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Chlorinated briarane-type diterpenoids from the gorgonian coral *Ellisella robusta* (Ellisellidae)

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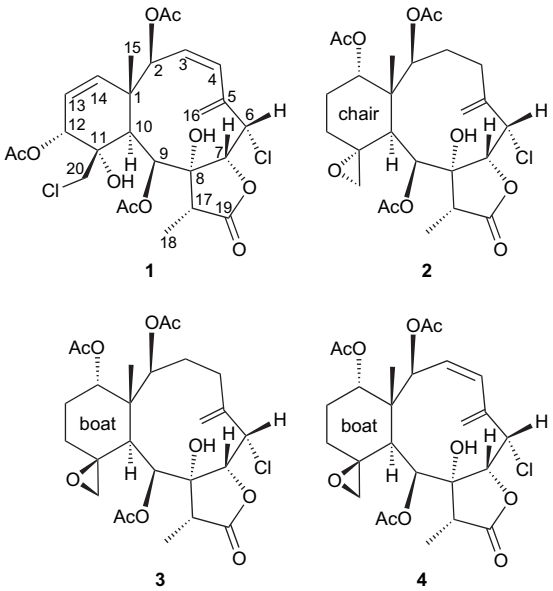
Abstract—Four chlorinated metabolites featuring briarane carbon skeletons have been isolated from the gorgonian coral *Ellisella robusta*, which was collected off the coast of southern Taiwan: two new natural products, robustolides D (**1**) and E (**2**), and two known metabolites, robustolides F (**3**) and G (**4**). The structures of metabolites **1**–**4** were determined by spectroscopic methods, using 1D and 2D NMR in particular. The structures and absolute stereochemistry of robustolides D (**1**), F (**3**), and G (**4**) were directly established by X-ray diffraction analysis. Robustolide D (**1**) is the first metabolite of briarane-related natural products found to possess two halogen atoms.

1. Introduction

Since 1977, when the first briarane-type natural product, briarein A, was obtained from the Caribbean gorgonian coral *Briareum asbestinum*,[1] a series of 3,8-cyclized cembranoid (briarane) compounds have been reported, and the number is still increasing. Most compounds of this type contain a bicyclo[8.4.0] system and include a γ-lactone moiety in their structure. These compounds continue to attract investigation because of their structural novelty, complexity, and interesting biological activities.[2,3] We recently reported the isolation of three new briaranes, robustolides A–C,[4] from the gorgonian coral *Ellisella robusta* (Anthozoa, Gorgonacea, Ellisellidae).[5–7] In continuation of our study of the structural diversity of Taiwanese marine invertebrates, we have further isolated four highly functionalized diterpenoids with a C_{20} briarane carbon skeleton, named as robustolides D–G (**1**–**4**), from *E. robusta*. The structures of briaranes **1**–**4** were elucidated by spectral data analyses and by comparison with the spectral data of related metabolites.[8] The absolute configurations of briaranes **1**, **3**, and **4** were directly established by X-ray diffraction analysis.



2. Results and discussion

Robustolide D (**1**) was obtained as a white powder and recrystallized as colorless needles. The molecular formula for **1** was determined to be $C_{26}H_{32}Cl_2O_{10}$ (10 degrees of

Keywords: Briarane; Robustolide; *Ellisella robusta*; X-ray diffraction.
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Table 1. ^{1}H and ^{13}C NMR data for diterpenoids **1** and **2**

C/H	**1**		**2**	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1		45.6 s[e]		48.0 s
2	6.01 d (9.2)[c]	77.5 d	5.83 d (8.4)	73.4 d
3α/β	5.54 dd (12.0, 9.2)	128.6 d	1.60 m; 2.66 br s	28.5 t
4/4′	5.95 d (12.0)	130.0 d	2.44 m (2H)	33.2 t
5		137.3 s		148.0 s
6	5.07 br s	61.6 d	4.66 d (2.8)	n.o.[f]
7	5.03 d (4.4)	79.1 d	4.48 br s	81.2 d
8		82.8 s		81.3 s
9	5.60 d (6.0)	68.8 d	5.72 s	69.5 d
10	3.48 d (6.0)	39.1 d	3.19 s	40.6 d
11		77.4 s		57.1 s
12α/β	5.48 dd (5.2, 1.2)	68.2 d	2.13 m; 1.20 m	30.4 t
13/13′	5.79 dd (10.4, 5.2)	121.0 d	1.91 m (2H)	24.5 t
14	5.79 d (10.4)	143.1 d	4.94 dd (2.8, 2.8)	74.1 d
15	1.07 s	15.8 q	1.15 s	14.5 q
16a/b	5.95 d (2.0); 6.18 br s	117.1 t	5.48 s; 5.77 s	121.0 t
17	2.28 q (7.2)	45.7 d	2.98 q (7.2)	51.5 d
18	1.20 d (7.2)	7.2 q	1.26 d (7.2)	6.6 q
19		174.9 s		174.8 s
20a/b	3.79 d (11.6); 4.10 d (11.6)	49.0 t	2.23 dd (3.6, 2.0); 2.66 dd (3.6, 1.6)	51.5 t
OH-8	5.01 br s[d]		3.32 s	
OH-11	3.79 br s[d]			
Acetate methyls	2.17 s	22.1 q	2.21 s	21.2 q
	2.12 s	21.3 q	2.02 s	21.2 q
	2.11 s	21.0 q	2.02 s	21.2 q
Acetate carbonyls		172.0 s		170.9 s
		170.6 s		170.7 s
		169.8 s		169.6 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 (Hz) in parentheses. The values are downfield in parts per million from TMS.
[d] Data exchangeable.
[e] Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.
[f] n.o.=not observed (broad signal).

unsaturation) by analyses of ^{1}H and ^{13}C NMR data (Table 1) in conjunction with DEPT results, and this conclusion was further confirmed by HRESIMS analysis ($C_{26}H_{32}Cl_2O_{10}$+Na: found, 597.1272; calcd, 597.1270). Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be two exchangeable protons, requiring the presence of two hydroxy groups, and this deduction was supported by a broad absorption in the IR spectrum at 3334 cm^{-1}. The IR spectrum of **1** also showed strong bands at 1770 and 1739 cm^{-1}, consistent with the presence of γ-lactone and ester groups. From the ^{13}C NMR data of **1** (Table 1), the presence of two disubstituted olefins and an exocyclic olefin was deduced from the signals of carbon resonating at δ 143.1 (d, CH-14), 137.3 (s, C-5), 130.0 (d, CH-4), 128.6 (d, CH-3), 121.0 (d, CH-13), and 117.1 (t, CH_2-16), and further supported by six olefin proton signals at δ 6.18 (1H, br s, H-16b), 5.95 (1H, d, *J*=2.0 Hz, H-16a), 5.95 (1H, d, *J*=12.0 Hz, H-4), 5.79 (1H, dd, *J*=10.4, 5.2 Hz, H-13), 5.79 (1H, d, *J*=10.4 Hz, H-14), and 5.54 (1H, dd, *J*=12.0, 9.2 Hz, H-3) in the ^{1}H NMR spectrum of **1** (Table 1). Moreover, four carbonyl resonances were apparent at δ 174.9 (s, C-19), 172.0 (s, acetate carbonyl), 170.6 (s, acetate carbonyl), and 169.8 (s, acetate carbonyl), confirming the presence of a γ-lactone group and three ester groups in **1**; three acetate methyls (δ 2.17, 3H, s; 2.12, 3H, s; 2.11, 3H, s) were also observed. So, from the NMR data, seven degrees of unsaturation were accounted for, and **1** must be a tricyclic compound. In addition, a methyl singlet (δ 1.07, 3H, s, H_3-15), a methyl doublet (δ 1.20, 3H, d, *J*=7.2 Hz, H_3-18), two aliphatic protons (δ 3.48, 1H, d, *J*=6.0 Hz, H-10; 2.28, 1H, q, *J*=7.2 Hz, H-17), four oxymethine protons (δ 6.01, 1H, d, *J*=9.2 Hz, H-2; 5.60, 1H, d, *J*=6.0 Hz, H-9; 5.48, 1H, dd, *J*=5.2, 1.2 Hz, H-12; 5.03, 1H, d, *J*=4.4 Hz, H-7), a chlorinated methine proton (δ 5.07, 1H, br s, H-6), a pair of chlorinated methylene protons (δ 3.79, 1H, d, *J*=11.6 Hz, H-20a; 4.10, 1H, d, *J*=11.6 Hz, H-20b), and two hydroxy protons (δ 5.01, 1H, br s; 3.79, 1H, br s, OH-8 and OH-11) were observed in the ^{1}H NMR spectrum of **1**.

The gross structure of **1** and all ^{1}H and ^{13}C NMR data associated with the molecule were determined and verified by 2D NMR studies. ^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of the C2–C3–C4, C6–C7, C9–C10, and C12–C13–C14 units. From these data and the HMBC correlations (Fig. 1 and Table 2), the connectivity from C-1 to C-14 could be established. An exocyclic double bond attached at C-5 was confirmed by the allylic coupling between H_2-16 and H-6 in the ^{1}H–^{1}H COSY experiment of **1** and by the HMBC correlations between H_2-16/C-4, C-5, C-6 and H-4/C-16. The presence of a chlorinated methylene attached at oxygenated quaternary carbon C-11 was confirmed by the connectivity between H_2-20/C-11, C-12, and H-10/C-20. The ring junction C-15 methyl group was positioned at C-1 from key HMBC correlations between H_3-15/C-1, C-2, C-10, C-14, H-2/C-15, and H-10/C-15. Furthermore, the acetate esters positioned at C-2 and C-9 were established by key correlations between H-2 (δ 6.01), H-9 (δ 5.60) and the

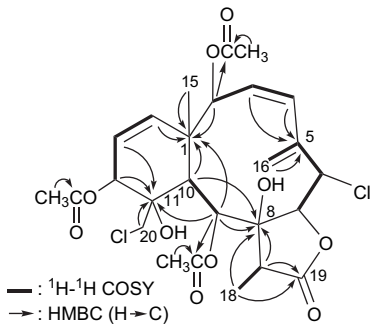

Figure 1. The ^{1}H–^{1}H COSY and selective HMBC correlations (protons and quaternary carbons) of **1**.

Table 2. The HMBC correlations for diterpenoids **1** and **2** (H→C)

Position	1	2
H-2	C-1, 4, 10, 15, acetate carbonyl	C-15, acetate carbonyl
H-3	C-5	n.o.
H-4	C-2, 3, 5, 6, 16	C-2, 3, 16
H-6	n.o.[a]	n.o.
H-7	C-6	n.o.
H-9	C-1, 7, 8, 10, 11, 17, acetate carbonyl	C-1, 7, 8, 10, 17, acetate carbonyl
H-10	C-1, 8, 9, 14, 15, 20	C-1, 8, 11, 12, 14
H-12	C-10, 11, 13, 14	C-11, 20
H-13	C-11, 12	n.o.
H-14	C-1, 10, 12	C-10, 12, acetate carbonyl
H-15	C-1, 2, 10, 14	C-1, 2, 10, 14
H-16	C-4, 5, 6	C-4
H-17	C-8, 9, 19	C-7, 8, 18, 19
H-18	C-8, 17, 19	C-8, 17, 19
H-20	C-11, 12	n.o.
OH-8	n.o.	C-7, 8, 9
OH-11	n.o.	

[a] n.o.=not observed.

acetate carbonyls observed in the HMBC spectrum of **1**. The remaining acetoxy group was positioned at C-12, an oxygen-bearing methine (δ_H 5.48, 1H, dd, J=5.2, 1.2 Hz; δ_C 68.2, d), as indicated by analysis of key ^{1}H–^{1}H COSY correlations and characteristic NMR signals, although no HMBC correlation was observed between H-12 and the acetate carbonyl. Thus, the hydroxy groups had to be positioned at C-8 and C-11. These data, together with the ^{1}H–^{1}H COSY correlation between H-17 and H_3-18, and HMBC correlations between H-17/C-8, C-9, C-19 and H_3-18/C-8, C-17, C-19, were used to establish the molecular framework of **1**.

The relative stereochemistry of **1** was deduced mainly from the NOE interactions observed in a NOESY experiment (Fig. 2) and by vicinal ^{1}H–^{1}H coupling constant analysis. As per convention, when analyzing the stereochemistry of briarane-type diterpenoids, H-10 and the ring junction C-15 methyl group were assigned to the α and β faces, respectively, anchoring stereochemical analysis, as no NOE correlation was observed between H-10 and H_3-15. In the NOESY experiment of **1**, an NOE correlation of H-10 with H-2 suggested that these two protons are located on the same face and can be assigned as α protons, as C-15 methyl is β-oriented and H-10 did not show correlation with H_3-15. The chlorinated C-20 methylene protons were found to exhibit NOE responses with H-12 and H_3-15, but not with H-10, showing that the C-20 methylene and 12-acetoxy

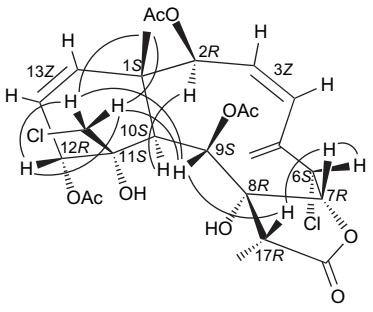

Figure 2. Selective NOESY correlations of **1**.

groups in **1** were β- and α-oriented, respectively. H-9 was found to show NOE correlations with H-17 and H_2-20. From molecular models, H-9 was found to be reasonably close to H-17 and H_2-20 and can therefore be placed on the α face in the 10-membered ring of **1**, H-17 is β-oriented in the γ-lactone ring. Furthermore, H-7 exhibited strong NOE interactions with H-17 and H-6, indicating that these protons are on the β face of **1**. The *cis* geometry of the C-3/C-4 double bond was indicated by a 12.0 Hz coupling constant between H-3 (δ 5.54) and H-4 (δ 5.95); a 10.4 Hz coupling constant observed between H-13 (δ 5.79) and H-14 (δ 5.79) confirmed that the C-13/C-14 double bond is also of *cis* geometry. However, no NOE response was observed between OH-8 and any proton in the NOESY experiment of **1**, so the stereochemistry of the hydroxy group at C-8 cannot be determined by this method.

Single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1**. The X-ray structure (Fig. 3) demonstrates the location of the hydroxy group on the α-orientation of C-8, and the $\Delta^{3,5(16)}$-butadiene system was found to be present in an *s-cis* system. Based on the X-ray diffraction analysis, the chiral centers in **1** were assigned as 1*S*, 2*S*, 3*Z*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 13*Z*, and 17*R*. From the above findings, the structure, including

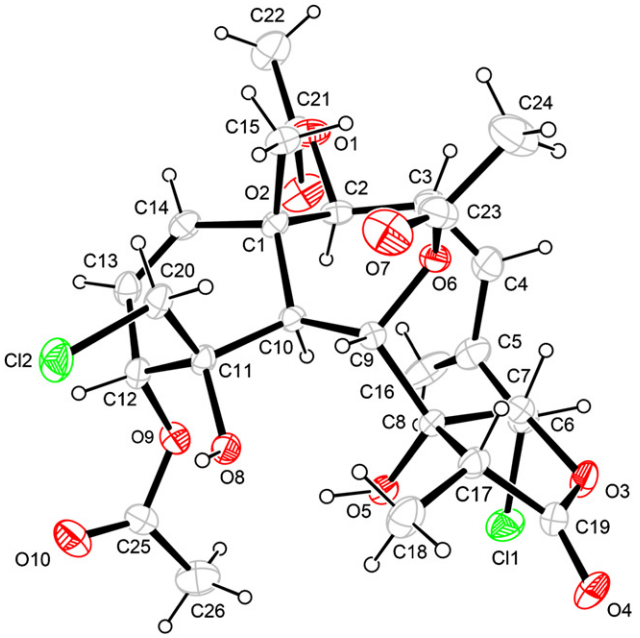

Figure 3. Computer-generated ORTEP plot of **1** showing the absolute configuration.

the absolute configuration, of **1** was therefore elucidated unambiguously.

Robustolide E (**2**) was isolated as a white powder that gave an $(M+Na)^+$ ion with *m/z* 565.1819 in the HRESIMS analysis, appropriate for the molecular formula $C_{26}H_{35}ClO_{10}Na$ (calcd for $C_{26}H_{35}ClO_{10}$+Na, 565.1816). Thus, nine degrees of unsaturation were determined for **2**. Inspection of the IR spectrum revealed strong absorptions indicative of hydroxy (3453 cm^{-1}), γ-lactone (1789 cm^{-1}), and ester (1727 cm^{-1}) groups. From the ^{13}C NMR data of **2** (Table 1), an exocyclic carbon–carbon double bond was deduced from the signals of two carbons resonating at δ 148.0 (s, C-6) and 121.0 (t, CH_2-16) and further supported by two olefin proton signals at δ 5.77 (1H, s, H-16b) and 5.48 (1H, s, H-16a) in the ^1H NMR spectrum of **2** (Table 1). Moreover, four carbonyl resonances appeared at δ 174.8 (s, C-19), 170.9 (s, ester carbonyl), 170.7 (s, ester carbonyl), and 169.6 (s, ester carbonyl), confirming the presence of a γ-lactone and three other ester groups in **2**. In the ^1H NMR spectrum of **2**, three acetate methyls (δ 2.21, 3H, s; 2.02, 2×3H, s) were observed. Thus, from the NMR data, five degrees of unsaturation were accounted for, and **2** must be tetracyclic. An exocyclic epoxy group was confirmed from the signals of two oxygenated carbons at δ 57.1 (s, C-11) and 51.5 (t, CH_2-20). The proton chemical shifts of H_2-20 (δ 2.23 dd, *J*=3.6, 2.0 Hz, H-20a; 2.66, dd, *J*=3.6, 1.6 Hz, H-20b) confirmed the presence of this group. In addition, a methyl singlet (δ 1.15, 3H, s, H_3-15), a methyl doublet (δ 1.26, 3H, d, *J*=7.2 Hz, H_3-18), two aliphatic methine protons (δ 3.19, 1H, s, H-10; 2.98, 1H, q, *J*=7.2 Hz, H-17), four pairs of aliphatic methylene protons (δ 1.60, 1H, m; 2.66, 1H, br s, H_2-3; 2.44, 2H, m, H_2-4; 2.13, 1H, m; 1.20, 1H, m, H_2-12; 1.91, 2H, m, H_2-13), four oxymethine protons (δ 5.83, 1H, d, *J*=8.4 Hz, H-2; 5.72, 1H, s, H-9; 4.94, 1H, dd, *J*=2.8, 2.8 Hz, H-14; 4.48, 1H, br s, H-7), a chlorinated methine proton (δ 4.66, 1H, d, *J*=2.8 Hz, H-6), and a hydroxy proton (δ 3.32, 1H, s, OH-8) were observed in the ^1H NMR spectrum of **2**.

The gross structure of **2** was determined using 2D NMR studies. From the ^1H–^1H COSY experiment of **2** (Fig. 4), it was possible to establish the spin system that maps 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-7/H_3-18. The allylic coupling between H-6 and H_2-16 and the *w*-coupling between H-20b and H-10 were also observed in ^1H–^1H COSY spectrum of **2**. Based on these data and the HMBC correlations (Fig. 4 and Table 2), the carbon skeleton of **2** could be established. An exocyclic double bond attached at C-5 was confirmed by the HMBC correlations between H_2-16/C-4 and H_2-4/C-16. The epoxy group positioned at C-11/20 was confirmed by the connectivity between H_2-12/C-11, 20. The ring junction C-15 methyl group was positioned at C-1 from key HMBC correlations between H_3-15/C-1, 2, 10, 14, and H-2/C-15; the HMBC correlations also indicated that three acetoxy groups are attached at C-2, C-9, and C-14. Thus, the remaining hydroxy group is at C-8, an oxygen-bearing quaternary carbon resonating at δ 81.3 (s). This observation was further confirmed by the HMBC correlations observed between OH-8/C-7, 8, 9. These data, together with the HMBC correlations between H-17/C-7, 8, 18, 19 and H_3-18/C-8, 17, 19, were used to establish the molecular framework of **2**.

The chemical shifts of exocyclic 11,20-epoxy groups in briarane derivatives have been summarized, and although the ^{13}C NMR peaks for C-11 and C-20 appear at δ 55–61 and 47–52 ppm, respectively, the epoxy group is α-oriented (11*R**), and the cyclohexane ring is of a chair conformation.[9] Based on the above observations, the configuration of the 11,20-epoxy group in **2** (δ 57.1, s, C-11; 51.5, t, CH_2-20) should be α and the cyclohexane ring in **2** should be in a chair conformation. The relative stereochemistry of **2** was elucidated from the NOE interactions observed in a NOESY experiment (Fig. 5). Due to the α-orientation of H-10, the ring junction C-15 methyl group 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 gives NOE correlations to H-2, H-9 and one proton of the C-12 methylene (δ 2.13), and H-2 showed NOE correlation with OH-8, suggesting that these protons (H-2, OH-8, H-9, H-10, H-12α) are located on the same face and can be assigned as α protons, as the C-15 methyl group is β-oriented and H-10 did not show correlation with H_3-15. H-14 was found to exhibit a strong NOE response with H_3-15, but not with H-10, showing that this proton is of β-orientation. H-9 was found to show NOE correlations with H-10, H-17, and H_3-18, and, from molecular models, was found to be reasonably close to H-10, H-17, and H_3-18; therefore, H-9 should be placed on the α face in **2**, and H-17 and H_3-18 are β- and α-oriented in the γ-lactone ring, respectively. Furthermore, H-7 exhibited strong NOE correlations with H-17 and H-6, suggesting that these protons are on the β face of **2**. Based on the above findings, the configurations of all chiral centers of **2** were assigned to be 1*S**, 2*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 14*S**, and 17*R**.

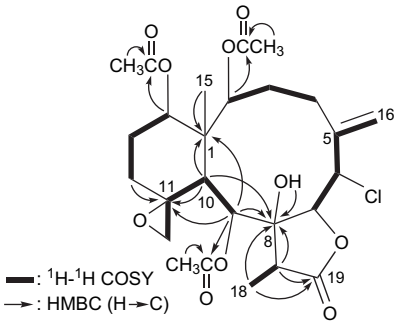

Figure 4. The ^1H–^1H COSY and selective HMBC correlations (protons and quaternary carbons) of **2**.

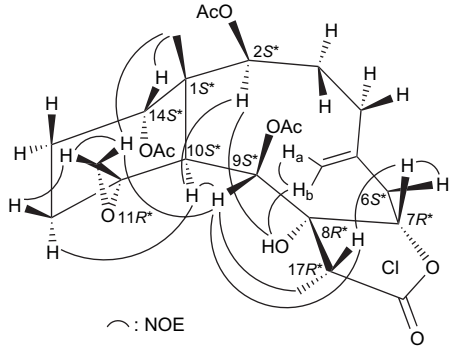

Figure 5. Selective NOE correlations of **2**.

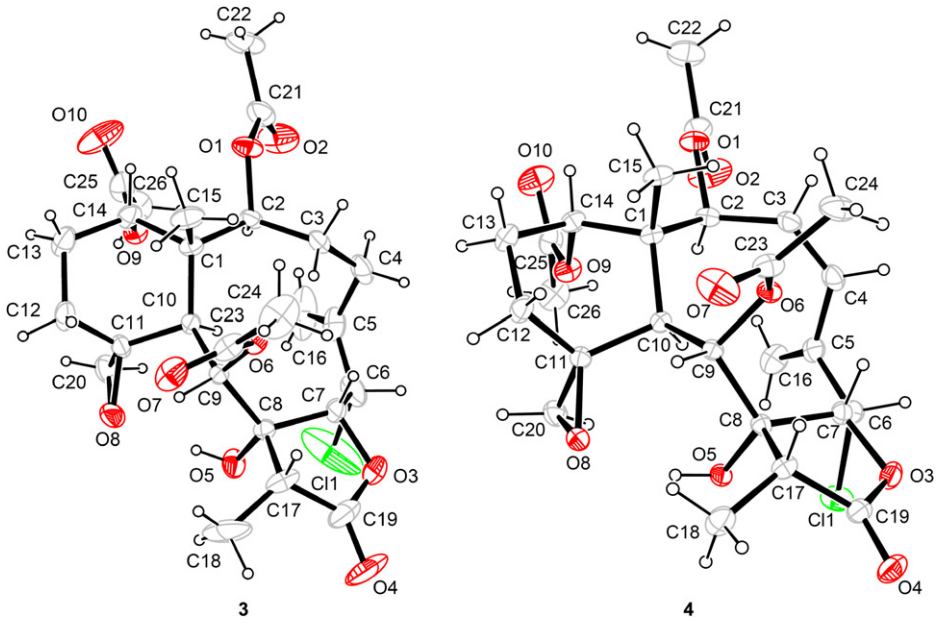

Figure 6. Computer-generated ORTEP plots of **3** and **4** showing the absolute configurations.

The two known chlorinated compounds **3** and **4**, which are designated as robustolides F and G, respectively, were first isolated from a Japanese gorgonian coral, *Ellisella* sp., and their structures, including the relative configurations, were elucidated by spectral data analysis.[8] In this study, we determined the absolute configurations for all chiral centers of **3** (1*S*, 2*S*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 14*S*, 17*R*) and **4** (1*R*, 2*S*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 14*S*, 17*R*) by X-ray diffraction analysis (Fig. 6). Moreover, based on the X-ray diffraction data, we found that the $\Delta^{3,5(16)}$-butadiene system in **4** exists in an *s-cis* form, as found in **1**. The authors suggest that the structure of compound **4** reported previously should be re-examined.[8]

It is noteworthy to mention that metabolite **1** represents the first example of a briarane possessing two halogen atoms. The cytotoxicity of **1**–**4** toward HepG2 (human hepatocellular carcinoma), Hep3B (human hepatocellular carcinoma), MDA-MB-231 (human breast carcinoma), MCF7 (human breast carcinoma), A549 (human lung adenocarcinoma), and Ca9-22 (oral squamous cell carcinoma) was assayed, and it was found that all four metabolites were inactive (ED_{50}'s>20 μg/mL) toward these cancer cell lines. Other possible biological activities of briaranes **1**–**4** will be assayed in the future.

3. Experimental

3.1. General experimental procedures

Melting points were determined using 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, and NMR spectra were recorded on a VARIAN MERCURY PLUS 400 FT-NMR at 400 MHz for ^{1}H and 100 MHz for ^{13}C, in $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26 ppm); ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1 ppm. ESIMS and HRESIMS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on silica gel (230–400 mesh, Merck, Darmstadt, Germany), and 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 using a system composed of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port, and a semi-preparative column (Hibar 250–25 mm, LiChrospher Si 60, 5 μm) was used.

3.2. Animal material

Specimens of the gorgonian coral *E. robusta* were collected by hand by divers equipped with SCUBA off the coast of southern Taiwan in August 2006, at a depth of 20 m. The organism was identified by comparison with previous descriptions.[5–7] Living reference specimens are being maintained in the authors' marine organisms cultivating tank 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 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 hexane/EtOAc (stepwise, 20:1–pure EtOAc) to yield fractions 1–25. Fraction 15 was purified by normal phase HPLC, and a mixture of hexane and EtOAc was used to afford briarane **2** (1.4 mg, 1:1). Fraction 18 was separated by normal phase HPLC, and a mixture of hexane and acetone was used to afford briaranes **3** (42.5 mg, 3:1) and **4** (6.2 mg, 3:1). A mixture from fraction 18 was repurified by normal phase HPLC, and a mixture of CH_2Cl_2 and EtOAc was used to afford briarane **1** (1.4 mg, 8:1).

3.3.1. Robustolide D (1). White powder; mp 171–172 °C; $[\alpha]_D^{25}$ −132 (*c* 0.07, $CHCl_3$); IR (neat) ν_{max} 3334, 1770, 1739 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data: see Table 1; ESIMS *m/z* 597 $(M+Na)^+$; HRESIMS *m/z* 597.1272 (calcd for $C_{26}H_{32}Cl_2O_{10}$+Na, 597.1270).

3.3.2. Robustolide E (2). White powder; mp 109–110 °C; $[\alpha]_D^{25}$ +37 (*c* 0.19, $CHCl_3$); IR (neat) ν_{max} 3453, 1789, 1727 cm^{-1}; ^{13}C ($CDCl_3$, 100 MHz) and ^1H ($CDCl_3$, 400 MHz) NMR data: see Table 1; ESIMS *m/z* 565 $(M+Na)^+$; HRESIMS *m/z* 565.1819 (calcd for $C_{26}H_{35}ClO_{10}$+Na, 565.1816).

3.3.3. Robustolide F (3). White powder; mp 124–126 °C; $[\alpha]_D^{25}$ −28 (*c* 0.24, $CHCl_3$) (Ref. 8 $[\alpha]_D^{26}$ −26.8 (*c* 1.038, $CHCl_3$)); IR (neat) ν_{max} 3311, 1781, 1738 cm^{-1}; ESIMS *m/z* 565 $(M+Na)^+$. The physical (rotation value) and spectral (ESIMS, ^1H, and ^{13}C NMR) data of **3** were in full agreement with those reported previously.[8]

3.3.4. Robustolide G (4). White powder; mp 112–113 °C; $[\alpha]_D^{25}$ −108 (*c* 0.28, $CHCl_3$) (Ref. 8 $[\alpha]_D^{26}$ −127 (*c* 0.175, $CHCl_3$)); IR (neat) ν_{max} 3287, 1781, 1740 cm^{-1}; ESIMS *m/z* 563 $(M+Na)^+$. The physical (rotation value) and spectral (^1H and ^{13}C NMR) data of **4** were in full agreement with those reported previously.[8]

3.4. Single-crystal X-ray crystallography of robustolide D (1)[10]

Suitable colorless prisms of **1** were obtained from a solution of EtOH/acetone (2:1). The crystal (0.6×0.5×0.4 mm) belongs to the monoclinic system, space group $P2_1$ (#4), with a=9.976(2) Å, b=22.918(6) Å, c=12.471(3) Å, V=2839(1) $Å^3$, Z=4, D_{calcd}=1.346 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 8308 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 $R1$=0.0373; $wR2$=0.0948 for 4983 observed reflections [$I>2\sigma(I)$] and 707 variable parameters. The absolute configuration of **1** was determined by Flack's method in which the fractional contribution of the inverted component of its racemic twin structure, expressed as Flack's parameter (zero for correct absolute configuration), was refined against data with Bijvoet pairs. In this case the Flack' parameter was determined to be 0.05(6).[11]

3.5. Single-crystal X-ray crystallography of robustolide F (3)[10]

Suitable colorless prisms of **3** were obtained from a solution of EtOH/acetone (2:1). The crystal (0.6×0.4×0.3 mm) belongs to the monoclinic system, space group $P2_1$ (#4), with a=11.429(2) Å, b=8.525(2) Å, c=14.079(4) Å, V=1351.3(6) $Å^3$, Z=2, D_{calcd}=1.334 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 4063 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 $R1$=0.0598; $wR2$=0.1577 for 2154 observed reflections [$I>2\sigma(I)$] and 340 variable parameters. The absolute configuration was determined by Flack's method with Flack's parameter determined as 0.13(7).[11]

3.6. Single-crystal X-ray crystallography of robustolide G (4)[10]

Suitable colorless prisms of **4** were obtained from a solution of EtOH/acetone (2:1). The crystal (0.8×0.6×0.6 mm) belongs to the monoclinic system, space group $P2_1$ (#4), with a=8.375(3) Å, b=18.830(6) Å, c=8.641(3) Å, V=1348.7(8) $Å^3$, Z=2, D_{calcd}=1.332 g/cm^3, λ (Mo Kα)=0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 26°. All 5842 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 $R1$=0.0411; $wR2$=0.1071 for 4157 observed reflections [$I>2\sigma(I)$] and 340 variable parameters. The absolute configuration was determined by Flack's method with Flack's parameter determined as 0.1(2).[11]

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