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Ten new antifouling briarane diterpenoids from the South China Sea gorgonian *Junceella juncea*

Shu-Hua Qi,[a,*] Si Zhang,[a] Pei-Yuan Qian,[b] Zhi-Hui Xiao[a] and Ming-Yi Li[a]

[a]*Guangdong Key Laboratory of Marine Materia Medica, South China Sea Institute of Oceanology, The Chinese Academy of Sciences, 164 West Xingang Road, Guangzhou, 510301 Guangdong, People's Republic of China*
[b]*Hong Kong University of Science and Technology/Department Biology—CML/Clearwater Bay, KLN, Hong Kong, China*

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Abstract—Ten new antifouling briarane diterpenoids, juncins R–ZI (**1–10**) were isolated from the South China Sea gorgonian coral *Junceella juncea*. The structures of these new compounds were established by extensive spectroscopic analysis, including 1D and 2D NMR data. Compounds **1–10** all showed potent antifouling activities against the larval settlement of barnacle *Balanus amphitrite* at nontoxic concentrations 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^{-1}. The structure–activity relationship was discussed.

1. Introduction

In the past, antifouling paints containing tributyltin (TBT), copper or organonitrogen compounds have been used to protect ship hulls. However, these substances, especially TBT, are highly toxic and persistent in the marine environment and can adversely affect nontarget organism, leading to a total ban on the production of the TBT-based coating since January 2003 and application in January 2008. The imminent prohibition of TBT-based coating means that there is a need to develop new environmentally compatible alternatives that would be equally efficient against several fouling organisms such as barnacles. In the marine environment, many sessile organisms such as gorgonians, soft corals, sponges, and seaweeds are known to elaborate chemical defense mechanisms against predation and epibiont growth. The use of natural marine products that are capable of inhibiting one or several stages of fouling on ships and other submerged structures may provide an acceptable solution from the aspect of environmental impact.

Gorgonians are a major source of unusual secondary metabolites that play important roles in protecting the colonies against grazing, feeding, and the settlement of both the adult and larval form of marine organisms such as barnacles.[1–3] Gorgonian *Junceella juncea* (Ellisellidae) belongs to the genus *Junceella* that is known to produce highly oxidized diterpenoids of the briarane class (3,8-cyclized cembranoids). In recent years, briarane-type diterpenoids continue to attract the attention of investigations because of the structural complexity and interesting biological activities, such as cytotoxicity, anti-inflammatory, antiviral, immunomodulatory activity, insect control, antifouling, biotoxin, and ichthyotoxicity.[4] Previous chemical investigations on *J. juncea* have yielded more than 20 briaranes.[5–11] During the course of our further investigation on the EtOH/CH_2Cl_2 extract of *J. juncea* by semi-preparative HPLC, 10 new briarane diterpenoids, juncins R–ZI (**1–10**) were obtained from an EtOAc soluble fraction of the EtOH/CH_2Cl_2 extract. Antifouling bioassay tests showed that the EtOAc soluble fraction led to 0% larval settlement and 10% mortality toward barnacle *Balanus amphitrite* (Cirripedia) larvae at concentration of 100.0 μg mL^{-1}, and compounds (**1–10**) all had potent antifouling activities against *B. amphitrite* larvae at nontoxic concentrations 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^{-1}. These EC_{50} values were lower than the standard requirement of an EC_{50} of 25 μg mL^{-1} established by the US Navy program as an efficacy level for natural antifoulants. In this paper, we report the isolation, structural elucidation, and antifouling activities of compounds **1–10**.

2. Results and discussion

The residue from the EtOH/CH_2Cl_2 extract of *J. juncea* was partitioned in H_2O and extracted with EtOAc. The EtOAc extract was chromatographed over silica gel to give 12 fractions, and then selected fractions were rechromatographed

Keywords: *Junceella juncea*; Briarane diterpenoids; Antifouling; Structure–activity relationship.
* Corresponding author. Tel.: +86 20 89023105; fax: +86 20 84458964; e-mail: shuhuaqi2001@yahoo.com

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Table 1. ^{13}C NMR spectral data of compounds **1–11**[a]

Carbon	1	2	3	4	5	6	7	8	9	10	11
1	46.5	46.5	46.4	46.5	47.5	47.4	47.5	47.5	47.9	47.1	48.3
2	74.0	74.0	75.5	74.1	75.5	75.1	72.8	72.0	72.9	72.8	75.8
3	131.8	131.8	131.4	131.2	131.7	132.5	38.1	38.2	37.4	40.5	132.5
4	128.2	128.2	128.5	128.6	128.0	127.1	69.2	69.3	67.6	97.2	128.3
5	140.1	140.1	139.6	141.6	141.2	139.3	140.1	141.9	136.8	138.0	147.0
6	125.9	125.9	122.7	122.8	123.4	123.4	123.7	122.5	139.1	55.3	121.6
7	78.5	78.5	78.8	78.9	79.1	79.2	76.4	76.4	76.7	78.6	81.8
8	81.2	81.0	81.2	81.1	81.2	81.3	80.3	83.2	82.9	81.4	82.0
9	63.7	63.7	63.8	63.9	64.2	64.2	71.4	73.3	72.2	71.7	65.7
10	32.6	32.7	32.7	32.7	30.7	30.6	39.6	42.4	43.3	40.5	31.3
11	58.4	58.3	58.3	58.4	60.4	60.3	62.3	151.0	150.7	56.2	60.4
12	73.3	72.8	73.3	73.3	76.0	75.9	23.5	25.6	29.3	24.7	76.6
13	66.5	66.4	66.6	66.3	68.1	68.2	24.5	27.6	27.6	29.7	70.0
14	73.3	73.6	73.8	73.9	76.5	77.0	73.2	70.9	74.1	73.2	77.3
15	14.2	14.4	14.4	14.4	13.7	13.6	15.1	15.2	14.4	15.5	13.8
16	44.5	44.5	63.0	72.2	73.1	63.9	65.6	65.9	166.6	117.8	63.0
17	44.0	44.0	44.1	44.2	44.3	44.3	42.2	42.4	42.7	49.9	45.6
18	6.3	6.3	6.3	6.3	6.3	6.3	6.6	6.6	6.4	7.2	7.4
19	175.0	175.0	176.9	175.3	175.6	175.7	176.4	176.4	175.4	174.2	176.9
20	49.1	49.1	48.7	48.9	48.5	48.3	58.9	113.0	112.4	51.2	47.5
CH_3COO	169.7	169.7	169.7	169.6	170.2	169.9	169.5	169.3	169.2	169.3	168.2
	169.8	169.8	170.0	169.6	170.4	170.1	170.1	169.9	169.6	169.9	170.3
	170.1	170.1	170.1	169.9	170.5	170.4	170.2	170.1	169.8	173.4	171.0
	172.3	171.8	170.1	170.2		170.5	170.7	170.7	170.5		
							170.8	170.9			
CH_3COO	20.5	20.5	20.5	20.8	21.1	20.8	20.8	20.7	20.8	20.8	20.8
	21.0	20.9	20.6	20.9	21.4	21.1	20.9	20.8	21.1	21.1	21.1
	21.3	21.1	20.7	21.3	21.5	21.3	20.9	20.9	21.2	21.8	21.4
	21.5	21.5	21.5	21.5		21.4	21.0	21.0	21.7		
							21.1	21.8			
1′	172.3	171.8	171.1	171.7							
2′	43.2	43.6	60.8	42.6							
3′	25.0	25.7	172.4	25.0							
4′	22.4	22.3	42.7	22.4							
5′	22.4	22.3	29.7	22.4							
6′			25.6								
7′, 8′			22.3								
OCH_3				53.0	53.2				53.0		

[a] All compounds were determined at 125 MHz with TMS as an internal standard, and measured in $CDCl_3$; chemical shifts are in parts per million.

on silica gel, Sephadex LH-20, and semi-preparative HPLC to yield compounds **1–10**. These compounds had a similar briarane core.

Juncin R (**1**) had a molecular formula of $C_{33}H_{43}ClO_{14}$ as deduced from its NMR spectra and ESIMS, which showed a pair of peaks at *m/z* 699/701 (3:1) $[M+H]^+$ suggesting one chlorine atom in **1**. Its IR and UV spectra indicated the presence of hydroxyls (3543 cm^{-1}), a γ-lactone (1790 cm^{-1}), esters (1750, 1739, and 1720 cm^{-1}), and a conjugated diene system (274 nm). The ^{13}C (DEPT) and ^1H NMR spectral data (Tables 1 and 2) showed signals for four acetate esters and an isovalerate ester [δ_C 172.3 (s), 43.2 (t), 25.0 (d), 22.4 (2q)], a tertiary methyl (δ_H 1.13, s), a secondary methyl (δ_H 1.13, d, J=7.0 Hz), a γ-lactone (δ_C 175.0), an exocyclic 11(20)-epoxide [δ_H 2.93, 3.60 (each br s), δ_C 49.1 (t), 58.4 (s)], a conjugated diene [δ_C 131.8 (d), 128.2 (d), 140.1 (s), 125.9 (d), δ_H 5.61 (t, J=9.8 Hz), 6.36 (d, J=10.5 Hz), 6.03 (d, J=8.6 Hz)], an exocyclic methylene [δ_C 44.2 (t), δ_H 4.58, 4.64 (each d, J=13.6 Hz)], an oxygenated quaternary carbon and six oxygenated methines. These data showed that **1** was a briarane-type diterpenoid with a 3,5(6)-conjugated diene and an exocyclic 11(20)-epoxide, similar to the structures of gemmacolide F,[12] juncenolides B–D,[8,12] juncins I–K,[9] and juncin Q (**11**), which we previously obtained from *J. juncea*.[11] Comparison of the ^1H and ^{13}C NMR spectral data of **1** with those of **11** revealed the difference between them, **1** had some additional signals for an acetate ester and an isovalerate ester, and a signal for a methylene bearing chlorine ($-CH_2Cl$)[13] [δ_C 44.2 (t), δ_H 4.58, 4.64 (each d, J=13.6 Hz)] in place of an oxymethylene ($-CH_2OH$) [δ_C 63.0(t), δ_H 4.63, 5.44 (each d, J=16.3 Hz) in **11**] at position C-16. The additional acetate and isovalerate groups were assigned to C-12 and C-14, respectively, because of HMBC correlations of H-12 with δ_C 170.1, and H-14/2′/3′ with C-1′ (δ_C 172.3) (Table 4). The skeletal structure of **1** was further deduced from the ^1H–^1H COSY and HMBC correlation data (Table 4).

According to a summary about the chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives, that while the ^{13}C NMR data for C-11 and C-20 appeared at δ_C 62–63 and 58–60 ppm, respectively, the epoxy group was β-oriented and the cyclohexane ring existed in a boat conformation; however, if the ^{13}C NMR data for C-11 and C-20 were shifted upfield and signals appeared at δ_C 55–61 and 47–52 ppm, respectively, the epoxy group was α-oriented and the cyclohexane ring had a chair conformation,[14] the stereochemistry of 11,20-epoxide [δ_C 58.4 (C-11), 49.1 (C-20)] in **1** should be α-oriented, and the cyclohexane ring was in a boat conformation. The *Z* configuration of the disubstituted double bond at C-3 was determined by the proton

Table 2. ^{1}H NMR spectral data of compounds **1–5**[a]

H	1	2	3	4	5
2	5.44 (d, 9.5)	5.51 (d, 9.5)	5.67 (d, 9.2)	5.57 (d, 9.0)	5.96 (d, 9.6)
3	5.61 (t, 9.8)	5.61 (t, 9.8)	5.63 (t, 9.5)	5.56 (t, 8.9)	5.69 (t, 10.2)
4	6.36 (d, 10.5)	6.36 (d, 10.5)	6.33 (d, 9.7)	6.28 (d, 8.1)	6.28 (d, 10.9)
6	6.03 (d, 8.6)	6.04 (d, 8.6)	5.70 (d, 8.4)	5.88 (d, 8.6)	5.84 (d, 8.0)
7	4.95 (d, 8.6)	4.96 (d, 8.6)	4.97 (d, 8.4)	5.00 (d, 8.6)	5.00 (d, 8.4)
9	4.73 (d, 4.6)	4.73 (d, 4.6)	4.74 (d, 4.1)	4.74 (d, 4.6)	4.80 (d, 4.3)
10	3.57 (d, 4.6)	3.56 (d, 4.6)	3.63 (d, 4.7)	3.61 (br s)	3.58 (d, 4.1)
12	4.88 (d, 2.6)	4.91 (d, 2.6)	4.86 (br s)	4.88 (d, 2.2)	3.59 (br s)
13	5.09 (t, 3.4)	5.08 (t, 3.4)	5.07 (br s)	5.09 (t, 3.7)	5.00 (t, 4.0)
14	5.24 (d, 3.0)	5.20 (d, 2.5)	5.20 (br s)	5.20 (t, 2.1)	3.74 (br s)
15	1.13 (s)	1.14 (s)	1.13 (s)	1.13 (s)	0.99 (s)
16	4.58, 4.64 (each d, 13.6)	4.55, 4.66 (each d, 13.6)	4.62, 5.41 (each d, 15.6)	4.23, 4.50 (each d, 14.9)	4.20, 4.30 (each d, 14.3)
17	2.30 (q, 7.0)	2.30 (q, 7.0)	2.30 (q, 7.0)	2.31 (q, 7.1)	2.33 (q, 7.0)
18	1.13 (d, 7.0)	1.13 (d, 7.0)	1.15 (d, 7.0)	1.16 (d, 7.1)	1.17 (d, 7.0)
20	2.93, 3.60 (each br s)	2.93, 3.60 (each br s)	2.92, 3.60 (each br s)	2.93, 3.60 (each br s)	2.76, 3.52 (each br s)
2-OAc	1.96 (s)	1.97 (s)		1.94 (s)	2.08 (s)
9-OAc	2.22 (s)	2.20 (s)	2.26 (s)	2.14 (s)	2.19 (s)
12-OAc	2.16 (s)		2.13 (s)	2.22 (s)	
13-OAc	1.95 (s)	1.94 (s)	1.96 (s)		2.17 (s)
14-OAc		2.07 (s)	2.10 (s)	2.06 (s)	
16-OAc					
2′	2.10 (2H, m)	2.11 (2H, m)	4.44, 4.54 (each d, 15.7)	2.09 (2H, m)	
3′	1.99 (1H, m)	1.99 (1H, m)		1.98 (1H, m)	
4′	0.97 (3H, d, 6.5)	0.97 (3H, d, 6.5)	2.29 (2H, m)	0.92 (6H, d, 6.5)	
5′	1.01 (3H, d, 6.5)	1.01 (3H, d, 6.5)	1.26 (2H, m)		
6′			2.14 (1H, m)		
7′ and 8′			0.98 (6H, 6.5)		
OCH_3				3.80 (3H, s)	3.81 (3H, s)

[a] All compounds were determined at 500 MHz, and measured in $CDCl_3$; chemical shift values δ are in parts per million, and coupling constant values J in hertz.

coupling constant (J=10.5 Hz) between the olefinic protons H-3 and H-4. The *E* configuration of the trisubstituted double bond at C-5 was demonstrated by the NOE correlation between H-6 and H-16 (as shown in Fig. 2). The relative stereochemistries of the 11 chiral centers of **1** were also deduced by the analysis of NOE correlations (Fig. 2). In the NOESY spectrum of **1**, NOE correlations between Me-15 with H-13/14/20, and H-20 with H-12 suggested that H-20, H-13, H-12, H-14, and Me-15 were all in the β-orientations; meanwhile, 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 α orientated, correspondingly, correlation of H-17 with H-7 suggested the β-orientations of H-17 and H-7. Based on above observation, the relative stereochemistry of juncin R (**1**) was assigned as 1*R**, 2*S**, 3*Z*, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 13*R**, 14*R**, and 17*R**, and its structure was proposed as shown in Figure 1.

Juncins S–ZI (**2–10**) were analogues of juncin R (**1**). Their stereostructure determinations were thus aided by comparison of their spectroscopic data with those of **1** and some known briarane-type diterpenoids. However, complete NMR studies (including HSQC, HMBC, ^{1}H–^{1}H COSY, and NOESY spectra) on each new compound were performed in order to unambiguously determine their structures and to assign all the proton and carbon resonances. HMBC, HSQC, and ^{1}H–^{1}H COSY spectra showed that compounds **1–6** and **7–10** contained similar oxygenation patterns meanwhile NOESY spectrum gave the relative stereochemistry information of chiral centers. Some key points for structure elucidations of compounds **2–10** are described below.

Juncin S (**2**) showed the same molecular formula of $C_{33}H_{43}ClO_{14}$ as compound **1**, which was deduced from the ESIMS and NMR data of **2**. There was almost no obvious difference between the ^{13}C and ^{1}H NMR spectral data of **2** and **1** (Tables 1 and 2), however, they were obtained from a same fraction by semi-preparative HPLC (Luna™ C18(2), 250×10 mm i.d., 5 ml min^{-1}) with different retention times (t_R) (20.3 min for **1**, and 17.3 min for **2**), using MeOH/H_2O (64:36) as eluent. Comparison of the HMBC spectrum of **2** with that of **1** revealed that the differences between them were the substituent positions of an isovalerate group and an acetate group. In **2**, an isovalerate group was attached to C-12 instead of C-14, and an acetate group was attached to C-14 instead of C-12. These were deduced from HMBC correlations of δ_H 4.91 (d, J=2.6 Hz, H-12), 2.11 (2H, m, H-2′) and 1.99 (1H, m, H-3′) with δ_C 171.8 (s, C-1′), and HMBC correlations of δ_H 5.20 (d, J=2.5 Hz, H-14) and 2.07 (s, 3H) with δ_C 170.1 (s). So, the structure of juncin S

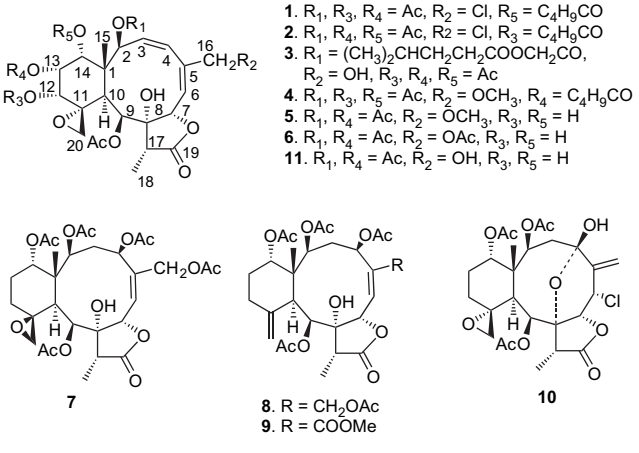

Figure 1. Structures of compounds **1–11**.

(**2**) was determined, and its relative stereochemistry was the same as that of **1**.

Juncin T (**3**) was assigned the molecular formula of $C_{36}H_{48}O_{17}$ on the basis of its ESIMS and NMR data. Comparison of overall ^1H and ^{13}C NMR spectral data (Tables 1 and 2) revealed similarities between **3** and **11**. The difference between them was the appearance of two additional ester signals in **3**. The ^{13}C (DEPT) and ^1H NMR spectral data of **3** (Tables 1 and 2) showed signals for four acetate groups assigned to C-9, C-12, C-13, and C-14 because their carbonyl carbons were correlated with the corresponding oxymethine protons in the HMBC spectrum of **3**. Other additional signals were assigned to be an $-OCOCH_2OCOCH_2CH_2CH(CH_3)_2$ unit, which was supported by the HMBC correlations of δ_H 4.44, 4.54 (each d, J=15.7 Hz, H-2′) with δ_C 171.1 (s, C-1′) and 172.4 (s, C-3′), of δ_H 2.29 (2H, m, H-4′) and 1.26 (2H, m, H-5′) with δ_C 172.4 (s, C-3′) and 25.6 (t, C-6′), and ^1H–^1H COSY spectrum showing correlations of δ_H 1.26 (2H, m, H-5′) with δ_H 2.29 (2H, m, H-4′), 2.14 (1H, m, H-6′), and δ_H 2.14 (1H, m, H-6′) with δ_H 0.98 (6H, d, J=6.5 Hz, H-7′ and 8′). NOESY spectrum proved that the relative stereochemistry of **3** was the same as that of **1**. So, the structure of juncin T (**3**) was determined as shown in Figure 1.

Juncin U (**4**) was found to have a molecular formula of $C_{34}H_{46}O_{15}$ as determined from its HRESIMS. Its ^{13}C and ^1H NMR data (Tables 1 and 2) were similar to those of **11** with the difference of some additional signals for an acetate ester, an isovalerate ester, and an oxymethyl (δ_C 53.0, q). In **4**, the ^1H and ^{13}C NMR spectra showed the existence of four acetate and one isovalerate groups which were assigned to C-2, C-9, C-12, C-14, and C-13 because their carbonyl carbons were correlated with the corresponding oxymethine protons in the HMBC spectrum. The additional oxymethyl was attached to the oxymethylene C-16 because of the HMBC correlation of δ_H 3.80 (3H, s) with δ_C 72.2 (t, C-16). On the basis of complete NMR studies, the structure of juncin U (**4**) was elucidated, and its relative stereochemistry was proved to be the same as that of **1**.

Juncin V (**5**) had a molecular formula of $C_{27}H_{36}O_{13}$ as deduced from its ESIMS and NMR data. Its ^{13}C and ^1H NMR data (Tables 1 and 2) were very similar to those of **11** with the only difference of an additional oxymethyl (δ_C 53.2, q) attached to C-16 because of the HMBC correlation of δ_H 3.81 (3H, s) with δ_C 73.1 (t, C-16). Based on complete NMR studies, the structure of juncin V (**5**) was elucidated as shown in Figure 1, and its relative stereochemistry was proved to be the same as that of **1**.

Juncin W (**6**) exhibited a molecular formula of $C_{28}H_{37}O_{14}$ as deduced from its ESIMS and NMR data. Its ^{13}C and ^1H NMR data (Tables 1 and 3) were closely similar to those of **11** with the only difference of an additional acetate group attached to the oxymethylene C-16 because of the HMBC correlations of δ_H 4.75, 5.04 (each d, J=15.6 Hz, H-16) and 2.12 (3H, s) with δ_C 170.5 (s). Based on complete NMR study, the structure of juncin W (**6**) was inferred as shown in Figure 1, and its relative stereochemistry was proved to be the same as that of **1**.

Juncin X (**7**) had a molecular formula of $C_{30}H_{40}O_{14}$ as deduced from its ESIMS and NMR data. Its ^{13}C and ^1H NMR spectral data (Tables 1 and 3) were similar to those of **6** with the obvious difference that three methylenes appeared and a C-3/4 double bond disappeared in **7**. Complete analysis of the HMBC and ^1H–^1H COSY spectral data (Table 4) showed a gross structure of **7** that was similar to

Table 3. ^1H NMR spectral data of compounds **6–10**[a]

H	6	7	8	9	10
2	5.91 (d, 10.0)	4.92 (d, 5.5)	5.24 (d, 7.2)	4.88 (d, 7.5)	5.30 (d, 7.5)
3	5.75 (t, 10.5)	1.90 (m), 2.75 (br d, 14.0)	1.81 (m), 2.80 (dd, 4.2, 14.5)	1.84 (m), 2.72 (br t, 14.5)	3.38 (dd, 7.7, 16.3), 1.59 (d, 16.3)
4	6.27 (d, 10.5)	5.01 (dd, 5.4, 12.2)	5.14 (dd, 5.6, 12.6)	5.91 (dd, 5.9, 12.2)	
6	5.69 (d, 8.6)	5.76 (d, 9.9)	5.78 (d, 10.2)	7.06 (d, 10.0)	4.90 (d, 2.4)
7	4.98 (d, 8.5)	5.53 (d, 10.0)	5.61 (d, 10.5)	5.63 (d, 10.0)	4.33 (d, 2.7)
9	4.82 (d, 4.5)	4.83 (d, 4.8)	4.82 (d, 4.8)	5.60 (d, 2.8)	5.62 (br s)
10	3.58 (d, 4.1)	3.29 (d, 2.9)	3.22 (d, 5.3)	3.29 (d, 2.4)	2.78 (br s)
12	3.61 (br s)	2.14, 2.21 (each m)	2.20 (m)	2.15 (m)	2.11, 2.04 (each m)
13	4.98 (t, 3.7)	1.93, 2.00 (each m)	1.73 (m)	1.80 (m)	2.01, 1.95 (each m)
14	3.80 (br s)	4.73 (d, 4.2)	4.73 (d, 4.6)	4.70 (br s)	5.05 (br s)
15	1.00 (s)	1.11 (1H, s)	1.11 (s)	1.03 (s)	1.22 (s)
16	4.75, 5.04 (each d, 15.6)	4.62, 5.36 (each br d, 16.3)	5.32, 4.78 (each br d, 17.3)		5.63, 5.92 (each br s)
17	2.35 (q, 7.0)	2.35 (q, 7.0)	2.48 (q, 7.0)	2.65 (q, 7.0)	2.74 (q, 7.0)
18	1.18 (d, 7.0)	1.15 (d, 7.0)	1.12 (d, 7.0)	1.20 (d, 7.0)	1.32 (d, 7.0)
20	2.76, 3.42 (each br s)	2.46 (d, 3.5), 2.80 (d, 4.0)	4.87, 4.98 (each s)	4.96, 5.0 (each s)	2.41 (d, 3.2), 2.64 (d, 2.8)
2-OAc	2.03 (s)	2.01 (s)	1.90 (s)	1.90 (s)	2.09 (s)
4-OAc		2.03 (s)	1.98 (s)	1.97 (s)	
9-OAc	2.18 (s)	2.26 (s)	2.26 (s)	2.22 (s)	2.24 (s)
12-OAc					
13-OAc	2.16 (s)				
14-OAc		2.04 (s)	2.05 (s)	2.07 (s)	2.09 (s)
16-OAc	2.12 (s)	2.13 (s)	2.13 (s)		
OCH_3				3.83 (s)	
OH					6.57 (s)

[a] All compounds were determined at 500 MHz, and measured in $CDCl_3$; chemical shift values δ are in parts per million, and coupling constant values J in hertz.

Table 4. HMBC and ^{1}H–^{1}H COSY correlation data of compounds **1**, **7**, and **10**

H	**1**		**7**		**10**	
	HMBC	^{1}H–^{1}H COSY	HMBC	^{1}H–^{1}H COSY	HMBC	^{1}H–^{1}H COSY
2	C-1, 3, 4, 10, 14, 15, Me*C*OO	H-3	C-1, 3, 4, 10, 14, 15, Me*C*OO	H-3	C-1, 3, 4, 10, 14, 15, Me*C*OO	H-3
3	C-1, 2, 5	H-2, 4	C-1, 2, 5	H-2, 4	C-1, 2, 5	H-2
4	C-2, 6	H-3	C-2, 6, Me*C*OO	H-3		
6	C-4, 5, 16	H-7	C-4, 5, 7, 16	H-7	C-5, 16	H-7
7	C-5, 6, 8, 9	H-6	C-5, 6, 8, 9	H-6	C-5, 6, 8	H-6
9	C-1, 7, 8, 10, 11, 17, Me*C*OO	H-10	C-1, 7, 8, 10, 11, 17, Me*C*OO	H-10	C-1, 7, 8, 10, 11, 17, Me*C*OO	H-10
10	C-1, 2, 8, 9, 11, 12, 14, 15, 20	H-9	C-1, 2, 8, 9, 11, 12, 14, 15, 20	H-9	C-1, 2, 8, 9, 11, 12, 14, 15, 20	H-9
12	C-11, 13, 14, Me*C*OO	H-13	C-10, 11, 13, 14	H-13	C-10, 11, 13, 14	H-13
13	C-11, 12, 14, Me*C*OO	H-12, 14	C-1, 11, 12, 14	H-12, 14	C-1, 11, 12, 14	H-12, 14
14	C-1, 1′	H-13	C-1, 2, 10, 12, 13, Me*C*OO	H-13	C-1, 2, 10, 12, 13, Me*C*OO	H-13
15	C-1, 2, 10, 14		C-1, 2, 10, 14		C-1, 2, 10, 14	
16	C-4, 5, 6		C-4, 5, 6, Me*C*OO		C-4, 5, 6	
17	C-8, 9, 18, 19	H-18	C-8, 9, 18, 19	H-18	C-8, 9, 18, 19	H-18
18	C-8, 17, 19	H-17	C-8, 17, 19	H-17	C-8, 17, 19	H-17
20	C-11, 12		C-11, 12		C-11, 12	
2′	C-1′, 3′, 4′, 5′	H-3′				
3′	C-1′, 2′, 4′, 5′	H-2′, 4′				
4′	C-2′, 3′, 5′	H-3′				
5′	C-2′, 3′, 4′	H-3′				

junceellolide I[14] and (+)-11α,20α,-epoxy-junceellolide D.[15] Comparison of the ^{1}H and ^{13}C NMR spectral data of **7** with those of (+)-11α,20α,-epoxy-junceellolide D revealed that the only difference between them is **7** had an acetoxymethylene group at C-16 instead of a methyl, which was proved by the HMBC spectrum (Table 4) showing correlations of δ_H 4.62, 5.36 (each br d, J=16.3 Hz, H-16) with δ_C 123.7 (d, C-6), 140.1 (s, C-5), and 170.7 (s).

Recently, the stereochemistry of 11,20-epoxy group in (+)-11α,20α,-epoxy-junceellolide D [δ_C 62.6 (C-11), 59.4 (C-20)] was corrected to be β-oriented.[14] Comparison of ^{13}C NMR chemical shifts of C-11 and C-20 of **7** [δ_C 62.3 (C-11), 58.9 (C-20)] with those of **1** [δ_C 58.4 (C-11), 49.1 (C-20)] and (+)-11α,20α,-epoxy-junceellolide D showed that the stereochemistry of 11,20-epoxy group in **7** should be β-oriented, leading to the configuration of cyclohexane ring in a boat form. The *E* configuration of the trisubstituted double bond at C-5 was determined by the NOE correlation between H-6 and H-16 (as shown in Fig. 2). The relative stereochemistry of the nine chiral centers of **7** was also deduced by the analysis of NOESY spectrum (Fig. 2), in which NOE correlations of H-2 with H-10/4, H-10 with H-9/20, and Me-18 with H-9/10 indicated that H-2, H-4, H-9, H-10, H-20, and Me-18 were all in the α-orientations. NOE correlations of Me-15 with H-14/17, and H-17 with H-7 suggested that H-7, 14, 17, and Me-15 were all in the β-orientations. Based on above observation, the relative stereochemistry of juncin X (**7**) was assigned as 1*R**, 2*S**, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 11*S**, 14*S**, and 17*R**, and its structure was elucidated as shown in Figure 1.

Juncin Y (**8**) analyzed for $C_{30}H_{40}O_{13}$ by HRESIMS and ^{13}C NMR spectrometry. The ^{13}C and ^{1}H NMR spectral data of **8** were closely similar to those of **7** (Tables 1 and 3). However, the ^{13}C and ^{1}H NMR spectra of **8** showed that an exocyclic 11(20)-epoxide was converted into a double bond [δ_C 150.1 (s) and 113.0 (t), δ_H 4.87, 4.98 (each 1H, s)]. This was supported by the HMBC spectrum that showed the correlations of δ_H 4.87, 4.98 (each 1H, s) with δ_C 150.1 (s, C-11) and 42.4 (d, C-10). Based on above data and the NOESY spectrum of **8**, the relative stereochemistry of **8** was assigned as 1*R**, 2*S**, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**, and its structure was elucidated as shown in Figure 1.

Juncin Z (**9**) exhibited the molecular formula of $C_{29}H_{38}O_{13}$ as deduced from its ESIMS and NMR data. Its ^{13}C and ^{1}H NMR spectral data (Tables 3 and 1) were very similar to those of **8**. The only difference between them was that an acetoxymethylene group at C-16 was transformed into a methyl esterified carboxyl group, which was supported by the HMBC spectrum with correlations of δ_H 7.06 (d, J=10.0 Hz, H-6), 3.83 (3H, s, –OMe) with δ_C 166.6 (s, C-16). It was rare that an esterified carboxyl group was placed at C-16 in briarane-type diterpenes. Based on the 1D and 2D NMR studies, the structure of juncin Z (**9**) was elucidated, and its relative stereochemistry was assigned as 1*R**, 2*S**, 5*E*, 7*S**, 8*R**, 9*S**, 10*S**, 14*S**, and 17*R**.

Juncin ZI (**10**) exhibited a molecular ion peak at *m*/*z* 556/558 (3:1) $[M+H]^+$ in its ESIMS. Together with ^{1}H and ^{13}C NMR spectral data, a molecular formula of $C_{26}H_{33}ClO_{11}$ was established and confirmed by HRESIMS. Its ^{13}C and

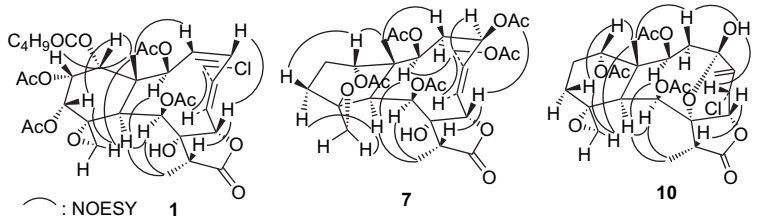

Figure 2. Key NOESY correlations for compounds **1**, **7**, and **10**.

^{1}H NMR spectral data (Tables 1 and 3) were similar to those of **7**. The main difference between them was the appearance of a characteristic quaternary hemiketal carbon (δ_C 97.4, s) and an exocyclic double bond [δ_C 138.0 (s), 117.8 (t), δ_H 5.63, 5.92 (each 1H, br s)] instead of a trisubstituted olefin in **10**. Complete analysis of the HMBC and ^{1}H–^{1}H COSY spectral data (Table 4) showed a gross structure of **10** that was similar to juncin P[11] and junceellolide A.[16] Comparison of the ^{1}H and ^{13}C NMR spectral data of **10** with those of junceellolide A revealed that the only difference between them is that a double bond was converted into an exocyclic 11(20)-epoxide [δ_C 56.2 (s) and 51.2 (t), δ_H 2.41 (1H, d, J=3.2 Hz), 2.64 (1H, d, J=2.8 Hz)] in **10**, which was supported by the HMBC spectrum with correlations of δ_H 2.41 (1H, d, J=3.2 Hz, H-20), 2.64 (1H, d, J=2.8 Hz, H-20) with δ_C 56.2 (s, C-11) and 40.5 (d, C-10).

Comparison of ^{13}C NMR chemical shifts of C-11 and C-20 of **10** with those of **1** [δ_C 58.4 (C-11), 49.1 (C-20)] showed that the stereochemistry of 11,20-epoxy group in **10** should be α-oriented, leading to the configuration of cyclohexane ring in a chair form. The relative stereochemistry of the 11 chiral centers of **10** was also deduced from its NOESY spectrum (Fig. 2). In the NOESY spectrum, NOE correlations between Me-15 with H-3β/14/20/9-OAc, and 4-OH with H-6/3β suggested that H-20, H-14, H-6, 4-OH, and Me-15 were all in the β-orientations, while 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-orientations, with corresponding correlation of H-17 with H-7 suggesting the β-orientations of H-17 and H-7. On the basis of above data, the structure of juncin ZI (**10**) was elucidated, and its relative stereochemistry was determined as 1*R**, 2*S**, 4*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 14*S**, and 17*R**.

2.1. Antifouling activity against barnacle larvae

Antifouling bioassay tests showed that the EtOAc soluble fraction of the EtOH/CH_2Cl_2 extract of *J. juncea* led to 0% larval settlement and 10% mortality toward barnacle *B. amphitrite* larvae at concentration of 100.0 μg mL^{-1}, and compounds (**1**–**10**) had potent antifouling activities at nontoxic concentrations 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^{-1}. The EC_{50} values of compounds (**1**–**10**) were lower than the standard requirement of an EC_{50} of 25 μg mL^{-1} established by the US Navy program as an efficacy level for natural antifoulants, indicating that compounds **1**–**10** are potential natural nontoxic antifouling agents. Besides renillafoulins A–C,[17] this is the second time for reporting the antifouling activities of briarane-type metabolites from marine organisms.

Based on the parent molecules of renillafoulins A–C, a preliminary structure–activity relationship study suggested that the antifouling functional group was the furan ring in briarane-type diterpenoids.[18] In our study, according to above observation, the structure–activity relationship was further summarized as follows. Comparison of the antifouling activities of compounds **1**–**6**, **8**, and **9** suggested that the potency of briarane-type diterpenoids inhibiting larval settlement could be increased as the exocyclic oxymethylene C-16 (such as –CH_2OH and –CH_2OCH_3 in **3**, **4**, and **5**) was substituted by a methylene bearing chlorine (–CH_2Cl in **1** and **2**), and decreased as the exocyclic oxymethylene C-16 was esterified (–CH_2OAc in **6**) or the acetoxymethylene C-16 (–CH_2OAc in **8**) was oxygenated to be an esterified carboxyl group (–COOMe in **9**). Further comparison of the antifouling activities of compounds **1**–**6** also indicated that the chain lengths of esters at C-1, 12, 13, and 14 could affect the potency of briarane-type diterpenoids. Moreover, compound **7** was more potent than compounds **8** and **9**, which suggested that the exocyclic 11,20-epoxy group was important for the antifouling activities of briarane-type diterpenoids.

3. Experimental

3.1. 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, and 2D NMR spectra were recorded on a Bruker DRX-500 MHz NMR spectrometer with TMS as an internal standard. MS spectral data were obtained on an LCQ^{DECA} XP HPLC/MS^n spectrometer for ESIMS. Semi-preparative HPLC was carried out on ODS columns (Phenomenex, 250×10 mm i.d., 5 ml min^{-1}) with a Waters 996 photodiode array detector. 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.

3.2. Animal material

The South China Sea gorgonian coral *J. juncea* (12 kg, wet weight) was collected in Sanya, Hainan Province, China in October 2003 and identified by Professor 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.

3.3. 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 three times. The EtOAc extract was concentrated in vacuo to afford 85 g of residue, and then 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. Fraction 5 was subjected to CC on silica gel, eluted with $CHCl_3$/Me_2CO (from 10:1 to 9:1) to give four sub-fractions (A–D). Sub-fraction A was subjected to CC on silica gel, eluted with $CHCl_3$/Me_2CO (10:1) to give **3** (3.2 mg); sub-fractions B–D were chromatographed over Sephadex LH-20 eluted with $CHCl_3$/MeOH (1:1) then purified with semi-preparative HPLC (Luna™ C18(2), 250×10 mm i.d., 5 ml min^{-1}), using MeOH/water (64:36) as eluent to yield **10** (retention time of 5.3 min, 5.9 mg), **8** (retention time of 8.3 min, 4.5 mg), **2** (retention time of 17.3 min, 4 mg), and **1** (retention time of 20.3 min, 11 mg) and MeOH/water (63:37) as eluent to yield

7 (retention time of 11.1 min, 6.0 mg), **9** (retention time of 12.8 min, 4.5 mg), and **4** (retention time of 15.3 min, 3.7 mg). Fraction 7 was repeatedly subjected to CC on silica gel and chromatographed over Sephadex LH-20, then purified with semi-preparative HPLC (Luna™ C18(2), 250×10 mm i.d., 5 ml min^{-1}), using MeOH/water (48:52) as eluent to yield **5** (retention time of 14.4 min, 3.2 mg) and **6** (retention time of 16.2 min, 14.5 mg).

3.3.1. Juncin R (1). White powder; $[\alpha]_D$ −36.2 (*c* 1.16, $CHCl_3$); IR (KBr) ν_{max} 3543, 1790, 1750, 1739, 1720, 1644 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 699 $[M+H]^+$; HRESIMS *m/z* 699.2412 $[M+H]^+$ (calcd for $C_{33}H_{44}ClO_{14}$, 699.2419).

3.3.2. Juncin S (2). White powder; $[\alpha]_D$ −32.8 (*c* 1.02, $CHCl_3$); IR (KBr) ν_{max} 3542, 1787, 1750, 1738, 1720, 1645 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 699 $[M+H]^+$; HRESIMS *m/z* 699.2413 $[M+H]^+$ (calcd for $C_{33}H_{44}ClO_{14}$, 699.2419).

3.3.3. Juncin T (3). White powder; $[\alpha]_D$ −14.0 (*c* 0.4, $CHCl_3$); IR (KBr) ν_{max} 3538, 1783, 1747, 1732, 1718, 1645 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 753 $[M+H]^+$; HRESIMS *m/z* 753.2963 $[M+H]^+$ (calcd for $C_{36}H_{49}O_{17}$, 753.2969).

3.3.4. Juncin U (4). White powder; $[\alpha]_D$ −18.9 (*c* 0.37, $CHCl_3$); IR (KBr) ν_{max} 3550, 1790, 1750, 1738, 1641 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 695 $[M+H]^+$; HRESIMS *m/z* 695.2908 $[M+H]^+$ (calcd for $C_{34}H_{47}O_{15}$, 695.2915).

3.3.5. Juncin V (5). White powder; $[\alpha]_D$ −13.1 (*c* 0.32, $CHCl_3$); IR (KBr) ν_{max} 3564, 3480, 1779, 1740, 1641 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 569 $[M+H]^+$; HRESIMS *m/z* 569.2230 $[M+H]^+$ (calcd for $C_{27}H_{37}O_{13}$, 569.2234).

3.3.6. Juncin W (6). White powder; $[\alpha]_D$ −11.7 (*c* 1.45, $CHCl_3$); IR (KBr) ν_{max} 3560, 3478, 1783, 1743, 1640 cm^{-1}; ^1H NMR spectral data, see Table 2; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 597 $[M+H]^+$; HRESIMS *m/z* 597.2178 $[M+H]^+$ (calcd for $C_{28}H_{37}O_{14}$, 597.2183).

3.3.7. Juncin X (7). White powder; $[\alpha]_D$ +21.34 (*c* 0.89, $CHCl_3$); IR (KBr) ν_{max} 3540, 1790, 1748, 1738, 1720 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 625 $[M+H]^+$; HRESIMS *m/z* 625.2492 $[M+H]^+$ (calcd for $C_{30}H_{41}O_{14}$, 625.2496).

3.3.8. Juncin Y (8). White powder; $[\alpha]_D$ +36 (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3542, 1780, 1750, 1732, 1720 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 609 $[M+H]^+$; HRESIMS *m/z* 609.2541 $[M+H]^+$ (calcd for $C_{30}H_{41}O_{13}$, 609.2547).

3.3.9. Juncin Z (9). White powder; $[\alpha]_D$ +31.57 (*c* 0.95, $CHCl_3$); IR (KBr) ν_{max} 3564, 1779, 1743, 1646 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 595 $[M+H]^+$; HRESIMS *m/z* 595.2385 $[M+H]^+$ (calcd for $C_{29}H_{39}O_{13}$, 595.2390).

3.3.10. Juncin ZI (10). White powder; $[\alpha]_D$ +9.3 (*c* 1.29, $CHCl_3$); IR (KBr) ν_{max} 3530, 1788, 1749, 1736, 1718 cm^{-1}; ^1H NMR spectral data, see Table 3; ^{13}C NMR spectral data, see Table 1; ESIMS(+) *m/z* 557 $[M+H]^+$; HRESIMS *m/z* 557.1782 $[M+H]^+$ (calcd for $C_{26}H_{34}ClO_{11}$, 557.1789).

3.4. Larval settlement bioassays

Adults of *B. amphitrite* Darwin were collected from the intertidal zone in Hong Kong (N: 22°22′, E: 114°16′) in October 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[19] and Harder et al.[20] Briefly, released nauplii were collected on sieves (90 μm). Larvae were reared to 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 six 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 one or two days, stored at 4 °C for two 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 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^{-1}). Twenty competent larvae were added to each well with 1 mL testing solution in four replicates, and wells containing 0.22-μm-sterile-filtered seawater (FSW) added with 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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