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Bioactive briarane diterpenoids from the South China Sea gorgonian *Dichotella gemmacea*

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ABSTRACT

Six new briarane diterpenoids, gemmacolides T–Y (**1**–**6**), were isolated together with three known analogs, juncenolide J (**7**), praelolide (**8**), and junceellolide C (**9**), from the South China Sea gorgonian *Dichotella gemmacea*. The structures of the new compounds were elucidated by detailed spectroscopic analysis and comparison with reported data. The absolute configuration was suggested based on biosynthetic considerations. In an in vitro bioassay, compounds **3** and **6** showed potent growth inhibition towards tumor cell lines of A549 and MG63, being stronger than the positive control of adriamycin. These compounds also exhibited weak antimicrobial activity against the bacterium *Escherichia coli* and the fungi *Microbotryum violaceum* and *Septoria tritici*.

Briarane-type diterpenoids are a group of highly oxidized secondary metabolites reported from marine organisms, particularly from octocorals.[1] This kind of molecules is proposed to be derived from cembrane diterpenoids by 3,8-cyclization, constructing of a fused six- and ten-membered bicyclic carbon skeleton, and characterized by an additional γ-lactone ring fused with the ten-membered ring.[2] These metabolites are reported to have a wide spectrum of interesting biological activities, including cytotoxic, anti-inflammatory, antiviral, antifouling, insecticidal and immunomodulatory effects.[1–3]

In the course of our ongoing screening for biologically active secondary metabolites from marine sources,[4–9] we made a collection of the gorgonian *Dichotella gemmacea* off the coast of Beihai, China. Chemical investigation on the species led to the isolation and structure elucidation of thirteen new briaranes, namely gemmacolides G–S, together with six known analogs, juncin O, junceellolide C, juncenolide D, and juncins R, S and U.[10,11] The absolute configuration of gemmacolide N was determined by a TDDFT calculation of its solution ECD spectrum. The determination of absolute configuration of other 11,20-epoxy-(3*Z*,5(6)*E*)-diene briarane analogs could then be achieved by comparison of their ECD spectra.[11]

In in vitro bioassays, these compounds exhibited different levels of growth inhibition activity against A549 and MG63 cells. In particular, gemmacolide J was more active than the positive control adriamycin against A549 cells,[10] demonstrating a potent activity in tumor cell growth inhibition. The interesting result encourages a systematic study on briarane diterpenoids concerning their chemistry and bioactivities. Our continuous investigation on the new collection of gorgonian *D. gemmacea* led to the isolation and structure elucidation of six new briaranes, namely gemmacolides T–Y (**1**–**6**), together with three known analogs, juncenolide J (**7**),[12] praelolide (**8**),[13] and junceellolide C (**9**) (Fig. 1).[14] The structures of the new compounds were elucidated by detailed analysis of spectroscopic data and comparisons with reported data. The isolated new compounds were tested in vitro for their antimicrobial and tumor cell growth inhibition activities.[15,16] We herein report on the isolation, structure elucidation, and bioactivities of these compounds.

Freshly collected specimens of *D. gemmacea* were immediately frozen to −20 °C and stored at this temperature before extraction. Frozen material was cut into little pieces and subsequently extracted with acetone. The acetone extract was evaporated and the residue was partitioned between EtOAc and H_2O. The EtOAc extract was subjected to sequential column chromatography on silicagel, Sephadex LH-20, and the RP-HPLC to afford night pure diterpenoids (**1**–**9**). Juncenolide J (**7**), praelolide (**8**), and junceellolide C (**9**) were previously reported from the South China Sea

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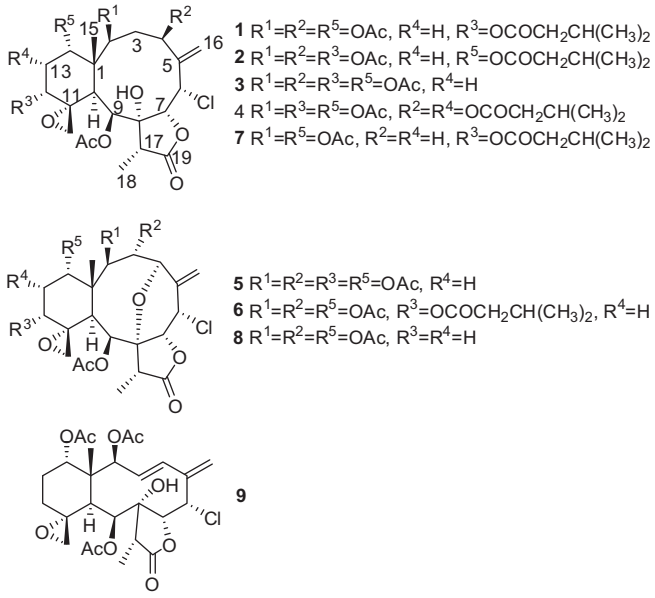

Figure 1. Structures of compounds **1**–**9**.

gorgonian *Junceella juncea*[12,14] and *Plexaureides praelonga*,[13] respectively. Junceellolide C (**9**) was also obtained from the earlier collection of the title animal.[10] The structures and relative stereochemistry of the known compounds were determined by extensive spectroscopic analysis and careful comparison with reported data.

Gemmacolide T (**1**) was isolated as a white amorphous powder and exhibited a molecular formula of $C_{33}H_{45}ClO_{14}$ as deduced from its NMR spectrum and HRESIMS data. An isotopic ration of 3:1 observed in the molecular ion peaks at *m*/*z* 723/725 $[M+Na]^+$ confirmed the presence of a chlorine atom in the molecule. The IR spectrum showed absorption bands of hydroxy (3368 cm^{-1}), γ-lactone (1786 cm^{-1}) and ester (1736 cm^{-1}) functionalities. This observation was in agreement with the signals in the ^{13}C NMR and DEPT spectra (Table 1) for 8 sp^2 carbon atoms ($6 \times OC{=}O$, $CH_2{=}C$) at lower field and 25 sp^3 carbon atoms at higher field ($2 \times OC$, $6 \times OCH$, $1 \times OCH_2$, $1 \times CHCl$, $1 \times C$, $3 \times CH$, $3 \times CH_2$, $8 \times CH_3$), accounting for seven double bond equivalents. The remaining double bond equivalents could be assigned to four rings in the molecule.

The ^1H and ^{13}C NMR spectra of **1** were almost identical to those of juncenolide *J* (**7**),[12] except for the appearance of signals for an additional acetyl moiety (δ_C 169.3, C and 21.1, CH_3; δ_H 2.05, s). The location of acetylation at C-4 was evident due to the significantly downfield shift of H-4 from δ (2.42–2.47) to δ 5.43 in the acetate-**7**. The assignment was confirmed by the diagnostic HMBC correlation from H-4 to the carbonyl atom at δ_C 169.3. The isovaleryl group remained the same at C-12 due to the diagnostic HMBC correlation from H-12 (δ 4.60) to δ_C 171.6 and the NOE cross peak between H-12 and δ_H 2.10. The established planar structure of **1** was further supported by the ^1H–^1H COSY and HMBC spectra as shown in Figure 2. The relative configuration of **1** at C-1, C-2, C-6, C-7, C-8, C-9, C-10, C-11, C-12, C-14, and C-17 was proven in the same way as those of **7** by a NOESY experiment (Fig. 3), showing a β orientation of H-6, H-7, H-12, H-14, Me-15, H-17, and H_2-20, and an α orientation of H-2, H-9, H-10, Me-18 and 8-OH. The diagnostic NOE correlations of H-4 with H-2, and H-16b, and H-16a with 8-OH indicated the α orientation of H-4. Thus, the relative configuration of **1** was determined as 1*S**,2*S**,4*R**,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,12*R**,14*S**,17*R**. The absolute configuration of **1** is suggested as (1*S*,2*S*,4*R*,6*S*,7*R*,8*R*,9*S*,10*S*,11*R*,12*R*,14*S*,17*R*)-**1** due to the established absolute configuration of gemmacolide N, an analog isolated from the same species of animals with its absolute stereochemistry being determined by a TDDFT/ECD approach.[11]

Gemmacolide U (**2**), a white amorphous powder, showed the same molecular formula of $C_{33}H_{45}ClO_{14}$ as that of **1** as deduced from its HRESIMS. ^1H and ^{13}C NMR spectroscopic data of **2** were almost identical to those of **1** (Tables 1 and 2), showing the same functional groups for both compounds. However, based on the analysis of HMBC spectra, the isovaleryl group was found to be attached to C-14 in **2** instead of C-12 in **1**. The structure of **2** had the same relative stereochemistry as that of **1** assessed by the NOESY measurements.

Gemmacolide V (**3**) was obtained as a white amorphous powder. The molecular formula $C_{30}H_{39}ClO_{14}$ was established by HRESIMS. Comparison of overall ^1H and ^{13}C NMR data (Tables 1 and 2) of **3** with those of **1** revealed great similarity. However, the isovaleryl group in **1** was replaced by an acetyl groups in **3**. The relative configuration of all the asymmetric centers remained intact, which was supported by a NOESY experiment.

Gemmacolide W (**4**) was isolated as a white amorphous powder with a molecular formula of $C_{38}H_{53}ClO_{16}$ as established by HRESIMS. The structure of **4** differed from that of **1** only by the presence of an additional isovaleryl group rather than an hydrogen in the molecule (Tables 1 and 2). The HMBC correlation of H-4 and H-13 with the carbonyl atom of two isovaleryl groups led the location of these substitutions at C-4 and C-13. The four acetyl groups were thus assigned to C-2, C-9, C-12, and C-14, which was supported by the ^1H–^1H COSY and HMBC experiments. A distinct NOE correlation between H-13 and Me-15 indicated a β configuration of these protons. The relative configuration of **4** was thus determined as 1*S**,2*S**,4*R*,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,12*R**,13*R**,14*R**, 17*R** based on the NOESY spectrum.

Gemmacolide X (**5**) was found to be a white amorphous powder, having the molecular formula of $C_{30}H_{37}ClO_{14}$ based on the HRESIMS data. The ^1H NMR spectrum of **5** showed great similarity to that of praelolide (**8**),[13] except for the presence of an additional signal at δ 2.05 (s) (Table 1), which suggested the presence of an additional acetyl group in **5**, consistent with two carbon resonances at δ 169.4 (C) and 21.0 (CH_3) in its ^{13}C NMR spectrum (Table 1). The established planar structure of **5** was further supported by the ^1H–^1H COSY and HMBC spectra (Fig. 4). The NOESY experiment of **5** (Fig. 5) revealed an α orientation of H-2, H-9, and H_3-18 due to the correlations of H-10 with H-2 and H-9. The NOESY correlations of H-15 with H-3, H-14, H-20 and 9-OAc, H-20 with H-12, H-4 with H-6, and H-7 with H-17 were in agreement with the β orientation of these protons. Consequently, the relative configuration of **5** was proven to be 1*R**,2*R**,3*S**,4*R**,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**, 12*R**,14*S**,17*R**.

Gemmacolide Y (**6**) was isolated as a white amorphous powder. The molecular formula $C_{33}H_{43}ClO_{14}$ was established by HRESIMS. Comparison of overall ^1H and ^{13}C NMR data (Tables 1 and 2) of **6** with those of **5** revealed great similarity. However, one of the acetyl groups in **5** was replaced by an isovaleryl group in **6**. The location of the isovaleryl group was supported by the obvious HMBC correlations of both H-12 and H-2′ (δ_H 2.15) with C-1′ (δ_C 171.7). The relative configuration of all the asymmetric centers remained intact, which was supported by a NOESY experiment.

The absolute configurations of **2**–**6** were suggested as depicted above based on isolation from the same source and unambiguous determination of the absolute configuration of gemmacolide N.[11]

The antimicrobial and tumor cell growth inhibition activities of the new compounds were evaluated.[14] In in vitro bioassays, compounds **1**–**8** exhibited potential growth inhibition against tumor cell lines with IC_{50} values of 16.9, 18.0, <1.5, 19.1, >45.7, <0.3, >46.7, >50.1 μM for A549 cells, and 18.0, 15.1, 20.5, 17.4, >45.7,