Robustolides H and I, Chlorinated Briaranes from the Gorgonian *Ellisella robusta* (Ellisellidae)

Ping-Jyun Sung,*[1,2,3] Wei-Tse Tsai,[1,2] Mei-Ru Lin,[1,4] Yin-Di Su,[1,2] Chen-Hao Pai,[1,2]
Hsu-Ming Chung,[4] Jui-Hsin Su,[3,4] and Michael Y. Chiang*[5]
[1]*National Museum of Marine Biology and Aquarium, Checheng, Pingtung 944, Taiwan*
[2]*Institute of Marine Biotechnology, National Dong Hwa University, Checheng, Pingtung 944, Taiwan*
[3]*Asian Pacific Ocean Research Center, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[4]*Department of Marine Biotechnology and Resources, National Sun Yat-sen University, Kaohsiung 804, Taiwan*
[5]*Department of Chemistry, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

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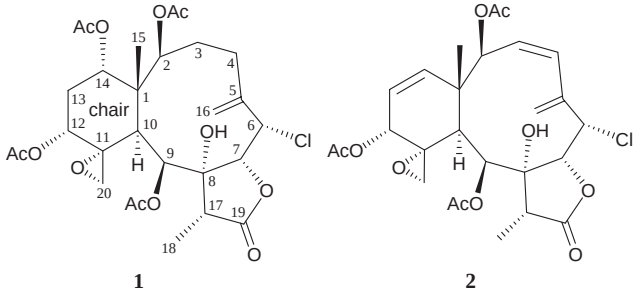
Two chlorinated briaranes have been isolated from the gorgonian *Ellisella robusta*: a new briarane, robustolide H (**1**) and a known briarane, robustolide I (**2**). The structure of **1** was determined by spectroscopic methods and the absolute configuration of **2** was established by X-ray diffraction analysis.

In continuation of our study on the chemical constituents of gorgonian *E. robusta*,[1–3] two chlorinated briaranes, robustolides H (**1**) and I (**2**) (Chart 1), were further isolated from *E. robusta*. The structures of **1** and **2** were elucidated by spectral data analysis. The absolute configuration of **2** was directly established by X-ray diffraction analysis.

Specimens of the gorgonian *E. robusta* (wet weight 664 g) were minced and extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The extract 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 25 fractions. Fraction 19 was purified by normal phase HPLC, and a mixture of hexane and acetone was used to afford **2** (9.1 mg, 3:1). Fraction 20 was separated by normal phase HPLC, and a mixture of CH_2Cl_2 and EtOAc was used to afford **1** (0.9 mg, 8:1).

Robustolide H (**1**) was obtained as a white powder, mp 136–138 °C, $[\alpha]_D^{25} + 202$ ($c = 0.05$, $CHCl_3$). The molecular formula for **1** was elucidated as $C_{28}H_{37}ClO_{12}$ (ten degrees of unsaturation) by HR-ESI-MS ($C_{28}H_{37}ClO_{12}$ + Na: m/z; found, 623.1874; calcd. 623.1871). The IR spectrum showed bands at 3472, 1784, and 1731 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups in **1**. From the ^{13}C NMR data of **1** (Table 1), an exocyclic carbon–carbon double bond was deduced from the signals of two carbons resonating at δ 146.7 (s, C-5) and 121.3 (t, CH_2-16), and further supported by two olefin proton signals at δ 5.82 (1H, s, H-16b) and 5.52 (1H, s, H-16a) in the ^1H NMR spectrum of **1** (Table 1). In the ^{13}C NMR spectrum of **1**, five carbonyl resonances appeared at δ 174.5 (s, C-19), 170.9 (s, ester carbonyl), 170.4 (s, ester carbonyl), and 169.4 (2 × s, ester carbonyls), confirming the presence of a γ-lactone and four other ester groups. In the ^1H NMR spectrum of **1**, four acetate methyls (δ 2.22, 3H, s; 2.01, 3H, s; 2.00, 3H, s; 1.98, 3H, s) were observed. Thus, from the NMR data, six degrees of unsaturation were accounted for, and **1** must be tetracyclic.



1 **2**

Chart 1.

The gross structure of **1** was determined using 2D NMR studies. From the ^1H–^1H COSY experiment of **1**, it was possible

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

C/H	$^1H^a/\delta$	$^{13}C^b/\delta$	HMBC (H→C)
1		47.8 (s)[d]	
2	5.97 d (8.4)[c]	73.1 (d)	C-1, 15, acetate carbonyl
3α/β	1.65 m; 2.72 m	28.2 (t)	C-2
4/4′	2.46 m (2H)	33.6 (t)	C-16
5		146.7 (s)[e]	
6	4.63 d (3.2)	52.7 (d)[e]	n.o.[f]
7	4.44 br s	81.2 (d)	n.o.
8		81.4 (s)	
9	5.76 s	72.1 (d)	C-1, 7, 8, 10, 11, 17, acetate carbonyl
10	3.70 s	35.5 (d)	C-1, 2, 8, 11, 14, 20
11		57.6 (s)	
12	4.54 dd (3.2, 2.0)	73.5 (d)	n.o.
13α	2.24 ddd (16.4, 2.8, 2.0)	29.1 (t)	C-1, 11, 12, 14
β	2.05 ddd (16.4, 3.2, 2.8)		
14	4.91 dd (2.8, 2.8)	73.3 (d)	C-2, 10, 12
15	1.16 s	14.0 (q)	C-1, 2, 10, 14
16a/b	5.52 s; 5.82 s	121.3 (t)	C-4, 5, 6
17	2.96 q (7.2)	51.5 (d)	C-7, 8, 18, 19
18	1.27 d (7.2)	5.9 (q)	C-8, 17, 19
19		174.5 (s)	
20a/b	2.36 d (3.2)	50.3 (t)[e]	C-12
	2.83 dd (3.2, 1.2)		
OH-8	3.43 s		C-7, 8, 9
Acetate	2.22 s	21.3 (q)	acetate carbonyl
methyls	2.01 s	21.2 (q)	acetate carbonyl
	2.00 s	21.1 (q)	acetate carbonyl
	1.98 s	20.9 (q)	acetate carbonyl
Acetate		170.9 (s)	
carbonyls		170.4 (s)	
		169.4 (s)	
		169.4 (s)	

Spectra recorded at [a]400 and [b]100 MHz in $CDCl_3$ at 25 °C, respectively. [c]J values (in Hz) in parentheses. [d]Multiplicity deduced by DEPT and indicated by usual symbols. [e]Due to the broad signals, the ^{13}C chemical shifts for C-5, 6, and 20 were assigned by the assistances of HMBC correlations. [f]n.o. = not observed.

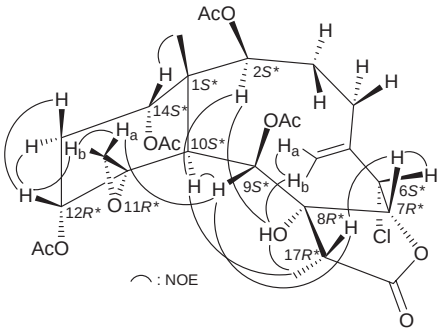


Figure 1. The selective NOESY correlations of **1**.

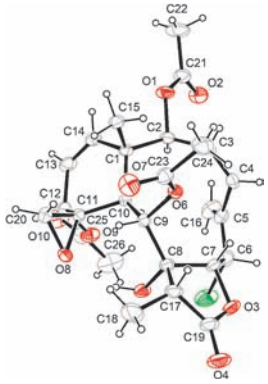


Figure 2. Computer-generated ORTEP plot of **2** showing the absolute configuration.

to establish a spin system from H-2/H_2-3; H_2-3/H_2-4; H-6/H-7; H-9/H-10, H-12/H_2-13; H_2-13/H-14; and H-17/H_3-18. The allylic coupling between H_2-4/H_2-16 and H-6/H_2-16, and *w*-coupling between H-20b with H-10 were also observed in the ^{1}H–^{1}H COSY spectrum of **1**. Based on these data and HMBC correlations (Table 1), the carbon skeleton of **1** could be established. An exocyclic double bond attached at C-5 was confirmed by HMBC correlations between H_2-16/C-4, 5, 6 and H_2-4/C-16. The epoxy group positioned at C-11/20 was confirmed by the connectivity between H_2-20/C-12 and H-10/C-20. The ring junction C-15 methyl group was positioned at C-1 from HMBC correlations between H_3-15/C-1, 2, 10, 14, and H-2/C-15. Furthermore, the HMQC and ^{1}H–^{1}H COSY correlations also revealed that the chlorine atom could attach at C-6 methine (δ_H 4.63, 1H, d, $J = 3.2$ Hz; δ_C 52.7, d). The acetate esters positioned at C-2 and C-9 were established by key correlations between H-2 (δ 5.97), H-9 (δ 5.76), and the acetate carbonyls observed in HMBC spectrum of **1**. The other acetoxy groups were positioned at C-12 and C-14, as indicated by analysis of key ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis, although no HMBC correlations were observed between H-12, H-14, and the acetate carbonyls. Thus, the remaining hydroxy group had to be positioned at C-8. These data together with 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 **1**.

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, respectively, the epoxy group is α-oriented, and the cyclohexane ring is of a chair conformation.[4] Based on the above observations, the configuration of the 11,20-epoxy group in **1** (δ 57.6, s, C-11; 50.3, t, CH_2-20) should be α and the cyclohexane ring in **1** should be in a chair conformation.

The relative stereochemistry of **1** was elucidated by a NOESY experiment (Figure 1). Because of 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 **1**, H-10 exhibited NOE responses with H-2, H-9, H_3-18, and H-2 showed an NOE correlation with OH-8, suggesting that these protons (H-2, OH-8, H-9, H-10, H_3-18) are located on the same face and can be assigned as α protons, as the C-15 methyl group is β-oriented. H-12 was found to exhibit NOE correlations with H_2-13 and one proton of C-20 methylene (δ 2.83, H-20b), indicating that the 12-acetoxy group was α-oriented. H-14 was found to exhibit an NOE response with H_3-15, showing that this proton is of β-orientation. H-9 was found to show NOE correlations with H-10, H-17, and H-20a and, from molecular models, was found to be reasonably close to H-10, H-17, and H-20a; therefore, H-9 should be placed on the α face in **1**, and H-17 is β-oriented in the γ-lactone ring. Furthermore, H-7 exhibited NOE correlations with H-17 and H-6, suggesting that these protons are on the β face of **1**. Based on above findings, the configurations of all chiral centers of **1** were assigned to be 1*S**, 2*S**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*R**, 12*R**, 14*S**, 17*R**.

By detailed analysis, the structure of **1** was found to be very similar to that of a known metabolite, gemmacolide C,[5,6] however, by comparison the physical and spectral data of **1** with those of gemmacolide C, particularly in ^{13}C NMR data and NOE correlations, the authors suggested the structure as we presented here as **1** is correct.

Briarane **2**, which is designated as robustolide I, was first isolated from a Japanese gorgonian coral, *Ellisella* sp.,[7] and its structure, including the absolute configuration for this metabolite was determined by X-ray diffraction analysis for the first time in this study (Figure 2), and the chiral centers of this compound were assigned as 1*R*, 2*S*, 3*Z*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*, 13*Z*, 17*R*.[8] Moreover, based on X-ray diffraction data, we found the $\triangle^{3,5(16)}$-butadiene system in **2** exists in an *s-cis* form. The authors suggest that the structure of compound **2** reported previously should be re-examined.[7]

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7 C. Tanaka, Y. Yamamoto, M. Otsuka, J. Tanaka, T. Ichiba, G. Marriott, R. Rachmat, T. Higa, *J. Nat. Prod.* **2004**, *67*, 1368. The known compound **2** (robustolide I) was reported as compound **1** in this article.
8 Crystallographic data of the structure of robustolide I (**2**) have been deposited with Cambridge Crystallographic Data Center as supplementary publication number CCDC 664825. Cpoies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44 (0) 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

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