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Robustolides A–C, three new briarane-type diterpenoids from the female gorgonian coral *Ellisella robusta* (Ellisellidae)

Ping-Jyun Sung,[a,b,*] Wei-Tse Tsai,[a,b] Michael Y. Chiang,[c] Yu-Mine Su[a,b] and Jimmy Kuo[a,b]

[a]*National Museum of Marine Biology and Aquarium, Checheng, Pingtung 944, Taiwan, ROC*
[b]*Institute of Marine Biotechnology, National Dong Hwa University, Checheng, Pingtung 944, Taiwan, ROC*
[c]*Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan, ROC*

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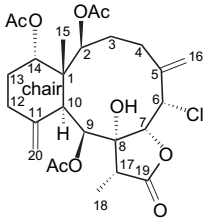
Abstract—Three new briarane-type diterpenoids, designated as robustolides A–C (**1**–**3**), were obtained from the female gorgonian coral *Ellisella robusta*. The structures of briaranes **1**–**3** were elucidated by the interpretation of spectral data analysis and the structure of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Briaranes **1** and **2** were found to show weak activity against the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

1. Introduction

Gorgonian corals are recognized as rich sources of diterpenoid derivatives with the well-known briarane carbon skeleton. Briarane metabolites are a group of diterpenoids having highly oxidized bicyclo[8.4.0] system with a γ-lactone group and compounds of this type exist only in marine organisms, particularly in octocorals.[1,2] In our screening for bioactive substances from the marine invertebrates collected at Taiwan coast, we have discovered a series of briaranes from the gorgonian corals including *Briareum* sp.,[3] *Briareum excavatum*,[4] *Junceella fragilis*,[5] and *Junceella juncea*[5c,6] and some of these metabolites have been reported to exhibit interesting biological activity. Previous studies on the gorgonian coral belonging to the genus *Ellisella* have resulted in the isolation of six briarane-type derivatives (including four new compounds).[7] In this paper, we wish to describe the isolation, structure determination, and antibacterial activity of three new briarane-type diterpenoids, robustolides A–C (**1**–**3**), from the female gorgonian coral *Ellisella robusta* (phylum Cnidaria, order Gorgonacea, family Ellisellidae)[8–10] and revise the structure of a known briarane diterpene, umbraculolide C.[11] The structures of briaranes **1**–**3** were established by spectroscopic methods and the configuration of **1** was further confirmed by a single-crystal X-ray diffraction analysis. Antibacterial activity of briaranes **1**–**3** toward the bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*, is also reported.



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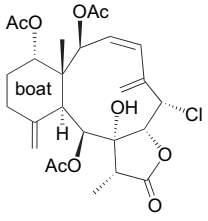

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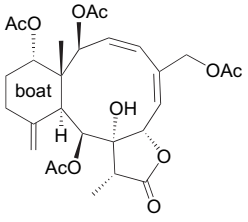

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2. Results and discussion

Specimens of the female gorgonian coral *E. robusta*, collected off southern Taiwan coast, were minced and

Keywords: Robustolide; Briarane; Diterpenoid; Gorgonian; *Ellisella robusta*; Umbraculolide C; Antibacterial activity.
* Corresponding author. Tel.: +886 8 8825037; fax: +886 8 8825087; e-mail: pjsung@nmmba.gov.tw

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extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The extract was concentrated and partitioned between EtOAc and H_2O. The EtOAc layer was successively subjected to silica gel gravity column chromatography and normal phase HPLC to afford briaranes **1**–**3**.

Robustolide A (**1**) was crystallized as colorless needles during slow evaporation of a normal phase HPLC fraction (*n*-hexane/acetone, 7:1), and had the molecular formula $C_{26}H_{35}ClO_9$ (HRESIMS, see Section 3), *m*/*z* 549/551 [3:1, $(M+Na)^+$, ESIMS]. Thus, nine degrees of unsaturation were determined for **1**. The IR spectrum showed bands at 3425, 1767, and 1728 cm^{-1}, consistent with the presence of hydroxyl, γ-lactone, and ester groups in **1**. From the ^{13}C NMR data of **1** (Table 1), two exocyclic carbon–carbon double bonds were deduced from the signals of four carbons resonating at δ 150.0 (s, C-11), 146.7 (s, C-5), 121.2 (t, C-16), and 110.2 (t, C-20). Furthermore, in the ^{13}C NMR spectrum of **1**, four carbonyl resonances appeared at δ 175.0 (s, C-19), 171.3 (s, ester carbonyl), 171.0 (s, ester carbonyl), and 170.2 (s, ester carbonyl), confirming the presence of a γ-lactone and three other ester groups. It was found that the ^1H and ^{13}C NMR spectra of **1** in $CDCl_3$ revealed several broad peaks when measured at 25 °C. However, the NMR signals for these protons and carbons of the molecule were sharpened and could be assigned by the assistance of 2D NMR spectral data analysis in cases where the NMR spectra were measured at −35 °C in $CDCl_3$. In the ^1H NMR spectrum of **1** (Table 1), three acetate methyls (δ 2.24, 3H, s; 2.01, 3H, s; 1.99, 3H, s) were observed. Based on the above data, briarane **1** was found to be tricyclic. The gross structure of **1** was determined by the assistance of 2D NMR (^1H–^1H COSY, HMQC, and HMBC) studies. From the ^1H–^1H COSY experiment of **1**, it was possible to establish the separate spin systems that map out the proton sequences from H-2/H_2-3, H_2-3/H_2-4, H-6/H-7, H-9/H-10, H_2-12/H_2-13, H_2-13/H-14, and H-17/H_3-18. From the data of an HMBC experiment of **1** (Table 1), the major molecular framework of **1** could be further established. However, as the chemical shift of C-6 methine in **1** was not observed in the ^{13}C NMR spectrum and no HMBC correlation was recorded for C-5 and C-6, the molecular framework between C-5 and C-6 cannot be fully determined by this way.

Furthermore, the acetate esters positioned at C-9 and C-14 were established by the key correlations observed in the

Table 1. ^1H and ^{13}C NMR data and HMBC correlations for diterpenoid **1** and the ^1H and ^{13}C NMR data for umbraculolide C

C/H	Robustolide A (**1**)						Umbraculolide C	
	δ_H[a]	δ_H[b]	δ_C[c]	δ_C[d]		HMBC (H→C)	δ_H[e]	δ_C[e]
1			48.4	48.8	(s)[h]			48.4
2	5.81 br s	5.92 d (8.8)	73.4	73.5	(d)	n.o.	5.65 d (8.4)	74.2
3	1.61 m	1.60 m	28.9	28.8	(t)	C-2	n.r.[i]	31.9
3′	2.53 m	2.56 dd (12.0, 12.0)						
4	2.43 br d (5.2)[f]	2.44 m	33.4	34.2	(t)	C-2, C-16	n.r.	30.4
4′		2.35 m						
5			146.7	147.8	(s)			142.5
6	4.71 br s	4.62 br s	n.o.[g]	53.4	(d)	n.o.	5.14 s	50.6
7	4.59 br s	4.45 d (3.6)	81.6	82.2	(d)	n.o.	5.03 s	78.0
8			81.5	81.8	(s)			82.0
9	5.87 br s	5.84 s	80.2	79.3	(d)	C-1, C-11, acetate carbonyl	5.81 s	75.6
10	3.41 s	3.39 s	44.0	44.4	(d)	C-1, C-2, C-8, C-11, C-12, C-14, C-15, C-20	3.48 s	43.0
11			150.0	150.1	(s)			149.6
12/12′	2.20 m	2.17 m	33.0	33.7	(t)	C-10, C-11, C-13, C-14, C-20	n.r.	27.4
13/13′	1.79 m	1.78 m	27.5	27.8	(t)	C-1, C-11	n.r.	33.3
14	4.87 br s	4.87 s	74.9	75.1	(d)	C-10, C-12, acetate carbonyl	4.88 s	74.7
15	1.03 s	0.99 s	14.5	15.0	(q)	C-1, C-2, C-10, C-14	1.05 s	14.2
16a	5.79 s	5.79 s	121.2	121.8	(t)	C-4	5.53 s	120.4
16b	5.50 s	5.50 s					n.r.	
17	2.98 q (6.8)	2.97 q (6.8)	51.4	52.3	(d)	C-7, C-8, C-18, C-19	5.28 s	43.0
18	1.18 d (6.8)	1.14 d (6.8)	6.7	6.8	(q)	C-8, C-17, C-19	1.39 d (7.0)	8.6
19			175.0	175.6	(s)			173.9
20a	4.96 s	4.36 s	110.2	110.9	(t)	C-10, C-11, C-12	4.97 s	109.5
20b	4.45 s	4.95 s					4.44 s	
OH-8	3.32 s	3.32 s				C-7, C-8		
Acetate methyls	2.24 s	2.27 s	21.6	21.9	(q)	Acetate carbonyl	2.18 s	n.r.
	2.01 s	2.01 s	21.5	21.9	(q)	Acetate carbonyl	2.04 s	n.r.
	1.99 s	2.00 s	21.5	21.8	(q)	Acetate carbonyl	2.03 s	n.r.
Acetate carbonyls			171.3	172.2	(s)			170.8
			171.0	171.9	(s)			170.4
			170.2	171.1	(s)			169.7

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 400 MHz in $CDCl_3$ at −35 °C.
[c] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[d] Spectra recorded at 100 MHz in $CDCl_3$ at −35 °C.
[e] Data were reported by Subrahmanyam et al. (see Ref. 11). These data were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C in $CDCl_3$.
[f] *J* values (in hertz) in parentheses. The values are downfield in parts per million from TMS.
[g] n.o.=not observed (broad signal).
[h] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.
[i] n.r.=not reported.

HMBC spectrum of **1**. The hydroxyl group had to be positioned at C-8, an oxygen-bearing quaternary carbon resonating at δ 81.5 ppm was confirmed from the HMBC correlations observed between the hydroxyl proton (δ_H 3.32) and C-7, C-8 (Table 1). Thus, the remaining acetoxy group should be positioned at C-2, an oxygen-bearing methine (δ_H 5.81, 1H, br s; δ_C 73.4, d), as indicated by the key ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis, although no HMBC correlation was observed between H-2 and the acetate carbonyl.

The relative stereochemistry of **1** was deduced from an NOESY experiment (Fig. 1). As per convention, when analyzing the stereochemistry of briarane-type diterpenoids, H-10 and the ring junction C-15 methyl were assigned to the α- and β-face, anchoring the stereochemical analysis because no NOE correlation was observed between H-10 and H_3-15. In the NOESY experiment of **1**, the NOE correlations of H-10 with H-2, H-9, H_3-18, and OH-8, indicated that these protons are situated on the same face and were assigned as α protons. H-14 was found to exhibit NOE responses with H-2 and H_3-15, but not with H-10, revealing the β-orientation of this proton. It was found that H-17 showed strong correlations with H-7 and H-9. Consideration of molecular models revealed that H-17 is reasonably close to H-7 and H-9 when H-17 and H-7 are β-oriented and H-9 is placed on the α face. In addition, the NOE correlation between H-6 and H-7 suggested that the chlorine atom attached at C-6 was α-oriented. Furthermore, a proton of the C-20 methylene (δ 4.45, s, H-20b) was found to exhibit a strong NOE response with H-9, but not with H-10, and H_3-15 did not show NOE response with the protons of methylene CH_2-12, indicating that the methylenecyclohexane ring in **1** should be presented as a chair rather than a boat conformation for briarane **1**.

A single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1**. The X-ray structure (Fig. 2) demonstrates the linkage between C-5/C-6, the location of an acetoxy group in β-orientation on C-2, and further confirms the chair conformation of methylenecyclohexane ring. The chiral centers in **1** were assigned as 1*R**,2*S**,6*S**,7*R**,8*R**,9*S**,10*S**,14*S**,17*R**. Based on the above findings, the structure, including the relative stereochemistry of **1**, was elucidated unambiguously.

In a previous study, the structure of **1** as we presented in this paper had been reported and named as umbraculolide C.[11] However, by detailed comparison of the NMR data of **1** with those of umbraculolide C, we found that the NMR data (^{1}H and ^{13}C) for umbraculolide C differ significantly from those of robustolide A (**1**) that we reported herein (Table 1). For example, the NMR chemical shifts, including

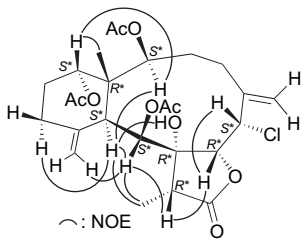

Figure 1. Selective NOESY correlations of **1**.

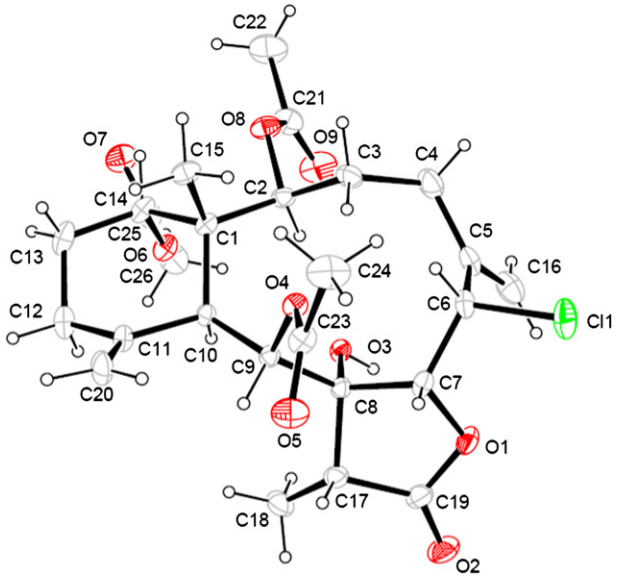

Figure 2. Computer-generated ORTEP plot of **1** showing the relative configuration.

the coupling patterns and coupling constants of C-17 methine (δ_H 5.28, s; δ_C 43.0, d) and C-18 methyl (δ_H 1.39, d, J=7.0 Hz; δ_C 8.6, q) for umbraculolide C are problematic and unreasonable by comparison with those of **1** (δ_H 2.98, q, J=6.8 Hz, H-17; δ_C 51.4, d, C-17; δ_H 1.18, d, J=6.8 Hz, H_3-18; δ_C 6.7, q, C-18). In addition, the ^{1}H NMR chemical shifts of H-2, H-6, H-7, H_2-16, and the ^{13}C NMR chemical shifts of C-2, C-3, C-4, C-5, C-6, C-7, C-9, C-10, C-12, C-13, C-19 for umbraculolide C are also found to be very different from those of **1**. Because the structure of **1** has been established by a single-crystal X-ray diffraction analysis, the authors suggest that the structure for umbraculolide C should be re-examined.

The molecular formula of robustolide B (**2**) was determined as $C_{26}H_{33}ClO_9$ by its HRESIMS. Thus, 10 degrees of unsaturation were determined for **2**. The IR spectrum showed bands at 3462, 1772, and 1731 cm^{-1}, consistent with the presence of hydroxyl, γ-lactone, and ester groups in **2**. From the ^{13}C NMR data of **2** (Table 2), the presence of a disubstituted and two exocyclic carbon–carbon double bonds were deduced from the signals of six carbons resonating at δ 148.8 (s, C-11), 138.1 (s, C-5), 131.1 (d, C-3), 128.6 (d, C-4), 117.6 (t, C-16), and 113.0 (t, C-20), and further supported by six olefin proton signals at δ 5.96 (1H, dd, J=12.0, 0.8 Hz, H-4), 5.90 (1H, d, J=2.8 Hz, H-16a), 5.89 (1H, br s, H-16b), 5.66 (1H, dd, J=12.0, 8.8 Hz, H-3), 4.96 (1H, br s, H-20a), and 4.86 (1H, br s, H-20b) in the ^{1}H NMR spectrum of **2** (Table 2). Moreover, four carbonyl resonances appeared at δ 175.4 (s, C-19), 170.4 (s, ester carbonyl), 170.0 (s, ester carbonyl), and 169.8 (s, ester carbonyl), confirming the presence of a γ-lactone and three other esters in **2**. In the ^{1}H NMR spectrum of **2**, three acetate methyls (δ 2.15, 3H, s; 2.00, 3H, s; 1.96, 3H, s) were observed. Thus, from the NMR data, seven degrees of unsaturation were accounted for, and **2** must be tricyclic. In addition, a methyl singlet (δ 1.09, 3H, s, H_3-15), a methyl doublet (δ 1.16, 3H, d, J=7.2 Hz, H_3-18), two aliphatic methine protons (δ 3.49, 1H, br s, H-10; 2.56, 1H, q,

Table 2. ^{1}H and ^{13}C NMR data and HMBC correlations for diterpenoids **2** and **3**

C/H	**2**				**3**			
	δ_H[a]	δ_C[b]		HMBC (H→C)	δ_H[a]	δ_C[b]		HMBC (H→C)
1		47.9	(s)[d]			47.5	(s)	
2	6.20 d (8.8)[c]	71.3	(d)	C-1, C-3, C-4, C-10, C-15, acetate carbonyl	5.51 d (9.6)	74.7	(d)	C-1, C-3, C-4, C-15, acetate carbonyl
3	5.66 dd (12.0, 8.8)	131.1	(d)	C-2, C-5	5.65 dd (10.0, 9.6)	133.2	(d)	C-5
4	5.96 dd (12.0, 0.8)	128.6	(d)	C-2, C-16	6.30 d (10.0)	127.4	(d)	C-2
5		138.1	(s)			140.0	(s)	
6	5.16 br s	63.2	(d)	C-5, C-8	5.73 d (10.0, 2.0)	122.8	(d)	n.o.
7	4.97 d (3.6)	78.8	(d)	C-6, C-9, C-19	5.15 d (10.0)	79.0	(d)	C-5
8		83.8	(s)			83.0	(s)	
9	5.32 d (5.2)	75.4	(d)	C-7, C-8, C-10, C-11, C-17, acetate carbonyl	5.41 d (6.8)	69.0	(d)	C-7, C-8, C-11, acetate carbonyl
10	3.49 br s	42.9	(d)	C-1, C-2, C-8, C-11	3.47 d (6.8)	42.5	(d)	C-1, C-2, C-8, C-9, C-11, C-12, C-15, C-20
11		148.8	(s)			150.6	(s)	
12α	2.20 m	27.9	(t)	C-10, C-11, C-13, C-14, C-20	2.19 m	27.2	(t)	C-11, C-20
12β	2.29 m				2.26 m			
13α	1.82 m	27.1	(t)	C-1, C-11, C-14	1.81 m	28.1	(t)	C-1, C-14
13β	1.92 m				1.94 m			
14	4.78 br s	73.8	(d)	n.o.[e]	4.83 m	74.0	(d)	n.o.
15	1.09 s	15.1	(q)	C-1, C-2, C-10, C-14	1.09 s	15.1	(q)	C-1, C-2, C-10, C-14
16a	5.90 d (2.8)	117.6	(t)	C-4, C-5, C-6	5.31 d (16.0)	63.6	(t)	C-5, C-6, acetate carbonyl
16b	5.89 br s				4.59 d (16.0)			
17	2.56 q (7.2)	46.0	(d)	C-8, C-9, C-18, C-19	2.37 q (7.2)	43.4	(d)	C-18, C-19
18	1.16 d (7.2)	7.7	(q)	C-8, C-17, C-19	1.11 d (7.2)	6.6	(q)	C-8, C-17, C-19
19		175.4	(s)			175.9	(s)	
20a	4.96 s	113.0	(t)	C-10, C-11, C-12	5.14 s	114.0	(t)	C-10, C-11, C-12
20b	4.86 s				5.02 s			
OH-8	2.91 br s			C-7, C-8, C-9, C-17	n.o.[e]			n.o.
Acetate methyls	2.15 s	21.6	(q)	Acetate carbonyl	2.18 s	22.0	(q)	Acetate carbonyl
	2.00 s	21.2	(q)	Acetate carbonyl	2.15 s	21.4	(q)	Acetate carbonyl
	1.96 s	21.1	(q)	Acetate carbonyl	1.99 s	21.2	(q)	Acetate carbonyl
					1.97 s	21.1	(q)	Acetate carbonyl
Acetate carbonyls		170.4	(s)			170.9	(s)	
		170.0	(s)			170.4	(s)	
		169.8	(s)			170.1	(s)	
						169.9	(s)	

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C.
[b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C.
[c] *J* values (in hertz) in parentheses. The values are downfield in parts per million from TMS.
[d] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.
[e] n.o.=not observed.

J=7.2 Hz, H-17), two pairs of methylene protons (δ 2.29, 1H, m, H-12β; 2.20, 1H, m, H-12α; 1.92, 1H, m, H-13β; 1.82, 1H, m, H-13α), four oxymethine protons (δ 6.20, 1H, d, *J*=8.8 Hz, H-2; 5.32, 1H, d, *J*=5.2 Hz, H-9; 4.97, 1H, d, *J*=3.6 Hz, H-7; 4.78, 1H, br s, H-14), a chlorinated methine (δ 5.16, 1H, br s, H-6), and a hydroxyl proton (δ 2.91, 1H, br s, OH-8) were observed in the ^{1}H NMR spectrum of **2**.

Similar to that of **1**, the planar structure of **2** was determined by 2D NMR studies. From the ^{1}H–^{1}H COSY spectrum of **2**, the proton sequences from H-2 to H-3, H-3 to H-4, H-6 to H-7, H-9 to H-10, H_2-12 to H_2-13, and H_2-13 to H_2-14 were established. These data, together with the correlations observed in an HMBC experiment of **2** (Table 2), established the connectivity from C-1 to C-14. The exocyclic double bonds attached at C-5 and C-11, were confirmed by the HMBC correlations between H_2-16/C-4, C-5, C-6 and H_2-20/C-10, C-11, C-12, respectively. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, C-2, C-10, C-14 and H-2/C-15. In addition, the HMBC correlations also indicated that the acetoxy groups should attach at C-2 and C-9. The C-8 hydroxyl group was also confirmed from the HMBC correlations observed between the hydroxyl proton (δ_H 2.91) and C-7, C-8, C-9, and C-17. Thus, the remaining acetoxy group should be at C-14, an oxygen-bearing methine (δ_H 4.78, 1H, br s; δ_C 73.8, d), as indicated by the key ^{1}H–^{1}H COSY correlations and characteristic NMR signal analysis, although no HMBC correlation was observed between H-14 and the acetate carbonyl. These observations, together with the ^{1}H–^{1}H COSY correlations between H-17 and H_3-18 and the HMBC correlations between H-17/C-8, C-9, C-18, C-19 and H_3-18/C-8, C-17, C-19, unambiguously established the molecular framework of briarane **2**.

The relative stereochemistry of **2** was elucidated from the NOE interactions observed in an NOESY experiment (Fig. 3) and by the vicinal ^{1}H–^{1}H coupling constant analysis. Due to the α-orientation of H-10, the ring junction C-15 methyl should be β-oriented as no NOE correlation was observed between H-10 and H_3-15. In the NOESY spectrum of **2**, H-10 displayed correlations with H-2, H-9, and OH-8, and OH-8 displayed correlation with H_3-18, suggesting that these protons are located on the same face of the molecule and assigned as α protons. H-14 was found to exhibit NOE responses with H-2 and H_3-15, but not with H-10, showing that this proton was positioned on the equatorial direction and has a β-orientation at C-14. H-7 showed strong NOE

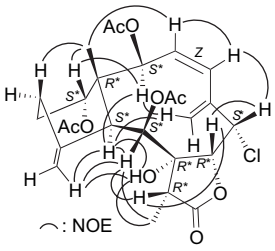

Figure 3. Selective NOESY correlations of **2**.

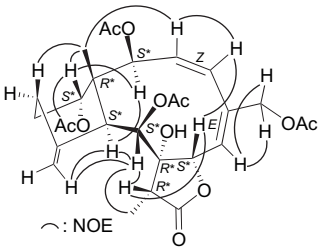

Figure 4. Selective NOESY correlations of **3**.

correlations with H-6 and H-17, suggesting that these protons are on the β-face of **2**. The *cis* geometry of the C-3/C-4 double bond is indicated by a 12.0 Hz coupling constant between H-3 (δ 5.66) and H-4 (δ 5.96) and by a strong NOE correlation between H-3 and H-4. Furthermore, the olefinic proton H-3 showed a strong NOE correlation with H_3-15, but not with H-2; the olefinic proton H-4 showed a strong response with H-6, and H_2-16 showed NOE correlations with H-2 and H-10, demonstrating the *s-cis* diene configuration of $\Delta^{3,5(16)}$. In the configuration of methylenecyclohexane ring of **2**, a proton of the C-20 methylene (δ 4.86, H-20b) was found to exhibit NOE correlations with H-9, H-10, and OH-8, and H_3-15 showed a strong NOE correlation with H-12β, indicating that the methylenecyclohexane ring of **2** should be presented as a boat rather than a chair conformation for briarane **2**, based on the consideration of a 3D model of **2**, and the chiral centers for briarane **2** are assigned as 1*R**,2*S**,3*Z*,6*S**,7*R**,8*R**,9*S**,10*S**,14*S**,17*R**.

The new briarane diterpene, robustolide C (**3**), had a molecular formula of $C_{28}H_{36}O_{11}$ as deduced from its HRESIMS. Thus, 11 degrees of unsaturation were deduced for **3**. The IR spectrum of **3** indicated the presence of hydroxyl (3465 cm^{-1}), γ-lactone (1767 cm^{-1}), and ester (1740 cm^{-1}) groups in **3**. From the ^{13}C NMR data of **3** (Table 2), a trisubstituted olefin, a disubstituted olefin, and an exocyclic carbon–carbon double bond were deduced from the signals of six carbons resonating at δ 150.6 (s, C-11), 140.0 (s, C-5), 133.2 (d, C-3), 127.4 (d, C-4), 122.8 (d, C-6), and 114.0 (t, C-20). Moreover, in the ^{13}C NMR spectrum of **3**, five carbonyl resonances appeared at δ 175.9 (s, C-19), 170.9 (s, ester carbonyl), 170.4 (s, ester carbonyl), 170.1 (s, ester carbonyl), and 169.9 (s, ester carbonyl), supporting the presence of a γ-lactone and four additional ester groups in **3**. The esters were identified as acetates by the presence of four methyl resonances in the ^1H NMR spectrum at δ 2.18 (3H, s), 2.15 (3H, s), 1.99 (3H, s), and 1.97 (3H, s) (Table 2). Based on the above data, briarane **3** was found to be a tricyclic compound. The planar structure of **3** was determined by the assistance of 2D NMR experiments. The coupling information in the ^1H–^1H COSY experiment of **3** enabled identification of the C-2/C-3/C-4, C-6/C-7, C-9/C-10, C-12/C-13/C-14, and C-17/C-18 units. From these data, together with the observations of an HMBC experiment of **3** (Table 2), the molecular framework of **3** could be further established. Furthermore, the HMBC correlations revealed that the acetate groups are attached at C-2, C-9, and C-16. Thus, the remaining hydroxyl and acetate groups should be positioned at C-8 or C-14.

The relative stereochemistry of **3** was also deduced from an NOESY experiment (Fig. 4) and by the vicinal ^1H–^1H coupling constant analysis. The *cis* geometry of the C-3/C-4 double bond is indicated by a 10.0 Hz coupling constant between H-3 (δ 5.65) and H-4 (δ 6.30) and by a strong NOE correlation between H-3 and H-4. The olefinic proton H-6 showed strong responses with H_2-16, demonstrating the *E*-configuration of $\Delta^{5,6}$. The boat conformation for the methylenecyclohexane unit of **3** was elucidated by the NOE correlations and by comparing the NMR (^1H and ^{13}C) data of **3** with those of briaranes **1** and **2**. Furthermore, by detailed analysis, H-14 was found to exhibit NOE correlations with H-2, H_3-15, and the proton signals of an acetate methyl. Thus, the remaining acetate group should be positioned in the α-orientation on C-14, and the hydroxyl group was attached at C-8. Based on the description in previous reviews,[1,2] in the briarane derivatives possessing the β-hydroxy-γ-lactone system, the C-8 hydroxyl group is almost α-oriented and only two 8-hydroxybriaranes, briaexcavatolides K and L,[12] were found. By comparing the ^{13}C NMR chemical shifts for C-8 of **3** (δ 83.0, s) with those of **2** (δ 83.8, s) and the known briaranes, briaexcavatolides K (δ 81.1, s) and L (δ 81.2, s), the 8-hydroxyl group in **3** should be α-oriented. Based on the above findings, the chiral centers for **3** were assigned as 1*R**,2*S**,3*Z*,5*E*,7*S**,8*R**,9*S**,10*S**,14*S**,17*R**, and the structure of **3** was elucidated unambiguously.

In the biological activity testing, compounds **1** and **2** exhibited weak activity in standard agar disk diffusion assay against the Gram-negative bacteria *P. aeruginosa*, each causing a 0.5 mm zone inhibition (200 μg/ml, diameter of the paper disk was 7.0 mm). The Gram-positive bacteria *S. aureus* was inhibited by **1**, the zone being 0.5 mm (200 μg/ml, diameter of the paper disk was 7.0 mm). Briarane **3** was not active toward the above two bacteria.

3. Experimental

3.1. General experimental procedures

Melting points were measured on a FARGO apparatus and were uncorrected. Optical rotation values were measured with a JASCO P-1010 digital polarimeter at 25 °C. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrometer. ESIMS and HRESIMS data were recorded by ESI FT-MS on a BRUKER APEX II mass spectrometer. NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^1H and 100 MHz for ^{13}C. Chemical shifts are given on a δ (ppm) scale with $CHCl_3$ (^1H, 7.26 ppm) and $CDCl_3$ (^{13}C, 77.0 ppm) as the internal standards. Column chromatography was performed on Si 60 (230–400 mesh) (Merck,

Darmstadt, Germany). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck, Darmstadt, Germany) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed by using a system comprised of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A semi-preparative column (Hibar 250×25 mm, LiChrospher Si 60, 5 μm) was used for HPLC. All solvents and reagents used were analytical grade.

3.2. Animal material

Specimen of the female gorgonian coral *E. robusta* was collected by hand using scuba off the coast of southern Taiwan in Aug 2006, at a depth of 20 m. This organism was identified by comparison with previous description.[8–10] Living reference specimens are being maintained in the authors' marine organism cultivating tanks and a voucher specimen was deposited in the National Museum of Marine Biology and Aquarium (NMMBA), Taiwan.

3.3. Extraction and isolation

The freeze-dried and minced material of the female gorgonian coral *E. robusta* (wet weight 664 g, dry weight 333 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1) at room temperature. The residue was partitioned between EtOAc and H_2O. The EtOAc layer was separated on silica gel and eluted using *n*-hexane/EtOAc (stepwise, 20:1–pure EtOAc) to yield fractions 1–25. Fractions 11 and 15 were purified by normal phase HPLC, using the mixtures of *n*-hexane and acetone as a mobile phase to afford briaranes **1** (7:1) and **2** (3:1), respectively, and the fraction 17 was purified by normal phase HPLC, using the mixtures of DCM and acetone as a mobile phase to afford briarane **3** (9:1).

3.3.1. Robustolide A (1). White powder (11.0 mg); mp 178–180 °C; $[\alpha]_D^{25}$ +78 (*c* 0.55, $CHCl_3$); IR (neat) ν_{max} 3425, 1767, 1728 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESIMS *m/z* 549 $(M+Na)^+$, 551 $(M+2+Na)^+$; HRESIMS *m/z* 549.1865 (calcd for $C_{26}H_{35}ClO_9$+Na, 549.1867).

3.3.2. Robustolide B (2). White powder (5.8 mg); mp 179–180 °C; $[\alpha]_D^{25}$ −124 (*c* 0.29, $CHCl_3$); IR (neat) ν_{max} 3462, 1772, 1731 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 2; ESIMS *m/z* 547 $(M+Na)^+$, 549 $(M+2+Na)^+$; HRESIMS *m/z* 547.1711 (calcd for $C_{26}H_{33}ClO_9$+Na, 547.1711).

3.3.3. Robustolide C (3). White powder (1.6 mg); mp 182–183 °C; $[\alpha]_D^{25}$ −83 (*c* 0.08, $CHCl_3$); IR (neat) ν_{max} 3465, 1767, 1740 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 2; ESIMS *m/z* 571 $(M+Na)^+$; HRESIMS *m/z* 571.2153 (calcd for $C_{28}H_{36}O_{11}$+Na, 571.2155).

3.4. Single-crystal X-ray crystallography of robustolide A (1)[13]

Suitable colorless prisms of **1** were obtained from a solution of EtOH. The crystal (0.80×0.60×0.25 mm) belongs to the monoclinic system, space group $P4_3$ (# 78) with a=11.5391(12) Å, b=11.5391(12) Å, c=20.494(4) Å, V=2728.8(7) $Å^3$, Z=4, D_{calcd}=1.283 g/cm^3, λ (Mo Kα)= 0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 52°. All 3872 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-squares procedure. The refined structural model converged to a final $R1$=0.0320, $wR2$=0.0814 for 2349 observed reflections [$I>2\sigma(I)$] and 331 variable parameters.

3.5. Antimicrobial assays

Briaranes **1–3** were assayed for antibacterial activity against the bacteria *P. aeruginosa* (Gram-negative) and *S. aureus* (Gram-positive). The standard agar diffusion assay was carried out according to the procedure described previously.[14]

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