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ANTIFEEDANT AND ANTIFOULING BRIARANES FROM THE SOUTH CHINA SEA GORGONIAN *Junceella juncea*

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*A new briarane diterpene, juncin ZII (**1**), along with three known briaranes (**2–4**), was isolated from the EtOH/CH_2Cl_2 extracts of the South China Sea gorgonian* Junceella juncea. *The structure of **1** was established by extensive spectroscopic analysis, including 1D and 2D NMR data. For compounds **1–4** and eight other briaranes (**5–12**) isolated from* J. juncea *previously, the antifeedant activity against second-instar larvae of* Spodoptera litura *and cytotoxicity against* S. litura *cells were investigated, and it was observed that they all exhibit medium antifeedant activity. Compounds **1**, **8**, **9**, and **12** also showed potent antifouling activity against the larval settlement of barnacle* Balanus amphitrite *at nontoxic concentrations with EC_{50} values of 0.004, 0.005, 2.82, and 0.447 μg/mL, respectively, while all compounds did not show obvious cytotoxicity against tumor cell lines K562, A549, Hela, and Hep-2. Their structure-activity relationship was discussed.*

Key words: *Junceella juncea*, briaranes, antifeedant, cytotoxicity, antifouling, structure–activity relationship.

Gorgonian *Junceella juncea* (Ellisellidae) belongs to the genus *Junceella,* which is known to produce highly oxidized diterpenoids of the briarane class (3,8-cyclized cembranoids). In recent years, briarane-type diterpenoids have continued to attract the attention of investigators because of their structural complexity and interesting biological activities such as cytotoxicity, anti-inflammatory, antiviral, immunomodulatory, insect control, antifouling, biotoxic, and ichthyotoxic activities [1]. More than 20 briaranes, such as juncins A–H [2, 3], (+)-gemmacolides A–B [3], juncenolides A–D [4, 5], and junceellolide C [5], juncins I–M [6], and juncin N [7], were isolated from *J. juncea*. In our previous investigation on the South China Sea gorgonian coral *J. juncea*, 13 new briarane diterpenoids, juncins O–Q (**5**–**7**) and juncins R–ZI, together with five known briaranes, gemmacolide B (**8**), gemmacolide A (**9**), praelolide (**10**), junceellin A (**11**), and junceellolide D (**12**), were obtained [8, 9]. Now, in our further chemical investigation on the EtOH/CH_2Cl_2 extract of *J. juncea*, a new briarane diterpene, juncin ZII (**1**), along with three known briaranes, gemmacolide C (**2**) [10], gemmacolide F (**3**) [10], and (+)-11α,20α-epoxy-junceellolide D (**4**) [11], was obtained.

In the marine environment, many sessile species such as gorgonians, soft corals, sponges, and seaweeds effectively defend against predators, competitors, and potential pathogens. For example, gorgonian corals produce chemicals with antimicrobial, antifouling, predator deterrent, and allelopathic properties [12–14]. In order to obtain bioactive compounds from *J. juncea* and investigate the potential chemical defensive roles of these secondary metabolites in gorgonians, we studied the antifeedant, cytotoxicity, and antilarval activities of compounds **1–12**.

This paper deals with the isolation and structural elucidation of compound **1**. For compounds **1–12**, antifeedant activity against second-instar larvae of *Spodoptera litura*, cytotoxicity against *S. litura* cell were reported, and antifouling activity of compounds **1**, **8**, **9**, and **12** against the larval settlement of barnacle *Balanus amphitrite* were investigated. Their structure–activity relationship was also discussed.

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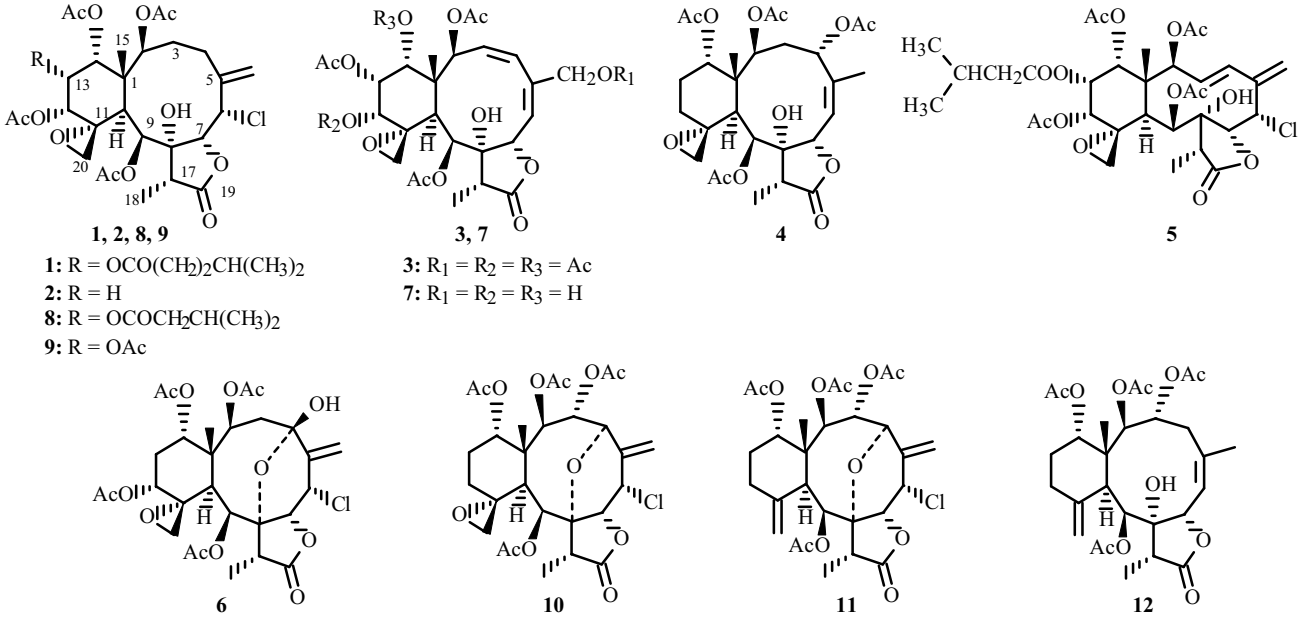

1, 2, 8, 9

1: R = $OCO(CH_2)_2CH(CH_3)_2$
2: R = H
8: R = $OCOCH_2CH(CH_3)_2$
9: R = OAc

3, 7

3: $R_1 = R_2 = R_3$ = Ac
7: $R_1 = R_2 = R_3$ = H

4

5

6

10

11

12

The residue from the $EtOH/CH_2Cl_2$ extracts of *J. juncea* was partitioned in H_2O and extracted with EtOAc and *n*-BuOH, respectively. The EtOAc-soluble fraction was chromatographed over silica, and selected fractions were rechromatographed on semi-preparative HPLC (Luna^{TM}C18(2), 250×10 mm i.d.) to yield compounds **1–4**. All the compounds possessed a briarane-type skeleton, and compounds **2–4** were identified as gemmacolide C (**2**) [10], gemmacolide F (**3**) [10], and (+)-11α,20α-epoxy-junceellolide D (**4**) [11] by comparison of their spectral data with literature values. The structure of **1** is described below.

Juncin ZII (**1**) has molecular formula $C_{34}H_{47}ClO_{14}$ as deduced from NMR spectra and ESIMS, which showed a pair of peaks at *m/z* 715/717 (3:1) $[M+H]^+$, suggesting one chlorine atom in **1**. Its IR spectrum showed strong absorptions at 3542, 1790, 1750, and 1732 cm^{-1}, indicating the existence of hydroxyl, γ-lactone, and ester groups. The ^1H and ^{13}C (DEPT) NMR spectra showed signals for four acetate esters and a long chain fatty acid ester, a tertiary methyl (δ_H 1.17, s), a secondary methyl (δ_H 1.26, d, J = 6.6 Hz), a γ-lactone (δ_C 174.3), an exocyclic 11(20)-epoxide [δ_H 2.85 (d, J = 3.2 Hz), 2.36 (d, J = 3.2 Hz), δ_C 50.4 (t), 57.5 (s)], an exocyclic double bond [δ_H 6.02, 5.79 (each 1H, s)], an oxygenated quaternary carbon, and six oxygenated methines (Table 1). These data showed that **1** was a briarane-type diterpene, similar to the structures of gemmacolide B (**8**), gemmacolide A (**9**), and gemmacolide C (**2**) [10]. Comparison of the ^1H and ^{13}C NMR spectral data of **1** with those of gemmacolide B (**8**) revealed that the only difference between them was that **1** showed an additional methylene [δ_H 1.27 (2H, m), δ_C 29.6 (t)] in high field. The additional methylene belongs to the group $-OCOCH_2CH_2CH(CH_3)_2$, which was supported by the HMBC correlations of δ_H 2.17 (2H, m, H-2′) and 1.27 (2H, m, H-3′) with δ_C 166.6 (s, C-1′) and 25.6 (t, C-4′), and ^1H–^1H COSY spectrum showing correlations of δ_H 1.27 (2H, m, H-3′) with δ_H 2.17 (2H, m, H-2′), 2.08 (1H, m, H-4′), and δ_H 2.08 (1H, m, H-4′) with δ_H 0.95 (6H, d, J = 6.5 Hz, H-5′ and 6′). The four acetate moieties were assigned to C-2, C-9, C-12, and C-14 because their carbonyl carbons were correlated with the corresponding methine protons in the HMBC spectrum, and the group $-OCOCH_2CH_2CH(CH_3)_2$ was attached to C-13 because of the existence of HMBC correlations between H-13/2′/3′ with C-1′ (δ_C 166.6) (Table 1).

In the NOESY spectrum of **1**, NOE correlations between Me-15 with H-13/14/20/9-OAc, H-20 with H-12, and H-6 with H-9-OAc suggested that H-20, H-13, H-12, H-14, and Me-15 were all in the β-orientation. NOE correlations of H-2 with H-10, H-9 with H-10, and Me-18 with H-9/10 indicated that H-2, H-9, H-10, and Me-18 were all in the α-orientation, with corresponding correlation of H-17 with H-7 suggesting the β-orientation of H-17 and H-7. On the basis of NOESY correlations (Table 1), the relative stereochemistry of juncin ZII (**1**) was determined as 1*S**, 2*S**, 6*S**, 7*R**, 8*S**, 9*S**, 10*S**, 11*S**, 12*R**, 13*R**, 14*R**, and 17*R**.

TABLE 1. NMR Spectral Data for Compound **1**, ppm*

C atom	δ_C	δ_H (J/Hz)	HMBC	^{1}H-^{1}H COSY	NOESY
1	47.8 q				
2	72.9	5.96 (d, J = 8.0)	C-1, 3, 4, 10, 14, 15, Me*C*OO	H-3	H-10
3	29.1	2.22, 2.07 (each 1H, m)	C-1, 2, 5	H-2, 4	
4	35.4	1.67, 3.01 (each 1H, m)	C-2, 6	H-3	
5	144.0 q				
6	51.0	4.78 (s)	C-4, 5, 7, 16	H-7	H-9-OAc
7	71.7	4.55 (s)	C-5, 6, 9	H-6	H-17
8	81.7 q				
9	81.5	4.47 (br.s)	C-1, 7, 8, 10, 11, 17, Me*C*OO	H-10	H-10
10	35.5	3.69 (s)	C-1, 2, 8, 9, 11, 12, 14, 15, 20	H-9	H-9, 18
11	57.5 q				
12	75.3	4.86 (br.s)	C-11, 13, 14, Me*C*OO	H-13	H-20
13	70.5	5.07 (br.s)	C-1, 12, 1′	H-12, 14	H-15
14	73.5	5.20 (br.s)	C-1, 12, 13, Me*C*OO	H-13	H-15
15	14.1	1.17 (s)	C-1, 2, 10, 14		H-13, 14, 20, 9-OAc
16	126.3	5.79, 6.02 (each 1H, s)	C-4, 5, 6		
17	51.2	3.01 (1H, overlap)	C-8, 9, 18, 19	H-18	H-7
18	6.8	1.26 (d, J = 6.6)	C-8, 17, 19	H-17	H-9, 10
19	174.3 q				
20	50.4	2.85, 2.36 (d, J = 3.2)	C-11, 12		H-12
OAc	169.3 q				
	169.4 q				
	170.2 q				
	171.3 q				
	20.8	1.97 (s)			
	21.0	2.25 (s)			
	21.1	2.04 (s)			
	21.2	2.00 (s)			
1′	166.6				
2′	43.5	2.17 (2H, m)	C-1′, 3′, 4′	H-3′	
3′	29.6	1.27 (2H, m)	C-1′, 2′, 4′	H-2′, 4′	
4′	25.6	2.08 (1H, m)	C-2′, 3′, 5′, 6′	H-3′, 5′, 6′	
5′	22.2	0.95 (d, J = 6.5)	C-3′, 4′, 6′	H-4′	
6′	22.4	0.95 (d, J = 6.5)	C-3′, 4′, 5′	H-4′	

*^{13}C and ^{1}H NMR spectra were determined at 125 MHz and 500 MHz, respectively, with TMS as internal standard and $CDCl_3$ as solvent; chemical shifts are in ppm, and coupling constant values in J/Hz.

Antifeedant and Cytotoxicity against *S. litura*. *S. litura* is a kind of main vegetable pest in summer and fall in China. For compounds **1–12**, the antifeedant activity against second-instar larvae of *S. litura* at a concentration of 500 μg/mL and cytotoxicity against *S. litura* cell at a concentration of 100 μg/mL are shown in Table 2. It was observed that compounds **1–12** all exhibited medium antifeedant activity lower than that of the model compound azadirachtin, and compounds **1–7**, **9**, and **12** also showed medium cytotoxicity against *S. litura* cell, while compounds **8**, **10**, **11** showed negative cytotoxicity activity. The purpose of the cytotoxicity bioassay was to assess the insecticidal activity of the tested samples. By comparison with the conventional leaf disk method, the method has several advantages. For example, it requires a short time and smaller amounts of samples.

TABLE 2. Antifeedant and Toxicity Activity of Compounds **1-12**

Tested compounds	Antifeeding rate AR* (100%)	Cell mortality** (100%)	
		24 h	48 h
1	84.50±2.12	20.51±7.19	43.15±9.98
2	40.50±9.45	5.73±3.53	13.20±5.64
3	58.60±6.36	37.16±6.04	48.61±9.35
4	82.12±8.04	53.21±8.33	56.08±7.84
5	90.70±1.98	8.69±4.87	11.88±9.75
6	68.99±4.07	25.32±4.81	29.72±7.03
7	46.51±9.29	31.25±9.57	44.04±9.40
8	86.82±4.68	-12.99±4.34	-17.83±13.53
9	59.69±10.29	21.96±5.06	21.01±3.04
10	91.47±1.90	-2.13±5.24	-18.01±9.58
11	68.99±6.93	-25.11±1.72	-44.27±10.36
12	65.12±2.74	49.42±4.62	56.25±7.72
Azadirachtin	96.90±1.45		

*AR represents the antifeeding rate calculated from AR = [(C-T)/C]×100%. C and T represent the areas eaten by the larvae of the control and treatment disks, respectively.

**Mortality of *S. litura* cell was calculated from [1-(OD_{570nm} test product/OD_{570nm} negative control)]×100%. The data in the table are mean±SE.

Antifouling Activity against Barnacle Larvae. Compounds **1**, **8**, **9**, and **12** all showed potent antifouling activities against the larval settlement of barnacle *B. amphitrite* at nontoxic concentrations with EC_{50} values of 0.004, 0.005, 2.82, and 0.447 μg/mL, respectively, which were lower than the standard requirement of EC_{50} of 25 μg/mL established by the US Navy program as an efficacy level for natural antifoulants, indicating that compounds **1**, **8**, **9**, and **12** are potential natural non-toxic antifouling agents. We have reported 10 new antifouling briaranes with EC_{50} values of 0.004, 0.34, 2.65, 1.61, 3.77, 21.06, 0.004, 0.14, 1.47, and 0.51 μg/mL from *J. juncea* [9]. The results further support the conjecture that briarane-type diterpenoids play important chemical defensive roles against epibiosis, competitors, and potential pathogens.

According to the cytotoxicity bioassay, compounds **1–12** did not show obvious cytotoxicity against tumor cell lines K562, A549, Hela, and Hep-2.

Based on the above antifeedant bioassay results, the structure–activity relationship was summarized as follows. Comparison of the antifeedant activity of compounds **1**, **2**, **8**, and **9** indicated that the chain lengths of esters at C-13 could improve the potency of briarane-type diterpenoids. Moreover, compounds **4** and **10** were more potent than compounds **12** and **11**, respectively, which suggested that the exocyclic 11,20-epoxy group was important for the antifeedant activity of briarane-type diterpenoids. This deduction was consistent with the conjecture that we got from 10 antifouling briaranes [9].

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured with a Shimadzu double-beam 210A spectrophotometer in MeOH solution. IR (KBr) spectra were obtained on a Bio-Rad FTS-135 infrared spectrophotometer. ^{1}H, ^{13}C NMR, and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as internal standard. MS spectral data were obtained on an LCQ^{DECA} XP $HPLC/MS^{n}$ spectrometer for ESIMS. Si gel (200–300 mesh) for column chromatography and GF_{254} for TLC were obtained from the Qindao Marine Chemical Factory, Qindao, People's Republic of China.

Animal Material. The South China Sea gorgonian coral *Junceella juncea* (Ellisellidae) (12 kg, wet weight) was collected in Sanya, Hainan province, China in October 2003 and identified by Prof. Zou R. L., the South China Sea Institute of Oceanology, Academia Sinica. A voucher specimen (No. 0310) was deposited in the South China Sea Institute of Oceanology, Academia Sinica, Guangzhou, China.

Extraction and Isolation. The frozen specimen was extracted with EtOH–CH_2Cl_2 (2:1) three times at room temperature, and the solvent was evaporated *in vacuo*. The residue was partitioned in H_2O and extracted with EtOAc and *n*-BuOH three times. The EtOAc and *n*-BuOH extracts were concentrated *in vacuo* to afford 85 and 56 g of residue, respectively. The EtOAc portion was subjected to column chromatography (CC) on silica, using petroleum ether–EtOAc (from 10:1 to 0:10) as eluent. By combining the fractions with TLC (GF_{254}) monitoring, 12 fractions were obtained. Fractions 3 and 4 were subjected to CC on silica gel, eluted with $CHCl_3$–Me_2CO (from 11:1 to 8:2), and then purified with semi-preparative HPLC (Luna^{TM}C18(2), 250×10 mm i.d., 5 mL/min), using MeOH-water as eluent to afford **1** (12 mg), **2** (6 mg), **3** (7 mg), and **4** (7 mg).

Juncin ZII (1): white powder; $[\alpha]_D$ 20.9° (*c* 1.1, $CHCl_3$); IR (KBr, ν_{max}, cm^{-1}) 3542, 1790, 1750 and 1732; ^1H NMR spectral data, see Table 1; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 716 $[M+H]^+$; HRESIMS *m/z* 715.2650 $[M+H]^+$ (calcd for $C_{28}H_{36}ClO_{13}$, 715.2654).

Antifeedant Bioassays. Antifeedant activities of compounds **1**–**12** against second-instar larvae of *Spodoptera litura* were tested by the conventional leaf disk method [15].

Cytotoxic Bioassays. Cytotoxicity of compounds **1**–**12** against *S. litura* cells was tested by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method as described previously for assessing their insecticidal activity [15, 16].

Larval Settlement Bioassays. Antifouling activities of compounds **1**, **8**, **9**, and **12** against the larval settlement of barnacle *B. amphitrite* were tested as follows. Adults of *B. amphitrite* Darwin were collected from the intertidal zone in Hong Kong (N: 22°22′, E: 114°16′) in Oct. 2005. After 12 h of exposure to air, several hundred adults were placed in a container filled with (FSW) (30 ppt salinity) to induce the release of larvae. Larval culture was maintained according to the method described by Thiyagarajan et al. [17]. Briefly, released nauplii were collected on sieves (90 μm). Larvae were reared to the cyprid stage at a density of about 2 larvae mL^{-1}. When kept at 26°C and fed with *Isochrysis galbana*, larvae developed to the cyprid stage within 6 days. The culture medium was changed daily. The newly molted cyprid larvae were filtered onto a 100 μm sieve and were then washed with FSW to remove algae and detritus. Only cyprids at an age of 1 or 2 days, stored at 4°C for 2 days at most, were used in the experiments.

In vitro still water larval settlement assays were performed using 24-well polystyrene plates. The tested samples (**1**, **8**, **9**, **12**) were soluble in DMSO and added to autoclaved 0.22 μm filtered seawater (FSW) with different concentrations (0.05, 0.2, 1, 10, and 50 μg/mL). Twenty competent larvae were added to each well with 1 mL testing solution in 4 replicates, and wells containing 0.22 μm sterile-filtered seawater (FSW) with added DMSO were served as control. The 24-well plates were incubated at 28°C for 24 h. The effects of the tested samples activating antagonists on the larvae were determined by examining the plates with the aid of a dissecting microscope to check for 1) attached larvae, 2) unattached larvae, and 3) dead larvae. The percentage of larval settlement was determined by counting the number of attached individuals and expressed as a proportion of the total number of larvae in the well. The EC_{50} (inhibits 50% of settlement of *B. amphitrite* larvae in comparison with the control) was the mean of three repeated experiments with different batches of larvae, and calculated by using the Probit software program.

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REFERENCES

1. P. J. Sung, J. H. Sheu, S. W. Wu, and J. P. Xu, *Heterocycles,* **57**, 535 (2002).
2. S. Isaacs, S. Carmely, and Y. Kashman, *J. Nat. Prod.,* **53**, 596 (1990).
3. A. S. R. Anjaneyulu and N. S. K. Rao, *J. Chem. Soc., Perkin Trans. 1*, 959 (1997).

4. Y. C. Shen, Y. C. Lin, and M. Y. Chiang, *J. Nat. Prod.,* **65**, 54 (2002).
5. Y. C. Shen, Y. C. Lin, C. L. Ko, and L. T. Wang, *J. Nat. Prod.,* **66**, 302 (2003).
6. A. S. R. Anjaneyulu, V. L. Rao, V. G. Sastry, M. J. R. V. Venugopal, and F. J. Schmitz, *J. Nat. Prod.,* **66**, 507 (2003).
7. P. J. Sung, T. Y. Fan, L. S. Fang, J. H. Sheu, S. W. Wu, G. H. Wang, and M. R. Lin, *Heterocycles,* **61**, 587 (2003).
8. S. H. Qi, S. Zhang, H. Huang, Z. H. Xiao, J. S. Huang, and J. Wu, *J. Nat. Prod.,* **67**, 1907 (2004).
9. S. H. Qi, S. Zhang, P. Y. Qian, Z. H. Xiao, and M. Y. Li, *Tetrahedron,* **62**, 9123 (2006).
10. H. Y. He and D. J. Faulkner, *Tetrahedron,* **47**, 32710 (1991).
11. M. Garcia, J. Rodriguez, and C. Jimenez, *J. Nat. Prod.*, **62**, 257 (1999).
12. P. Devi, J. Vennam, C. G. Naik, P. S. Parameshwaran, T. V. Raveendran, and K. S. Yeshwant, *J. Mar. Biotechnol*., **6**, 229 (1998).
13. P. R. Jensen, C. D. Harvell, K. Wirtz, and W. Fenical, *Mar. Biol*., **125**, 411 (1996).
14. K. Kim, P. D. Kim, A. P. Alker, and C. D. Harvell, *Mar. Biol.*, **137**, 393 (2000).
15. Z. X. Zhang, H. H. Xu, D. M. Cheng, Y. L. Wu, and J. F. Fan, *J. South Chin. Agric. Univ.*, **21**, 29 (2000).
16. T. Mosmann, *J. Immunol. Methods*, **65**, 56 (1983).
17. V. Thiyagarajan, T. Harder, and P. Y. Qian, *J. Exp. Mar. Biol. Ecol.,* **287**, 223 (2003).