (50:1 to 0:100), to obtain 15 fractions (Fr.1–Fr.15). Fr.6 (2.8 g) was divided into five subfractions (Fr.6-1–Fr.6-5) by medium pressure liquid chromatography eluting with PE/EtOAc (90:10 to 0:100). Fr.6-4 was further applied on silica gel CC, eluted using hexane/EtOAc, to afford compound 5 (8.7 mg). Fr.6-3 was subjected to CC over ODS (MeOH/H₂O, 30:70 to 100:0) and LH-20 (CHCl₃/MeOH, 1:1), followed by repeated preparative TLC (one plate, 20 cm × 20 cm × 0.05 cm) using CHCl₃/MeOH (20:1) and/or n-hexane/EtOAc (1:1) leading to the isolation of compounds 1 (9.0 mg) and 3 (15.0 mg). Fr.6-5 was subjected to CC on silica gel, eluted with CHCl₃/Me₂CO (20:1 to 6:1) to give three subfractions Fr.6-5a–Fr.6-5c; Fr.6-5a was further applied on silica gel CC, eluted using hexane/EtOAc, to afford compound 2 (7.2 mg). A further isolation of Fr.6-5c yielded compound 4 (6.3 mg) by preparative TLC with PE/EtOAc (1:1) as developer.

3.3.1 Dichotellide V (1)
White amorphous powder; [α]²⁰D −11.3 (c = 0.55, acetone); IR (KBr) νmax 3418, 1780, 1734, 1252 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz), δ 6.33 (1H, d, J = 10.0 Hz, H-4), 5.73 (1H, d, J = 8.5 Hz, H-6), 5.72 (1H, d, J = 10.0 Hz, H-2), 5.63 (1H, t, J = 10.0 Hz, H-2), 5.48 (1H, d, J = 15.5 Hz, H-16a), 5.18 (1H, d, J = 2.5 Hz, H-14), 5.09 (1H, t, J = 3.0 Hz, H-13), 4.96 (1H, d, J = 8.8 Hz, H-7), 4.91 (1H, d, J = 2.0 Hz, H-12), 4.74 (1H, d, J = 4.5 Hz, H-7), 4.63 (1H, d, J = 15.5 Hz, H-16b), 3.60 (1H, d, J = 2.0 Hz, H-20a), 3.60 (1H, d, J = 4.5 Hz, H-10), 2.94 (1H, d, J = 2.0 Hz, H-20b), 2.32 (2H, m, OCOCH₂CH(CH₃)₂), 2.29 (1H, q, J = 7.0 Hz, H-17), 2.28 (2H, m, OCOCH₂CH(CH₃)₂), 2.24 (2H, m, OCOCH₂CH(CH₃)₂), 2.19 (3H, s, OAc), 2.11 (3H, s, OAc), 2.07 (1H, m, OCOCH₂CH(CH₃)₂), 2.05 (3H, s, OAc), 1.99 (1H, m, OCOCH₂CH(CH₃)₂), 1.97 (1H, m, OCOCH₂CH(CH₃)₂), 1.14 (3H, s, H-15), 1.13 (3H, d, J = 7.0 Hz, H-18), 0.99 (3H, d, J = 7.0 Hz, OCOCH₂CH(CH₃)₂), 0.98 (3H, d, J = 7.0 Hz, OCOCH₂CH(CH₃)₂), 0.91 (6H, d, J = 6.5 Hz, OCOCH₂CH(CH₃)₂), 0.90 (6H, d, J = 7.0 Hz, OCOCH₂CH(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz), see Table S1; HR-ESI-MS m/z 829.3618 [M + Na]⁺ (cald for C₄₄H₅₈O₁₆Na, 829.3623).

3.4. Cytotoxicity assay
Cytotoxicity was assayed with the CCK8 (Dojindo, Japan) method. Cell lines H1975, U937, BGC823, MCF-7, A549 and Hela were purchased from Shanghai Cell Bank, Chinese Academy
of Sciences. Cells were routinely grown and maintained in mediums RPMI or DMEM with 10% FBS and with 1% penicillin/streptomycin. All cell lines were incubated in a Thermo/Forma Scientific CO₂ Water Jacketed Incubator with 5% CO₂ in air at 37°C. Cell viability assay was determined by the CCK8 (Dojindo, Japan) assay. Cells were seeded at a density of 400–800 cells/well in 384-well plates and treated with various concentrations of compounds or solvent control. After 72 h incubation, CCK8 reagent was added, and absorbance was measured at 450 nm using Envision 2104 multi-label Reader (Perkin Elmer, USA). Dose response curves were plotted to determine the IC₅₀ values using Prism 5.0 (Graphpad Software Inc., USA).

4. Conclusions
The investigation of bioactive natural products from the EtOH extract of D. gemmacea has led to the isolation of one new briarane-type diterpenoid, dichotellide V (1), along with four known analogues, gemmacolide N (2), dichotellide J (3), junceelin A (4) and junceellolide A (5). All compounds were tested in vitro for cytotoxic activity against six human tumour cell lines by the CCK8 (DOjinDo, Kumamoto, Japan) method. However, none of the compounds exhibited cytotoxic effects on the tested cancer cell lines (IC₅₀(100 μM)).

Supplementary material
Supplementary material relating to this article is available online, alongside Tables S1–S2 and Figures S1–S7.

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