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NOTE

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Bioactive 11,20-Epoxy-3,5(16)-diene Briarane Diterpenoids from the South China Sea Gorgonian *Dichotella gemmacea*

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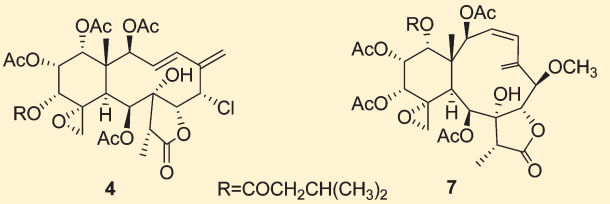
Supporting Information

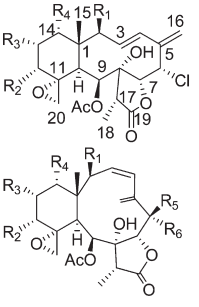
ABSTRACT: Seven new briarane diterpenoids, gemmacolides G−M (**1**−**7**), were isolated together with two known analogues, juncin O and junceellolide C, from the South China Sea gorgonian *Dichotella gemmacea*. The structures of the new compounds were elucidated by detailed analysis of spectroscopic data and comparison with reported data. In an *in vitro* bioassay, these compounds exhibited different levels of growth inhibition activity against A549 and MG63 cells. In particular, compound **4** was more active than the positive control adriamycin against A549 cells. Compounds **4** and **7** also exhibited weak antimicrobial activity against the bacterium *Bacillus megaterium* and the fungus *Septoria tritici*, respectively.



Gorgonians of the family Ellisellidae (Gorgonaceae) are well known to be a rich source of several closely related briarane diterpenoids. The briarane diterpenoids isolated from this family are characterized by 11,20-epoxy, 8-hydroxy, and 3,5(6)-diene/or 3,5(16)-diene with 6-chlorine functionalities, displaying various biological activities, such as cytotoxic, anti-inflammatory, antiviral, antifouling, insecticidal, and immunomodulatory effects.[1−3] Chemical investigation of the genus *Dichotella* has only been reported for the species *Dichotella gemmacea*, which was identified previously as *Junceella gemmacea*. To date, 18 briarane diterpenoids have been isolated and characterized, displaying similar chemical features to those from other gorgonians of the family Ellisellidae.[4−6] Among them, dichotellide C exhibited marginal activity against SW1990 cells (human pancreatic cancer).[6]

In the course of our ongoing screening for biologically active secondary metabolites from marine sources,[7−10] we investigated the gorgonian *D. gemmacea*, collected from the South China Sea, leading to the isolation and structure elucidation of seven new 11,20-epoxy-3,5(16)-diene briaranes, namely, gemmacolides G−M (**1**−**7**), together with two known analogues, juncin O (**8**)[11] and junceellolide C(**9**).[12] 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. We herein report on the isolation, structure elucidation, and bioactivities of these compounds.

Freshly collected specimens of *D. gemmacea* were immediately frozen and stored at −20 °C before extraction. The EtOAc-soluble portion of the acetone extract was partitioned between MeOH and *n*-hexane. The MeOH extract was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and RP-HPLC to afford nine pure diterpenoids (**1**−**9**). The structures of the known compounds were determined as juncin O (**8**) and junceellolide C (**9**) by extensive spectroscopic analysis combined with careful comparisons with the reported spectroscopic data. These two briaranes were previously reported from the South China Sea gorgonians *Junceella juncea*[11] and *J. fragilis*,[12] respectively.



1 R_1=R_2=R_3=R_4=OAc
2 R_1=R_2=R_4=OAc, R_3=H
3 R_1=R_4=OAc, R_2=$OCOCH_2CH(CH_3)_2$, R_3=H
4 R_1=R_3=R_4=OAc, R_2=$OCOCH_2CH(CH_3)_2$
8 R_1=R_2=R_4=OAc, R_3=$OCOCH_2CH(CH_3)_2$
9 R_1=R_4=OAc, R_2=R_3=H

5 R_1=$OCOCH_2OH$, R_2=$OCOCH_2CH(CH_3)_2$, R_3=R_4=OAc, R_5=H, R_6=Cl
6 R_1=$OCOCH_2OCOCH_2CH(CH_3)_2$, R_2=$OCOCH_2CH(CH_3)_2$, R_3=R_4=OAc, R_5=H, R_6=Cl
7 R_1= R_2=R_3=OAc, R_4=$OCOCH_2CH(CH_3)_2$, R_5=OCH_3, R_6=H

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Table 1. ^{13}C NMR Data for Gemmacolides G−M (1−7)[a]

position	1[b]	2[b]	3[c]	4[d]	5[d]	6[d]	7[d]
1	48.3, C	49.1, C	49.3, C	48.3, C	47.1, C	46.9, C	47.6, C
2	75.5, CH	75.8, CH	76.0, CH	75.5, CH	71.9, CH	71.8, CH	71.3, CH
3	129.7, CH	130.4, CH	130.6, CH	130.1, CH	129.1, CH	129.3, CH	129.3, CH
4	133.4, CH	132.7, CH	132.9, CH	133.7, CH	129.3, CH	129.0, CH	128.4, CH
5	141.9, C	142.1, C	142.2, C	141.9, C	136.9, C	136.8, C	139.1, C
6	65.0, CH	65.0, CH	65.1, CH	65.0, CH	64.3, CH	64.2, CH	84.1, CH
7	80.4, CH	80.6, CH	80.9, CH	80.5, CH	78.7, CH	78.7, CH	82.2, CH
8	82.7, C	82.9, C	83.1, C	82.8, C	80.0, C	80.0, C	80.2, C
9	72.5, CH	72.2, CH	72.4, CH	72.5, CH	76.2, CH	75.8, CH	66.4, CH
10	33.6, CH	33.8, CH	34.1, CH	33.7, CH	32.8, CH	32.9, CH	32.3, CH
11	56.3, C	57.3, C	57.4, C	56.3, C	56.8, C	56.9, C	58.6, C
12	73.7, CH	73.8, CH	73.5, CH	73.3, CH	72.8, CH	72.8, CH	73.7, CH
13	66.5, CH	29.0, CH_2	29.5, CH_2	66.4, CH	66.5, CH	66.5, CH	66.6, CH
14	73.0, CH	73.1, CH	73.4, CH	73.0, CH	72.9, CH	72.8, CH	73.3, CH
15	14.7, CH_3	14.4, CH_3	14.7, CH_3	14.7, CH_3	14.6, CH_3	14.7, CH_3	14.4, CH_3
16	115.2, CH_2	115.1, CH_2	115.5, CH_2	115.3, CH_2	116.9, CH_2	117.1, CH_2	119.5, CH_2
17	50.2, CH	50.0, CH	50.3, CH	50.3, CH	48.6, CH	48.5, CH	46.8, CH
18	7.0, CH_3	6.9, CH_3	7.3, CH_3	7.1, CH_3	8.7, CH_3	8.7, CH_3	6.5, CH_3
19	174.5, C	174.6, C	174.8, C	174.5, C	175.6, C	175.6, C	174.9, C
20	49.3, CH_2	49.2, CH_2	49.5, CH_2	49.4, CH_2	49.8, CH_2	49.7, CH_2	49.2, CH_2
OCH_3							56.5, CH_3
9-OAc	170.2, C	170.3, C	170.5, C	170.2, C	169.6, C	169.6, C	170.3, C
	20.7, CH_3	21.4, CH_3	21.6, CH_3	20.9, CH_3	21.0, CH_3	21.0, CH_3	21.5, CH_3
R_1	170.1, C	170.0, C	170.1, C	170.2, C	171.6, C	166.3, C	170.1, C
	21.3, CH_3	21.1, CH_3	21.3, CH_3	21.3, CH_3	61.3, CH_2	61.3, CH_2	21.3, CH_3
						172.4, C	
						42.8, CH_2	
						25.7, CH	
						22.3, CH_3	
						22.3, CH_3	
R_2	169.4, C	169.6, C	172.0, C	171.7, C	171.6, C	171.6, C	169.5, C
	20.8, CH_3	20.9, CH_3	43.8, CH_2	43.4, CH_2	43.6, CH_2	43.6, CH_2	21.0, CH_3
			25.7, CH	25.6, CH	25.8, CH	25.8, CH	
			22.7, CH_3	22.4, CH_3	22.4, CH_3	22.4, CH_3	
			22.7, CH_3	22.4, CH_3	22.4, CH_3	22.4, CH_3	
R_3	169.7, C	n.o.	n.o.	169.7, C	169.6, C	169.6, C	169.8, C
	20.5, CH_3			20.5, CH_3	20.5, CH_3	20.5, CH_3	20.6, CH_3
R_4	170.0, C	169.9, C	170.2, C	170.2, C	170.9, C	170.5, C	172.2, C
	21.4, CH_3	21.1, CH_3	21.4, CH_3	21.4, CH_3	21.0, CH_3	20.9, CH_3	43.4, CH_2
							25.2, CH
							22.5, CH_3
							22.5, CH_3

[a] In $CDCl_3$, assignments made by DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC. [b] Measured at 150 MHz. [c] Measured at 125 MHz. [d] Measured at 100 MHz.

Gemmacolide G (**1**) was isolated as a white, amorphous powder and exhibited a molecular formula of $C_{30}H_{37}ClO_{14}$, as deduced from its NMR spectrum and HRESIMS data. An isotopic ratio of 3:1 observed in the molecular ion peaks at m/z 679/681 $[M + Na]^+$ confirmed the appearance of a chlorine atom in the molecule. The IR spectrum showed absorption bands of hydroxy (3448 cm^{-1}), γ-lactone (1780 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 10 sp^2 carbon atoms (6 × OC═O, CH═CH, CH_2═C) at lower field and 20 sp^3 carbon atoms at higher field (2 × OC, 6 × OCH, 1 × OCH_2, 1 × CHCl, 1 × C, 2 × CH, 7 × CH_3), accounting for eight double-bond equivalents. The remaining double-bond equivalents were due to the presence of four rings in the molecule.

The ^{1}H and ^{13}C NMR spectra of **1** showed great similarity to those of juncin O (**8**),[11] except that the isovaleryl group in **8** was replaced by an acetyl group in **1**. The established planar structure of **1** was further supported by the ^{1}H−^{1}H COSY and HMBC spectra (Figure S1). The relative configuration of **1** at the stereogenic centers was proven to be the same as that of **8** by a NOESY experiment (Figure 1), showing a β-orientation of H-7, H-12, H-13, H-14, Me-15, H-17, and H_2-20 and an α-orientation

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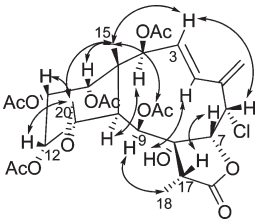

Figure 1. Key NOESY correlations for compound **1**.

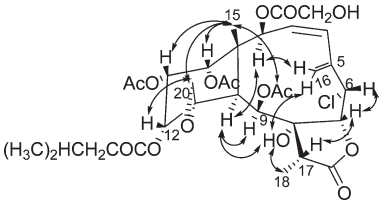

Figure 2. Key NOESY correlations for compound **5**.

of H-2, H-9, H-10, Me-18, and 8-OH. The geometry of the Δ^3 double bond was assigned as *E* on the basis of the large coupling constant (J = 16.0 Hz) between H-3 and H-4. Thus, the relative configuration of **1** was determined as 1*S**,2*S**,3*E*,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,12*R**,13*R**,14*R**,17*R**.

Gemmacolide H (**2**), a white, amorphous powder, had the molecular formula $C_{28}H_{35}ClO_{12}$ on the basis of its HRESIMS. Its ^{1}H and ^{13}C NMR data (Table 1) showed great similarity to those of **1** except that one of the acetoxy groups in **1** (δ_H 1.96, δ_C 169.7 and 20.5) was replaced by a hydrogen in **2**. This proton was assigned as part of CH_2-13 (δ_H 2.31, 2.04) due to the proton sequence of H-12/H_2-13/H-14, established by the ^{1}H−^{1}H COSY experiment. The determination of the structure of **2** was further supported by detailed analysis of its 2D NMR data, and thus its relative configuration was assigned as 1*R**,2*S**,3*E*,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,12*R**,14*S**,17*R**.

Gemmacolide I (**3**) was obtained as a white, amorphous powder with the molecular formula $C_{31}H_{41}ClO_{12}$ being established by HRESIMS. The structure of **3** differed from that of **2** only by the presence of an isovaleryl group rather than an acetyl group at C-12 (Table 1). The assignment of the isovalerate ester at C-12 was based on HMBC correlations from both H-12 and H-2′ (δ_H 2.17) to the isovaleryl carbonyl carbon. Its relative configuration was proven the same as that of **2** on the basis of a NOESY experiment.

Gemmacolide J (**4**) was isolated as a white, amorphous powder. The molecular formula $C_{33}H_{43}ClO_{14}$ was established by HRESIMS. Comparison of the overall ^{1}H and ^{13}C NMR data (Table 1) of **4** with those of **1** revealed great similarity. However, one of the acetyl groups in **1** was replaced by an isovaleryl group in **4**. The location of the isovaleryl group was supported by the obvious HMBC correlations of both H-12 and H-2′ (δ_H 2.22) 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 complete NMR data assignments of analogues **1**−**4** require that the reported ^{13}C NMR values for C-7 (δ_C 72.5) and C-9 (δ_C 80.5) in juncin O are interchanged, whereas the ^{1}H NMR values reported for both oxygenated methines were assigned correctly.[11] Moreover, the reported H-6 value of δ_H 5.29 in juncin O should be reassigned to δ_H 5.06 (see SI). Inspection of the experimental (see SI) and reported data of junceellolide C (**9**)[12] further supported the above conclusion.

Gemmacolide K (**5**) was a white, amorphous powder. Its molecular formula was established as $C_{33}H_{44}ClO_{15}$ by HRESIMS. The ^{1}H and ^{13}C NMR spectra of **5** showed similarity to those of compound **1**. However, the ^{1}H NMR data of **5** indicated the *Z* geometry of the Δ^3 double bond (J = 11.4 Hz) instead of the *E* geometry in **1**. Two acetyl groups at C-2 and C-12 in **1** were replaced by a glycolyl group and an isovaleryl group in **5**, respectively. The assignments were supported by the HMBC correlations of both H-2′ (δ_H 4.16, 4.01) and H-2 (δ_H 6.33) with C-1′ (δ_C 171.6) and by both H-12 (δ_H 4.92) and H-2″ (δ_H 2.26, 2.20) with the isovaleryl carbonyl carbon (δ_C 171.6) (Table 1). The NOESY experiment of **5** (Figure 2) revealed an α-orientation of H-2, H-9, OH-8, and H_3-18 due to the correlations of H-10 with H-2, H-9, and OH-8, and OH-8 with H-16a and H_3-18. The NOESY correlations of H-15 with H-13, H-14, H-20, and 9-OAc, H-20 with H-12, and H-7 with H-6 and H-17 were in agreement with the β-orientation of these protons. Consequently, the relative configuration of **5** was proven to be 1*S**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,11*R**,12*R**,13*R**,14*R**,17*R**.

Gemmacolide L (**6**) was found to be a white, amorphous powder, having the molecular formula $C_{38}H_{51}ClO_{16}$ based on the HRESIMS data. The ^{1}H and ^{13}C NMR data of **6** greatly resembled those of **5**. The primary alcohol of the glycolyl group, however, was further esterified with isovaleric acid. This conclusion was proven by the long-range correlation from both H_2-2′ (δ_H 4.16, 4.01) and H_2-4′ (δ_H 2.30) to C-3′ (δ_C 172.4) and from both H-2 and H_2-2′ (δ_H 4.16, 4.01) to C-1′ (δ_C 166.3). The relative configuration of all the stereogenic centers remained intact, which was supported by a NOESY experiment.

Gemmacolide M (**7**) was a white, amorphous powder. Its molecular formula was established as $C_{34}H_{46}O_{15}$ by HRESIMS, revealing the absence of the chlorine in contrast to those of **1**−**6**. The C-6 chlorine in **1**−**6** was replaced by a methoxy group in **7** (Table 1), which was confirmed by the HMBC correlation from the OMe (δ_H 3.38, s) to C-6 (δ_C 84.1, CH). Detailed analysis of the ^{1}H−^{1}H COSY and HMBC spectra clarified the isovaleryl group at C-14 and the four acetyl groups at C-2, C-9, C-12, and C-13, respectively. The configurations at these carbons were proven to be the same as those of **5** and **6** due to a NOESY experiment (Figure 3). Also, the *Z* geometry of the Δ^3 double bond in **7** was the same as those of **5** and **6** on the basis of the coupling constant (J = 11.3 Hz) between H-3 and H-4. However, the configuration of H-6 was assigned as α instead of β on the basis of the diagnostic NOESY correlation of H-6 with H-2 and H-10 (Figure 3). The relative configuration of **7** was therefore determined as 1*S**,2*S**,6*R**,7*S**,8*R**,9*S**,10*S**,11*R**,12*R**,13*R**,14*R**,17*R**.

The antimicrobial and tumor cell growth inhibition activities of the new compounds were evaluated. In *in vitro* bioassays, compounds **1**−**5** exhibited potential growth inhibition against tumor cell lines with IC_{50} values of 8.4, 47.3, 20.6, <1.4, and 38.2 μM for A549 cells, and 38.4, 54.0, 25.0, 79.8, and 45.9 for MG63 cells, respectively. Compound **7** showed some activity only against A549 (IC_{50} 27.4 μM). It is very interesting to observe that compound **4** displayed stronger activity than those of analogues **1**−**3** and also the positive control (adriamycin, IC_{50} 2.8 μM). The interesting discovery may encourage further investigations on briaranes and their activity of tumor cell growth inhibition.

In addition, compound **4** exhibited weak antibacterial activity against the Gram-negative bacterium *Bacillus megaterium*

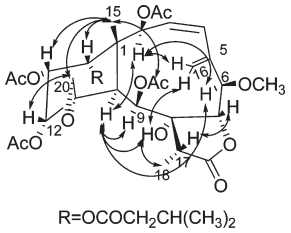

Figure 3. Key NOESY correlations for compound **7**.

(Φ = 16.0 mm), while compound **7** displayed weak antifungal activity against *Septoria tritici* (Φ = 15.0 mm) in the antimicrobial biotest. Compounds **1**, **2**, **3**, **5**, **8**, and **9** were inactive.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ on an Autopol IV polarimeter at the sodium D line (590 nm). UV absorption spectra were recorded on a Varian Cary 100 UV−vis spectrophotometer; peak wavelengths are reported in nm. Circular dichroism (CD) spectra were recorded on a JASCO J-715 CD spectropolarimeter. Infrared spectra were recorded in thin polymer films on a Nexus 470 FT-IR spectrophotometer (Nicolet); peaks are reported in cm^{-1}. The NMR spectra were recorded at 300 K on Bruker DRX 400 and Avance 600 spectrometers. Chemical shifts are reported in parts per million (δ), with use of the residual $CDCl_3$ signal (δ_H = 7.27 ppm) as an internal standard for ^1H NMR and $CDCl_3$ (δ_C = 77.0 ppm) for ^{13}C NMR; coupling constants (J) are in Hz. ^1H NMR and ^{13}C NMR assignments were supported by $^1H-^1H$ COSY, HSQC, HMBC, and NOESY experiments. The mass spectra and high-resolution mass spectra were performed on a Q-TOF Micro mass spectrometer, resolution 5000. An isopropyl alcohol solution of sodium iodide (2 mg/mL) was used as a reference compound. Semipreparative RP-HPLC was performed on an Agilent1100 system equipped with a refractive index detector using an YMC Pack ODS-A column (particle size 5 μm, 250 × 10 mm). Commercial silica gel (Yantai, P. R. China, 200−300; 400−500 mesh) was used for column chromatography. Precoated silica gel plates (Yantai, P. R. China, HSGF-254) were used for analytical thin-layer chromatography (TLC). Spots were detected on TLC under UV or by heating after spraying with anisaldehyde-sulfuric acid reagent.

Animal Material. The gorgonian coral *Dichotella gemmacea* (3.5 kg, wet weight) was collected from the South China Sea in August 2007 and identified by one of the authors (X.-B.L.). A voucher specimen (ZS-3) was deposited in the Second Military Medical University.

Extraction and Isolation. The frozen specimen was extracted ultrasonically with acetone (2.0 L × 3) and MeOH (1.5 L × 3), respectively. The combined residue was partitioned between H_2O and EtOAc to afford 16.1 g of an EtOAc extract. The EtOAc extract was further partitioned between MeOH and hexane, affording 11.2 g of MeOH-soluble residue. The MeOH extract was subjected to column chromatography (CC) on silica to give 16 fractions, using hexane/acetone (from 100:0 to 0:100) as eluent. Fraction 5 was subjected to repeated CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and normal-phase (gradient *n*-hexane/acetone, from 40:1 to 1:4) and reversed-phase silica gel (gradient MeOH/H_2O, from 1:9 to 4:1), followed by HPLC (MeOH/H_2O, 75:25; 1.5 mL/min) to yield **5** (3.8 mg, 25.5 min), **6** (8.1 mg, 34.0 min), and **8** (1.4 mg, 31.7 min). Fraction 8 was purified by repeated CC on Sephadex LH-20 ($CHCl_3$/MeOH, 1:1) and silica gel (gradient *n*-hexane/acetone, from 20:1 to 1:2) and then fractionated by HPLC (MeOH/H_2O, 70:30; 1.0 mL/min), affording **3** (4.1 mg, 45.2 min), **4** (1.4 mg, 52.1 min), **9** (3.1 mg, 28.3 min), and **7** (1.7 mg, 40.3 min). Fraction 12 was chromatographed on a silica gel column (gradient *n*-hexane/acetone, from 4:1 to 1:1) and HPLC (MeOH/H_2O, 65:35; 1.5 mL/min) to give pure **10** (3.5 mg, 45.7 min). Finally, CC on reversed-phase silica gel (gradient H_2O/MeOH, from 7:2 to 1:2) and a subsequent HPLC (MeOH/H_2O, 60:40; 1.5 mL/min) step yielded **2** (4.7 mg. 35.6 min) and **1** (2.9 mg, 38.9 min).

*Gemmacolide G (**1**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ 0 (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 206 (2.12) nm; CD (CH_3CN, *c* 1.3 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 231(−4.01) nm; IR (film) ν_{max} 3448, 1780, 1736 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.89 (1H, d, *J* = 16.0 Hz, H-4), 5.95 (1H, dd, *J* = 16.0, 10.0 Hz, H-3), 5.60 (1H, d, *J* = 10.0 Hz, H-2), 5.35 (1H, br s, H-16a), 5.29 (1H, br d, *J* = 3.5 Hz, H-14), 5.26 (1H, br s, H-16b), 5.18 (1H, t, *J* = 3.5 Hz, H-13), 5.14 (1H, br s, H-9), 5.06 (1H, d, *J* = 3.7 Hz, H-6), 4.87 (1H, br d, *J* = 3.5 Hz, H-12), 4.13 (1H, d, *J* = 3.7 Hz, H-7), 3.77 (1H, br s, H-10), 3.06 (1H, s, 8-OH), 2.89 (1H, br d, *J* = 3.2 Hz, H-20a), 2.87 (1H, q, *J* = 7.0 Hz, H-17), 2.65 (1H, br d, *J* = 3.2 Hz, H-20b), 2.14 (3H, s, 9-OAc), 2.10 (3H, s, 12-OAc), 2.10 (3H, s, 14-OAc), 2.01 (3H, s, 2-OAc), 1.96 (3H, s, 13-OAc), 1.28 (3H, s, H-15), 1.23 (3H, d, *J* = 7.0 Hz, H-18); ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; ESIMS *m/z* 679 $[M + Na]^+$; HRESIMS *m/z* 679.1770 $[M + Na]^+$ (calcd for $C_{30}H_{37}ClO_{14}Na$, 679.1773).

*Gemmacolide H (**2**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ +6 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 223 (1.43), 204 (1.78) nm; CD (CH_3CN, *c* 4.9 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 232.5 (−5.41) nm; IR (film) ν_{max} 3327, 1770, 1738 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.89 (1H, d, *J* = 16.2 Hz, H-4), 6.03 (1H, dd, *J* = 16.2, 9.6 Hz, H-3), 5.72 (1H, d, *J* = 9.6 Hz, H-2), 5.34 (1H, br s, H-16a), 5.27 (1H, br s, H-16b), 5.19 (1H, br s, H-9), 5.08 (1H, d, *J* = 3.9 Hz, H-6), 4.99 (1H, br s, H-14), 4.53 (1H, br s, H-12), 4.17 (1H, d, *J* = 3.9 Hz, H-7), 3.86 (1H, br s, H-10), 3.09 (1H, s, 8-OH), 2.85 (1H, q, *J* = 6.9 Hz, H-17), 2.78 (1H, br d, *J* = 3.3 Hz, H-20a), 2.66 (1H, br d, *J* = 3.3 Hz, H-20b), 2.31 (1H, m, H-13α), 2.12 (3H, s, 9-OAc), 2.11 (3H, s, 14-OAc), 2.06 (3H, s, 12-OAc), 2.04 (1H, ov, H-13*β*), 2.03 (3H, s, 2-OAc), 1.26 (3H, d, *J* = 6.9 Hz, H-18), 1.18 (3H, s, H-15); ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; ESIMS *m/z* 621 $[M + Na]^+$; HRESIMS *m/z* 621.1710 $[M + Na]^+$ (calcd for $C_{28}H_{35}ClO_{12}Na$, 621.1715).

*Gemmacolide I (**3**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ 0 (*c* 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 224 (1.28), 204 (1.53) nm; CD (CH_3CN, *c* 3.2 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 226.5 (−6.09) nm; IR (film) ν_{max} 3463, 1785, 1736 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.88 (1H, d, *J* = 15.8 Hz, H-4), 6.03 (1H, dd, *J* = 15.8, 9.7 Hz, H-3), 5.70 (1H, d, *J* = 9.7 Hz, H-2), 5.34 (1H, br s, H-16a), 5.27 (1H, br s, H-16b), 5.18 (1H, br s, H-9), 5.07 (1H, d, *J* = 3.7 Hz, H-6), 4.98 (1H, br s, H-14), 4.57 (1H, br s, H-12), 4.16 (1H, d, *J* = 3.7 Hz, H-7), 3.82 (1H, br s, H-10), 3.06 (1H, s, 8-OH), 2.85 (1H, q, *J* = 7.1 Hz, H-17), 2.77 (1H, br d, *J* = 2.6 Hz, H-20a), 2.64 (1H, br d, *J* = 2.6 Hz, H-20b), 2.27 (1H, m, H-13α), 2.17 (2H, m, $OCOCH_2CH(CH_3)_2$), 2.17 (1H, m, $OCOCH_2CH(CH_3)_2$), 2.11 (3H, s, 9-OAc), 2.09 (3H, s, 14-OAc), 2.06 (1H, ov, H-13*β*), 2.02 (3H, s, 2-OAc), 1.26 (3H, d, *J* = 7.1 Hz, H-18), 1.18 (3H, s, H-15), 0.95 (6H, d, *J* = 6.3 Hz, $OCOCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1; ESIMS *m/z* 663 $[M + Na]^+$; HRESIMS *m/z* 663.2188 $[M + Na]^+$ (calcd for $C_{31}H_{41}ClO_{12}Na$, 663.2184).

*Gemmacolide J (**4**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ 0 (*c* 0.04, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 272 (0.09), 204 (1.51) nm; CD (CH_3CN, *c* 1.1 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 229 (−6.28) nm; IR (film) ν_{max} 3459, 1781, 1737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.90 (1H, d, *J* = 15.8 Hz, H-4), 5.97 (1H, dd, *J* = 15.8, 9.9 Hz, H-3), 5.60 (1H, d, *J* = 9.9 Hz, H-2), 5.36 (1H, br s, H-16a), 5.30 (1H, br d, *J* = 3.3 Hz, H-14), 5.27 (1H, br s, H-16b), 5.20 (1H, t, *J* = 3.3 Hz, H-13), 5.14 (1H, br s, H-9), 5.06 (1H, d, *J* = 3.8 Hz, H-6), 4.91 (1H, br d, *J* = 3.3 Hz, H-12), 4.13 (1H, d, *J* = 3.8 Hz, H-7), 3.75 (1H, br s, H-10), 3.06 (1H, s, 8-OH), 2.90 (1H, br d, *J* = 3.0 Hz, H-20a), 2.86 (1H, q, *J* = 7.0 Hz, H-17), 2.64 (1H, br d, *J* = 3.0 Hz, H-20b), 2.22 (2H, m, $OCOCH_2CH(CH_3)_2$), 2.16 (1H, m, $OCOCH_2CH(CH_3)_2$), 2.14 (3H, s, 9-OAc), 2.11 (3H, s, 14-OAc), 2.01 (3H, s, 2-OAc), 1.95 (3H, s, 13-OAc), 1.26 (3H, s, H-15), 1.23 (3H, d, *J* = 7.0 Hz, H-18), 0.97 (6H, d, *J* = 6.4 Hz, $OCOCH_2CH(CH_3)_2$); ^{13}C

NMR ($CDCl_3$, 100 MHz) see Table 1; ESIMS m/z 721 $[M + Na]^+$; HRESIMS m/z 721.2245 $[M + Na]^+$ (calcd for $C_{33}H_{43}ClO_{14}Na$, 721.2239).

*Gemmacolide K (**5**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ −79 (c 0.17, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 211 (2.48) nm; CD (CH_3CN, c 2.0 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 217.5 (−16.83), 191(−18.11) nm; IR (film) ν_{max} 3500, 1783, 1746 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.33 (1H, d, J = 10.0 Hz, H-2), 6.01 (1H, d, J = 11.4 Hz, H-4), 5.78 (1H, dd, J = 11.4, 10.0 Hz, H-3), 5.75 (1H, br s, H-16a), 5.69 (1H, br s, H-16b), 5.23 (1H, ov, H-14), 5.22 (1H, ov, H-13), 5.10 (1H, d, J = 1.9 Hz, H-6), 4.96 (1H, br s, H-9), 4.92 (1H, br s, H-12), 4.74 (1H, br d, J = 1.9 Hz, H-7), 4.16 (1H, d, J = 16.3 Hz, $OCOCH_2OH$), 4.01 (1H, d, J = 16.3 Hz, $OCOCH_2OH$), 3.98 (1H, br s, H-10), 3.04 (1H, s, 8-OH), 2.95 (1H, ov H-17), 2.92 (1H, br s, H-20a), 2.61 (1H, br s, H-20b), 2.26 (1H, m, $OCOCH_2CH(CH_3)_2$), 2.22 (1H, m, $OCOCH_2CH(CH_3)_2$), 2.18 (1H, ov, $OCOCH_2CH(CH_3)_2$), 2.17 (3H, s, 9-OAc), 2.07 (3H, s, 14-OAc), 1.93 (3H, s, 13-OAc), 1.33 (3H, s, H-15), 1.23 (3H, d, J = 7.3 Hz, H-18), 0.99 (6H, d, J = 6.2 Hz, $OCOCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; ESIMS m/z 737 $[M + Na]^+$; HRESIMS m/z 737.2181 $[M + Na]^+$ (calcd for $C_{33}H_{43}ClO_{15}Na$, 737.2188).

*Gemmacolide L (**6**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ −136 (c 0.50, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 205 (1.48) nm; CD (CH_3CN, c 4.7 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 218.5 (−22.41), 192 (−35.22) nm; IR (film) ν_{max} 3550, 1782, 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.31 (1H, d, J = 10.3 Hz, H-2), 6.02 (1H, d, J = 11.5 Hz, H-4), 5.77 (1H, dd, J = 11.5, 10.3 Hz, H-3), 5.73 (1H, br s, H-16a), 5.66 (1H, br s, H-16b), 5.23 (1H, ov, H-14), 5.23 (1H, ov, H-13), 5.09 (1H, d, J = 1.9 Hz, H-6), 4.94 (1H, br s, H-9), 4.91 (1H, br s, H-12), 4.73 (1H, br d, J = 1.9 Hz, H-7), 4.16 (1H, d, J = 16.3 Hz, $OCOCH_2OCOCH_2CH(CH_3)_2$), 4.01 (1H, d, J = 16.3 Hz, $OCOCH_2OCOCH_2CH(CH_3)_2$), 3.95 (1H, ov, H-10), 3.04 (1H, s, 8-OH), 2.92 (1H, q, J = 7.4 Hz, H-17), 2.91 (1H, br d, J = 2.6 Hz, H-20a), 2.64 (1H, br d, J = 2.6 Hz, H-20b), 2.30 (2H, ov, $OCOCH_2OCOCH_2CH(CH_3)_2$), 2.22 (2H, ov, $OCOCH_2CH(CH_3)_2$), 2.17 (3H, s, 9-OAc), 2.15 (1H, ov, $OCOCH_2CH(CH_3)_2$), 2.13 (1H, ov, $OCOCH_2OCOCH_2CH(CH_3)_2$), 2.05 (3H, s, 14-OAc), 1.94 (3H, s, 13-OAc), 1.31 (3H, s, H-15), 1.22 (3H, d, J = 7.4 Hz, H-18), 0.99 (6H, d, J = 6.6 Hz, $OCOCH_2CH(CH_3)_2$), 0.99 (6H, d, J = 6.6 Hz, $OCOCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; ESIMS m/z 821 $[M + Na]^+$; HRESIMS m/z 821.2769 $[M + Na]^+$ (calcd for $C_{38}H_{51}ClO_{16}Na$, 821.2763).

*Gemmacolide M (**7**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ −148 (c 0.28, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 220 (1.25), 205 (1.17) nm; CD (CH_3CN, c 1.3 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) = 232 (4.25), 186(−51.65) nm; IR (film) ν_{max} 3475, 1778, 1742 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.43 (1H, d, J = 9.5 Hz, H-2), 6.15 (1H, d, J = 11.3 Hz, H-4), 5.56 (1H, dd, J = 11.3, 9.5 Hz, H-3), 6.01 (1H, br s, H-16a), 5.56 (1H, br s, H-16b), 5.28 (1H, br d, J = 2.5 Hz, H-14), 5.13 (1H, br d, J = 2.5 Hz, H-13), 4.90 (1H, br d, J = 3.8 Hz, H-9), 4.87 (1H, br d, J = 2.5 Hz, H-12), 4.35 (1H, d, J = 7.3 Hz, H-7), 4.07 (1H, d, J = 7.3 Hz, H-6), 3.76 (1H, br d, J = 3.8 Hz, H-10), 3.38 (3H, br s, OCH_3), 3.38 (1H, br s, H-20a), 2.91 (1H, br s, H-20b), 2.72 (1H, s, 8-OH), 2.48 (1H, q, J = 7.2 Hz, H-17), 2.28 (1H, m, $OCOCH_2CH(CH_3)_2$), 2.15 (3H, s, 9-OAc), 2.14 (1H, ov, $OCOCH_2CH(CH_3)_2$), 2.14 (1H, ov, $OCOCH_2CH(CH_3)_2$), 2.13 (3H, s, 12-OAc), 1.98 (3H, s, 2-OAc), 1.96 (3H, s, 13-OAc), 1.19 (3H, d, J = 7.2 Hz, H-18), 1.13 (3H, s, H-15), 1.00 (3H, d, J = 6.0 Hz, $OCOCH_2CH(CH_3)_2$), 0.97 (3H, d, J = 6.0 Hz, $OCOCH_2CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) see Table 1; ESIMS m/z 717 $[M + Na]^+$; HRESIMS m/z 717.2736 $[M + Na]^+$ (calcd for $C_{34}H_{46}O_{15}Na$, 717.2734).

*Juncin O (**8**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ +30 (c 0.12, $CHCl_3$); lit.[11] +36 (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see SI.

*Junceellolide C (**9**):* white, amorphous powder (MeOH); $[\alpha]^{24}_D$ +32 (c 0.127, $CHCl_3$); lit.[12] +36 (c 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) see SI.

Cytotoxicity Assay. Cytotoxicity was tested against human lung adenocarcinoma (A549) and human osteosarcoma cells (MG63) using a modification of the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method.[13] Adriamycin was used as positive control, IC_{50} = 2.8 μM.

Agar Diffusion Test for Biological Activity. Compounds **1**−**5** and **7**−**9** were dissolved in acetone at a concentration of 2 mg/mL; 25 μL of the solution (0.05 mg) was pipetted onto a sterile filter disk (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism. The test organisms were the Gram-negative bacterium *Escherichia coli* (Coli), the Gram-positive bacterium *Bacillus megaterium* (Meg) (both grown on NB medium), and the fungi *Microbotryum violaceum* (Vio) and *Septoria tritici* (Tri) (both grown on MPY medium). Commencing at the outer edge of the filter disk, the diameter of zone of inhibition was measured in mm. Reference substances were ketoconazole (0.05 mg), penicillin (0.05 mg) and streptomycin (0.05 mg), with the diameter (Φ) of the zone of inhibition being 18.0, 18.0, 30.0, 25.0; 8.0, 26.0, 14.0, 12.0; and 18.0, 11.0, 16.0, 11.0 mm, respectively.

■ ASSOCIATED CONTENT

Supporting Information. NMR spectra for compounds **1**−**7** and NMR data for compounds **8** and **9** are available free of charge via the Internet at http://pubs.acs.org.

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