

New Polyoxygenated Briaranes from Octocorals *Briareum excavatum* and *Ellisella robusta*

Tsong-Long Hwang,[1] Mei-Ru Lin,[2,3] Wei-Tse Tsai,[2,4] Hsiao-Chien Yeh,[2,3] Wan-Ping Hu,[5] Jyh-Horng Sheu,*[3,6] and Ping-Jyun Sung*[2,4,6]

[1]Graduate Institute of Natural Products, Chang Gung University, Taoyuan 333, Taiwan

[2]Taiwan Coral Research Center (TCRC), National Museum of Marine Biology & Aquarium (NMMBA), Checheng, Pingtung 944, Taiwan

[3]Department of Marine Biotechnology and Resources, National Sun Yat-sen University (NSYSU), Kaohsiung 804, Taiwan

[4]Graduate Institute of Marine Biotechnology, National Dong Hwa University (NDHU), Checheng, Pingtung 944, Taiwan

[5]Faculty of Biotechnology, Kaohsiung Medical University (KMU), Kaohsiung 807, Taiwan

[6]Asia-Pacific Ocean Research Center (APORC), National Sun Yat-sen University (NSYSU), Kaohsiung 804, Taiwan

Received July 8, 2008; E-mail: pjsung@nmmba.gov.tw

Six new polyoxygenated briarane-type diterpenoids, briaexcavatins Q–T (**1**–**4**) and robustolides J (**5**) and K (**6**), were isolated from a cultured octocoral *Briareum excavatum* and a sea whip gorgonian coral *Ellisella robusta*, respectively. The structures of briaranes **1**–**6** were established by spectroscopic methods. Briarane **3** exhibited weak cytotoxicity toward CCRF-CEM tumor cells and briaranes **5** and **6** displayed inhibitory effects on superoxide anion generation by human neutrophils.

In our continuing search for novel natural products from marine invertebrates collected in Taiwanese waters as part of the National Science and Technology Program for Biotechnology and Pharmaceuticals (NSTPBP), Taiwan, we analyzed organic extracts from a cultured octocoral *Briareum excavatum* (Briareidae) and a gorgonian coral *Ellisella robusta* (Ellisellidae), in the hope of identifying extracts that exhibit interesting and meaningful signals in NMR studies. We describe herein the isolation, structure determination, and bioactivities of briaexcavatins Q–T (**1**–**4**) and robustolides J (**5**) and K (**6**), six new briarane derivatives obtained from *B. excavatum* and *E. robusta*, respectively (Chart 1).

Experimental

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. Infrared spectra were obtained on a VARIAN DIGLAB FTS 1000 FT-IR spectrophotometer. 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), and ^{13}C NMR spectra were referenced to the center peak of $CDCl_3$ at δ 77.1. ESI-MS and HR-ESI-MS data were recorded on a BRUKER APEX II mass spectrometer. Column chromatography was performed on Sephadex LH-20 (Amersham Biosciences, Sweden), normal phase silica gel (230–400 mesh, Merck, Darmstadt, Germany), and C-18 reverse phase silica gel (230–400 mesh, Silicycle, Quebec, Canada). TLC was carried out on precoated Kieselgel 60 F_{254} (0.25 mm, Merck) and spots were visualized by spraying with 10% H_2SO_4 solution followed by heating. HPLC was performed using a system comprised of HITACHI L-7100 and L-7110 pumps, a HITACHI photo diode array detector L-7455, and a RHEODYNE 7725 injection port. A semi-preparative normal phase column (Hibar 250–25 mm, LiChrospher Si 60, 5 μm) and a semi-preparative reverse phase column (Hibar 250–10 mm, Purospher STAR RP-18e, 5 μm) were used for HPLC. All solvents used were analytical grade.

Animal Material. ***B. excavatum*:** Specimens of cultured octocoral *B. excavatum* were collected by hand from 0.6-ton cultivating tanks located in the NMMBA, Taiwan, in December 2006. This organism was identified by comparison with previous descriptions.[1,2] Living reference specimens are being maintained in the authors' marine organisms cultivating tanks and a voucher specimen was deposited in the NMMBA, Taiwan.

***E. robusta*:** Specimens of gorgonian coral *E. robusta* were collected 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.[3] Living reference specimens are being maintained in the authors' marine organisms cultivating tanks and a voucher specimen was deposited in the NMMBA, Taiwan.

Extraction and Isolation. ***B. excavatum*:** The freeze-dried and minced material of *B. excavatum* (wet weight 672 g, dry weight 270 g) was extracted with a mixture of MeOH and CH_2Cl_2 (1:1). The residue was partitioned between EtOAc and H_2O, and the EtOAc layer was separated on a Sephadex LH-20 and eluted

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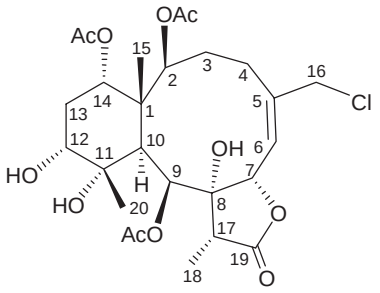
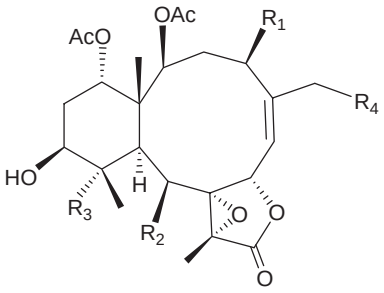
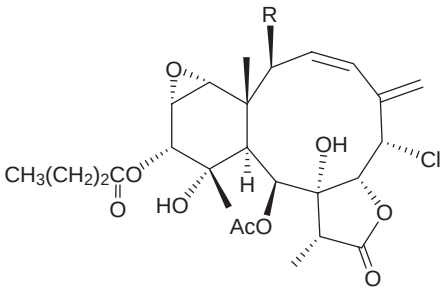


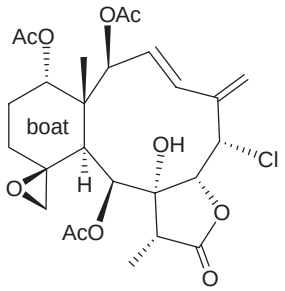
1



2 : R_1 = OH, R_2 = OAc, R_3 = R_4 = H
3 : R_1 = OAc, R_2 = R_3 = OH, R_4 = H
7 : R_1 = OH, R_2 = R_4 = OAc, R_3 = H
8 : R_1 = R_2 = OAc, R_3 = OH, R_4 = H



4 : R = OH
9 : R = OC(O)$(CH_2)_2CH_3$



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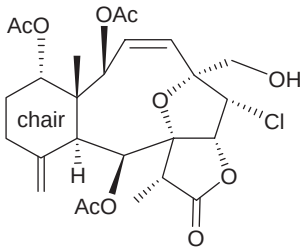

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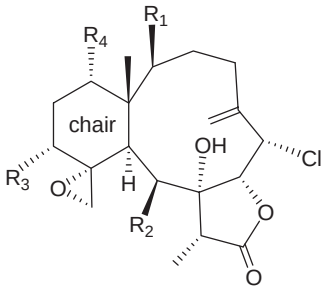

10 : R_1, R_2, R_3, R_4 = 3OAc + an isobutyroxy group
11 : R_1 = R_2 = R_3 = R_4 = OAc

Chart 1.

using MeOH/CH_2Cl_2 (2:1) to yield three fractions A–C. Fraction C was separated on silica gel and eluted using hexane/EtOAc (stepwise, 20:1–pure EtOAc) to yield fractions 1–9. Fraction C9 was separated by normal phase HPLC (NP-HPLC), using mixtures of CH_2Cl_2 and acetone to afford fractions from C9-1 to C9-8. Fraction C9-3 was separated on a C-18 gravity column using mixtures of CH_3CN and H_2O (stepwise, 1:3–1:2) to yield 8 fractions. Fractions C9-3-2, C9-3-3, and C9-3-4 were further separated by reverse phase HPLC (RP-HPLC), using mixtures of CH_3OH and H_2O to afford **2** (7:15), **4** (1:1), and **3** (1:1), respectively. Fraction C9-3-7 was eluted with a mixture of CH_3CN and H_2O by RP-HPLC to yield **1** (1:1).

Briaexcavatin Q (1); White powder (1.4 mg); mp 169–171 °C; $[\alpha]_D^{24}$ −27 (*c* 0.07, $CHCl_3$); IR (neat) ν_{max} 3439, 1766, 1735 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS *m*/*z* 583 $(M + Na)^+$, 585 $(M + 2 + Na)^+$; HR-ESI-MS *m*/*z* 583.1926 (calcd for $C_{26}H_{37}{}^{35}ClO_{11}$ + Na, 583.1922).

Briaexcavatin R (2); White powder (3.6 mg); mp 180–182 °C; $[\alpha]_D^{24}$ +99 (*c* 0.18, $CHCl_3$); IR (neat) ν_{max} 3444, 1774, 1735 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS *m*/*z* 547 $(M + Na)^+$; HR-ESI-MS *m*/*z* 547.2157 (calcd for $C_{26}H_{36}O_{11}$ + Na, 547.2155).

Briaexcavatin S (3); White powder (0.5 mg); mp 194–196 °C; $[\alpha]_D^{24}$ +226 (*c* 0.03, $CHCl_3$); IR (neat) ν_{max} 3463, 1770, 1737 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS *m*/*z* 563 $(M + Na)^+$; HR-ESI-MS *m*/*z* 563.2108 (calcd for $C_{26}H_{36}O_{12}$ + Na, 563.2104).

Briaexcavatin T (4); White powder (0.6 mg); mp 191–193 °C; $[\alpha]_D^{24}$ −140 (*c* 0.03, $CHCl_3$); IR (neat) ν_{max} 3431, 1774, 1735 cm^{-1}; ^1H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS *m*/*z* 565 $(M + Na)^+$, 567 $(M + 2 + Na)^+$; HR-ESI-MS *m*/*z* 565.1813 (calcd for $C_{26}H_{35}{}^{35}ClO_{10}$ + Na, 565.1816).

***E. robusta*:** The freeze-dried and minced material of 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). The residue was partitioned between EtOAc and H_2O, and the EtOAc layer was separated on silica gel and eluted using mixtures of hexane/EtOAc (stepwise, 20:1–pure EtOAc) to yield fractions 1–25. Fraction 15 was purified by NP-HPLC and a mixture of hexane and acetone was used to afford **6** (3:1). Fraction 19 was further

Table 1. ^{1}H and ^{13}C NMR Data for Diterpenoids **1–4**

Position	**1**		**2**		**3**		**4**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
1		47.1 (s)[d]		45.9 (s)		48.0 (s)		43.6 (s)
2	4.95 d (7.2)[c]	75.6 (d)	4.87 d (7.6)	74.3 (d)	4.94 d (8.4)	72.6 (d)	5.20 d (3.6)	72.6 (d)
3α	1.56 m	31.1 (t)	1.95 m	40.4 (t)	1.89 m	37.5 (t)	5.84 dd (12.0, 3.6)	134.1 (d)
β	2.90 td (11.2, 6.0)		2.84 dd (15.6, 11.6)		3.21 dd (14.8, 13.2)			
4α	2.18 m	26.1 (t)	4.19 dd (11.6, 5.6)	71.0 (d)	4.98 ddd (13.2, 5.2, 0.8)	72.5 (d)	5.88 d (12.0)	126.5 (d)
β	2.53 m							
5		142.6 (s)		146.7 (s)		143.7 (s)		136.9 (s)
6	5.87 d (9.2)	123.7 (d)	5.35 dd (8.8, 1.2)	121.8 (d)	5.36 ddd (8.8, 1.6, 0.8)	123.0 (d)	5.20 m	62.8 (d)
7	5.18 d (9.2)	77.4 (d)	5.83 d (8.8)	73.6 (d)	5.94 d (8.8)	74.6 (d)	5.05 d (4.0)	78.0 (d)
8		81.6 (s)		70.7 (s)		71.8 (s)		83.6 (s)
9	5.88 d (2.4)	70.1 (d)	5.00 br s	73.4 (d)	4.72 d (5.6)	66.8 (d)	5.78 d (6.0)	68.6 (d)
10	2.65 d (2.4)	40.4 (d)	2.38 dd (5.2, 2.0)	41.3 (d)	1.92 s	49.3 (d)	2.56 d (6.0)	36.3 (d)
11		75.9 (s)	2.05 m	44.3 (d)		78.3 (s)		73.5 (s)
12	3.62 m	73.9 (d)	4.05 m	66.7 (d)	3.69 br d (8.8)	73.6 (d)	4.58 d (5.6)	72.9 (d)
13α	2.14 m	26.9 (t)	1.82 m (2H)	28.9 (t)	2.00 m	30.2 (t)		54.6 (d)
β	2.06 m				1.71 m		3.68 dd (5.6, 3.6)	
14	4.92 dd (2.4, 2.0)	76.5 (d)	4.81 dd (3.2, 2.8)	76.1 (d)	4.78 m	74.9 (d)	3.14 d (3.6)	62.3 (d)
15	1.10 s	13.9 (q)	1.22 s	15.4 (q)	1.31 s	14.3 (q)	1.12 s	14.5 (q)
16a	4.55 d (11.6)	50.7 (t)	2.09 s	25.3 (q)	2.12 d (1.6)	25.3 (q)	5.86 d (1.2)	116.8 (t)
b	4.30 d (11.6)						5.57 d (1.2)	
17	2.41 q (6.8)	43.6 (d)		64.9 (s)		64.7 (s)	2.41 q (7.2)	45.8 (d)
18	1.26 d (6.8)	6.6 (q)	1.65 s	10.9 (q)	1.68 s	9.6 (q)	1.24 d (7.2)	7.0 (q)
19		175.8 (s)		170.7 (s)		171.7 (s)		174.4 (s)
20	1.39 s	24.6 (q)	1.06 d (7.6)	9.1 (q)	1.31 s	17.5 (q)	1.31 s	22.5 (q)
OH-8	3.87 s						4.29 s	
OH-9					3.03 d (5.6)			
OH-11	4.03 s				n.o.[e]		3.64 s	
OH-12	3.08 d (6.8)		n.o.[e]		n.o.[e]			
2-OAc	1.98 s	21.3 (q)	1.99 s	21.4 (q)	2.00 s	21.0 (q)		
		171.0 (s)		170.5 (s)		170.3 (s)		
4-OAc					2.00 s	21.2 (q)		
						170.4 (s)		
9-OAc	2.22 s	21.7 (q)	2.24 s	21.4 (q)			2.17 s	21.9 (q)
		168.6 (s)		168.4 (s)				170.0 (s)
14-OAc	2.04 s	21.5 (q)	1.98 s	21.2 (q)	2.02 s	21.5 (q)		
		169.3 (s)		170.3 (s)		170.3 (s)		
12-OCOPr							1.01 t (7.2)	13.8 (q)
							1.71 sext (7.2)	18.6 (t)
							2.37 t (7.2)	36.2 (t)
								173.0 (s)

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. c) J values (in hertz) in parentheses. d) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. e) n.o. = not observed.

separated by NP-HPLC, using a mixture of hexane and acetone (3:1) to yield 9 fractions. Fraction 19–9 was repurified by NP-HPLC and eluted with a mixture of CH_2Cl_2 and EtOAc to afford **5** (8:1).

Robustolide J (5); White powder (0.9 mg); mp 99–101 °C; $[\alpha]_D^{25}$ −11 (*c* 0.05, $CHCl_3$); IR (neat) ν_{max} 3452, 1782, 1738 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 3; ESI-MS m/z 563 $(M + Na)^+$, 565 $(M + 2 + Na)^+$; HR-ESI-MS m/z 563.1662 (calcd for $C_{26}H_{33}{}^{35}ClO_{10} + Na$, 563.1660).

Robustolide K (6); White powder (1.3 mg); mp 68–70 °C; $[\alpha]_D^{25}$ −7 (*c* 0.07, $CHCl_3$); IR (neat) ν_{max} 3436, 1788, 1738 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 3; ESI-MS m/z 563 $(M + Na)^+$, 565 $(M + 2 + Na)^+$; HR-ESI-MS m/z 563.1663 (calcd for $C_{26}H_{33}{}^{35}ClO_{10} + Na$, 563.1660).

Cytotoxicity Assay. The cytotoxicity of compounds **1–6** was assayed by a modified MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotoxicity assays were carried out according to the procedures described previously.[4]

Human Neutrophil Superoxide Anion Generation. Human neutrophils were obtained by means of dextran sedimentation and Ficoll centrifugation. Superoxide anion generation was carried out according to the procedures described previously.[5,6] Briefly, superoxide anion production was assayed by monitoring the superoxide dismutase-inhibitable reduction of ferricytochrome *c*.

Results and Discussion

Isolation and Structure Determination of Briaexcavatins Q–T from *B. excavatum*. Octocorals belonging to the genus *Briareum* are major sources of briarane-type natural products.[7–9] Cultured *B. excavatum* has been studied for its interesting and complex constituents and a series of new briaranes, including briaexcavatins I–P,[10,11] had been isolated from this organism. Sliced bodies of *B. excavatum* collected from the culturing tanks in NMMBA, Taiwan were extracted with a

Table 2. HMBC (H→C) Correlations for Diterpenoids **1**-**4**

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

a) n.o. = not observed.

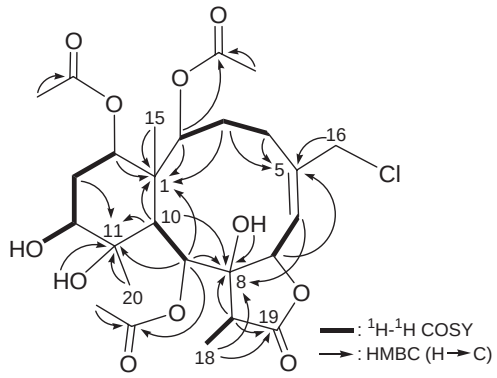

Figure 1. The ^{1}H–^{1}H COSY and HMBC correlations (protons and quaternary carbons) of **1**.

mixture of MeOH and CH_2Cl_2 (1:1). The extract was partitioned with EtOAc and H_2O, and separation of the EtOAc layer by gravity silica gel column chromatography followed by repeated HPLC yielded new briaranes **1**–**4**.

Briaexcavatin Q (**1**) was found to have the molecular formula $C_{26}H_{37}ClO_{11}$ (HR-ESI-MS, see Experimental), at m/z 583/585 [3:1, $(M + Na)^+/(M + 2 + Na)^+$, ESI-MS], which implied eight degrees of unsaturation. IR absorptions were observed at 3439, 1766, and 1735 cm^{-1}, suggesting the presence of hydroxy, γ-lactone, and ester groups in **1**. The ^{13}C NMR and DEPT spectra of **1** (Table 1) showed that this compound has 26 carbons, including six methyls, four sp^3 methylenes (including a chlorinated methylene), an sp^2 methine, seven sp^3 methines (including five oxymethines), three sp^3 quaternary carbons (including two oxygenated quaternary carbons), and five sp^2 quaternary carbons (including four ester carbonyls). From the ^{1}H and ^{13}C NMR spectra (Table 1), **1** was found to possess three acetyl groups (δ 2.04, 3H, s; δ 21.5, q; 169.3, s; δ 2.22, 3H, s; δ 21.7, q; 168.6, s; δ 1.98, 3H, s; δ 21.3, q; 171.0, s), a γ-lactone moiety (δ 175.8, s), and a trisubstituted olefin (δ 5.87, 1H, d, $J = 9.2$ Hz; δ 142.6, s; 123.7, d). The gross structure of **1** was determined by 2D NMR experiments. From the ^{1}H–^{1}H COSY spectrum of **1**, it was possible to identify five different structural units (Figure 1), which were assembled with the assistance of an HMBC experiment (Figure 1 and Table 2). The HMBC correlations between protons and quaternary carbons of **1**, such as H-2, -3, -9, -10, -14, -15/C-1; H-3, -4, -7, -16/C-5; H-6, -9, -10, -17, -18/C-8; H-9, -10, -13, -20/C-11; and H-17, -18/C-19, permitted elucidation of the carbon skeleton structure. The presence of acetoxy groups positioned at C-2 and C-9 was confirmed by the HMBC correlations from δ 4.95 (H-2) and 5.88 (H-9) to the acetate carbonyl carbons that appeared at δ 171.0 (s) and 168.6 (s), respectively. The remaining acetoxy group was positioned at C-14, an oxymethine (δ 4.92, 1H, dd, $J = 2.4$, 2.0 Hz; δ 76.5, d), as indicated by analysis of the ^{1}H–^{1}H COSY correlations and characteristic NMR signals, although no HMBC correlation was observed between H-14 and the acetate carbonyl. The hydroxy proton signals appearing at δ 4.03 (s) and 3.87 (s) were revealed by their HMBC correlations to the quaternary oxygenated carbons at δ 75.9 (s, C-11) and 81.6 (s, C-8), indicating their attachment to C-11 and C-8. The presence of a 12-hydroxy group was evidenced by an ^{1}H–^{1}H coupling and an HMBC correlation between a hydroxy proton (δ 3.08, 1H, d, $J = 6.8$ Hz, OH-12) and C-12 oxymethine (δ 3.62, 1H, m; δ 73.9, d). The intensity of the $(M + 2 + Na)^+$ isotope peak observed in ESI-MS [$(M + Na)^+$:$(M + 2 + Na)^+$ = 3:1] was strong evidence of the presence of a chlorine atom in **1**. The

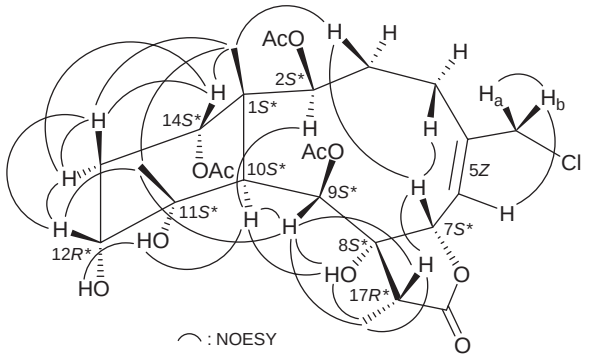

Figure 2. Selective NOESY correlations of **1**.

methylene unit at δ 50.7 (t) was more shielded than that expected for an oxygenated C-atom, and was correlated to the methylene protons at δ 4.55 and 4.30 in the HMQC spectrum. The latter methylene signals were ^2J-correlated with C-5 (δ 142.6, s) and ^3J-correlated with both C-4 (δ 26.1, t) and C-6 (δ 123.7, d), proving the attachment of a chloromethyl group at C-5 (Figure 1 and Table 2).

The relative stereochemistry of **1** was elucidated by analysis of NOESY correlations, as shown in Figure 2. The NOESY correlations between H-10 and H-2, H-9, OH-8, and OH-11 indicated that these protons are situated on same face; they were assigned as α protons, as C-15 methyl is β-oriented and H_3-15 did not show correlation with H-10. H-14 was found to exhibit a response with H_3-15 but not with H-10, revealing the β-orientation and equatorial direction of this proton. One of the methylene protons at C-3 (δ 2.90) exhibited a correlation with H_3-15 and was assigned as H-3β, while the other was denoted as H-3α (δ 1.56). The correlations observed between H-3β and H-7, and H-7 and H-17, reflected the β-orientation of both protons at C-7 and C-17. The NOESY spectrum showed a correlation of H-6 with one of the C-16 chloromethyl protons (δ 4.30, H-16b), revealing the Z geometry of the C-5/6 double bond. Furthermore, H_3-15 was found to correlate with H_3-20, and H-12 exhibited correlations with H_3-20 and C-13 methylene protons, indicating that both the hydroxy groups attached at C-11 and C-12 in the methylcyclohexane ring of **1** are α-oriented and are positioned on the equatorial and axial directions, respectively. Based on the above findings, the chiral centers of **1** were assigned as 1S*, 2S*, 7S*, 8S*, 9S*, 10S*, 11S*, 12R*, 14S*, and 17R*.

The HR-ESI-MS of **2** (briaexcavatin R) exhibited a pseudomolecular ion peak at m/z 547.2157 $(M + Na)^+$, with the molecular formula $C_{26}H_{36}O_{11}$, implying nine degrees of unsaturation. The IR absorptions of **2** showed the presence of hydroxy (3444 cm^{-1}), γ-lactone (1774 cm^{-1}), and ester carbonyl (1735 cm^{-1}) groups. The ^{13}C NMR spectrum of **2** at δ 170.7 (s), 170.5 (s), 170.3 (s), and 168.4 (s) (Table 1) confirmed the presence of a γ-lactone and three esters. From the ^1H NMR spectrum of **2** (Table 1), the presence of three acetyl methyls (δ 2.24, 1.99, and 1.98, each 3H × s) were deduced. The spectral data of **2** were found to be similar to those of a known briarane, briaexcavatin J (**7**).[10] By comparison of the NMR data of **2** with those of **7**, it was found that the 5-acetoxymethyl substituent in **7** was replaced by a methyl group in **2**. In addition, by comparison of the proton and carbon chemical shifts, coupling constants, and NOESY correlations of **2** with those of **7**, the relative stereochemistry of **2** was confirmed to be the same as that of **7**, and the configurations of the chiral centers of **2** were assigned as 1R*, 2S*, 4R*, 7S*, 8R*, 9S*, 10S*, 11R*, 12S*, 14S*, and 17R*.

Briaexcavatin S (**3**) was isolated as a white powder and had the molecular formula $C_{26}H_{36}O_{12}$ according to its HR-ESI-MS (see Experimental). The IR spectrum of **3** showed bands at 3463, 1770, and 1737 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester carbonyl groups. By comparison of the NMR data of **3** with those of other known briarane analogues, it was found that diterpenoid **3** is the 9-*O*-deacetyl derivative of a known briarane, briaexcavatolide U (**8**),[12] and possesses a structure as represented by formula **3**. The structure of **3** was further confirmed by 2D NMR experiments (Table 2) and the chiral centers of this compound were assigned as 1S*, 2S*, 4R*, 7S*, 8S*, 9S*, 10S*, 11S*, 12S*, 14S*, and 17R* by comparison of the proton and carbon chemical shifts, coupling constants, and NOESY correlations with those of **8**.

The molecular formula $C_{26}H_{35}ClO_{10}$ of **4** (briaexcavatin T) was proposed by examination of the ESI-MS pseudomolecular $(M + Na)^+$ ions at m/z 565/567 (in a ratio ca. 3/1) and verified by HR-ESI-MS. It was found that the NMR data of **4** were similar to those of a known briarane, 11-hydroxybrianthein V (**9**).[13,14] However, the ^1H and ^{13}C NMR spectra revealed that the signals corresponding to an *n*-butyryloxy group in **9** were not present, and had been replaced by those of a hydroxy group in **4**. In the HMBC experiment of **4** (Table 2), the carbon signal at δ 173.0 (s), which showed a correlation with H-12 (δ 4.58), was found to be correlated with the signals of the methylene protons at δ 2.37, and was consequently assigned as the carbon atom of an *n*-butyrate carbonyl. Thus, the *n*-butyrate ester could be positioned at C-12 in **4**. On the basis of above observations, **4** was found to be the 2-*O*-debutyryl derivative of **9**, and the chiral centers of **4** were assigned as 1S*, 2S*, 6S*, 7R*, 8R*, 9S*, 10S*, 11S*, 12R*, 13R*, 14R*, and 17R* by molecular models analysis.

Isolation and Structure Determination of Robustolides J and K from *E. robusta*. In the first study to have focused on the chemical constituents of a Japanese gorgonian coral identified as *Ellisella* sp. since 2004,[15] a series of briarane-type natural products, including robustolide D, the first briarane derivative possessing two halogen atoms in its structure,[16] were isolated from gorgonian corals belonging to the genus *Ellisella*, collected from Taiwanese and Japanese waters.[15–19] The minor components of extracts from a Taiwanese gorgonian coral *E. robusta* have been further studied for their interesting chemical structures.

Robustolide J (**5**) was isolated as a white powder that gave an $(M + Na)^+$ ion at m/z 563.1662 in the HR-ESI-MS, indicating the molecular formula $C_{26}H_{33}ClO_{10}$ (calcd for $C_{26}H_{33}ClO_{10} + Na$, 563.1660) and implying 10 degrees of unsaturation. Inspection of the IR spectrum revealed absorptions indicative of hydroxy (3452 cm^{-1}), γ-lactone (1782 cm^{-1}), and ester carbonyl (1738 cm^{-1}) groups. The presence of an exocyclic carbon–carbon double bond and a disubstituted olefin were deduced from the signals of four carbons resonating at δ 143.5 (s, C-5), 114.5 (t, CH_2-16), 130.7 (d, CH-3), and 128.8

Table 3. ^{1}H and ^{13}C NMR Data and HMBC Correlations for Diterpenoids **5** and **6**

Position	**5**			**6**		
	^{1}H[a)]	^{13}C[b)]	HMBC (H→C)	^{1}H[a)]	^{13}C[b)]	HMBC (H→C)
1		47.1 (s)[d)]			48.3 (s)	
2	5.25 d (7.2)[c)]	76.1 (d)	C-1, -4, -15, acetate carbonyl	6.51 dd (8.4, 0.8)	73.7 (d)	C-1
3	5.79 dd (16.0, 7.2)	130.7 (d)	C-5	5.45 dd (11.2, 8.4)	130.2 (d)	C-5
4	6.63 d (16.0)	128.8 (d)	C-2, -16	5.58 dd (11.2, 0.8)	130.6 (d)	C-5
5		143.5 (s)			85.1 (s)	
6	5.03 d (2.8)	62.4 (d)	C-5, -8, -16	4.26 d (5.6)	63.8 (d)	C-4
7	4.27 br s	82.2 (d)	n.o.[e)]	4.73 d (5.6)	82.0 (d)	C-5, -8
8		82.8 (s)			90.9 (s)	
9	5.65 d (7.6)	70.2 (d)	C-7, -8, -10, -11, -17, acetate carbonyl	5.73 s	78.5 (d)	C-1, -10, -11, -17, acetate carbonyl
10	2.62 d (7.6)	41.8 (d)	C-1, -2, -8, -9, -11, -15	3.40 s	45.5 (d)	C-1, -8, -11, -15, -20
11		61.0 (s)			148.0 (s)	
12α/β	1.87 m; 2.28 m	24.1 (t)	n.o.[e)]	2.29 m (2H)	32.8 (t)	C-11
13α/β	1.19 m; 2.23 m	31.9 (t)	n.o.[e)]	1.88 m (2H)	27.1 (t)	n.o.[e)]
14	4.88 d (4.4)	72.2 (d)	C-1, acetate carbonyl	5.12 dd (3.2, 2.8)	74.8 (d)	n.o.[e)]
15	1.12 s	16.4 (q)	C-1, -2, -10, -14	1.07 s	14.3 (q)	C-1, -2, -10, -14
16a/b	5.30 s; 5.15 s	114.5 (t)	C-4, -6	3.82 br s (2H)	66.2 (t)	n.o.[e)]
17	2.62 q (7.2)	48.5 (d)	C-8, -18, -19	2.81 q (7.2)	45.4 (d)	C-18, -19
18	1.30 d (7.2)	7.2 (q)	C-8, -17, -19	1.56 d (7.2)	9.6 (q)	C-8, -17, -19
19		174.6 (s)			174.8 (s)	
20a	2.99 dd (3.6, 2.4)	56.8 (t)	n.o.[e)]	5.05 s	111.1 (t)	C-10, -12
b	2.79 d (3.6)			4.73 s		
OH-8	4.65 br s		C-7, -8			
Acetates	2.11 s	21.3 (q)	Acetate carbonyl	2.14 s	21.5 (q)	Acetate carbonyl
		169.9 (s)			169.4 (s)	
	2.08 s	21.2 (q)	Acetate carbonyl	2.02 s	21.0 (q)	Acetate carbonyl
		169.9 (s)			170.2 (s)	
	2.05 s	20.9 (q)	Acetate carbonyl	1.99 s	21.3 (q)	Acetate carbonyl
		169.9 (s)			170.7 (s)	

a) Spectra measured at 400 MHz in $CDCl_3$ at 25 °C. b) Spectra measured at 100 MHz in $CDCl_3$ at 25 °C. c) J values (in hertz) in parentheses. d) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. e) n.o. = not observed.

(d, CH-4) in the ^{13}C NMR data of **5** (Table 3); this was further supported by four olefin proton signals at δ 6.63 (1H, d, $J = 16.0$ Hz, H-4), 5.79 (1H, dd, $J = 16.0$, 7.2 Hz, H-3), 5.30 (1H, s, H-16a), and 5.15 (1H, s, H-16b) in the ^{1}H NMR spectrum of **5** (Table 3). Moreover, four carbonyl resonances appeared at δ 174.6 (s, C-19) and 169.9 (ester carbonyls, 3 × s), confirming the presence of a γ-lactone and three other ester groups in **5**. In the ^{1}H NMR spectrum of **5**, three acetyl methyls (δ 2.11, 3H, s; 2.08, 3H, s; 2.05, 3H, s) were observed. Thus, from the NMR data, six degrees of unsaturation were accounted for, and **5** must be tetracyclic. The presence of an exocyclic epoxy group was deduced from the signals of two oxygenated carbons at δ 61.0 (s, C-11) and 56.8 (t, CH_2-20). The proton chemical shifts of H_2-20 (δ 2.99, 1H, dd, $J = 3.6$, 2.4 Hz; 2.79. 1H, d, $J = 3.6$ Hz) confirmed the presence of this group. In addition, a tertiary methyl (δ 1.12, 3H, s, H_3-15), a secondary methyl (δ 1.30, d, $J = 7.2$ Hz, H_3-18), two aliphatic methine protons (δ 2.62, 1H, d, $J = 7.6$ Hz, H-10; 2.62, 1H, q, $J = 7.2$ Hz, H-17), two pairs of aliphatic methylene protons (δ 2.28, 1H, m; 1.87, 1H, m, H_2-12; 2.23, 1H, m; 1.19, 1H, m, H_2-13), four oxymethine protons (δ 5.65, 1H, d, $J = 7.6$ Hz, H-9; 5.25, 1H, d, $J = 7.2$ Hz, H-2; 4.88, 1H, d, $J = 4.4$ Hz, H-14; 4.27, 1H, br s, H-7), a chlorinated methine proton (δ 5.03, 1H, d, $J = 2.8$ Hz, H-6), and a hydroxy proton (δ 4.65, 1H, br s, OH-8) were observed in the ^{1}H NMR spectrum of **5**.

From the ^{1}H–^{1}H COSY experiment of **5** (Figure 3), it was possible to establish the spin system that maps out the proton sequences from H-2/H-3, H-3/H-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. The allylic coupling between H-4/H-16a and H-6/H_2-16 and the *w*-coupling between H-20a/H-12β were also observed in ^{1}H–^{1}H COSY spectrum of **5**. Based on these data and the HMBC correlations (Figure 3 and Table 3), the carbon skeleton of **5** could be established. An exocyclic double bond attached at C-5 was confirmed by the HMBC correlations between H_2-16/C-4, -6; H-4/C-16; and H-6/C-16. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14; H-2/C-15; and H-10/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 positioned at C-8, an oxygenated quaternary carbon resonating at δ 82.8 (s). This observation was further confirmed by the HMBC correlations between OH-8/C-7, -8. These data, together with the HMBC correlations between H-17/C-8, -18, -19 and H_3-18/C-8, -17, -19, were used to establish the molecular framework of **5**.

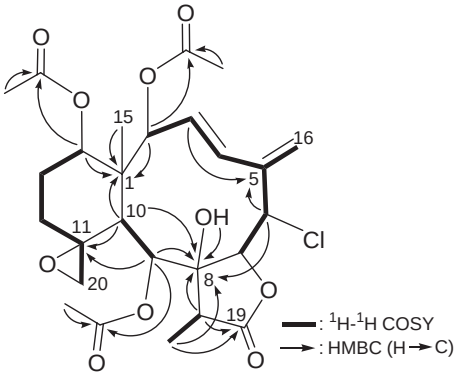

Figure 3. The ^{1}H–^{1}H COSY and HMBC correlations (protons and quaternary carbons) of **5**.

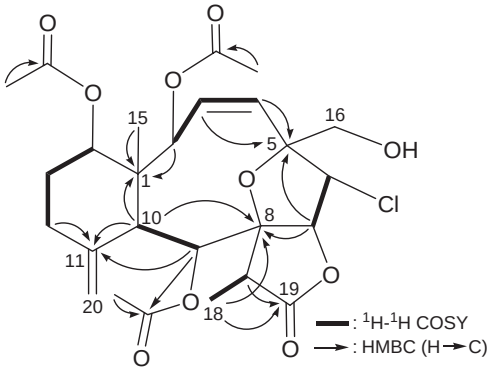

Figure 5. The ^{1}H–^{1}H COSY and HMBC correlations (protons and quaternary carbons) of **6**.

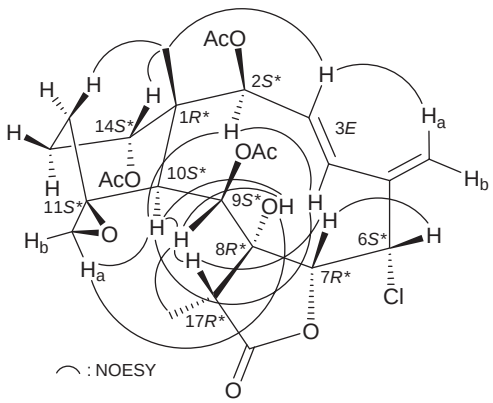

Figure 4. Selective NOESY correlations of **5**.

The relative stereochemistry of **5** was elucidated from the NOESY interactions observed in a NOESY experiment (Figure 4) and by the vicinal ^{1}H–^{1}H coupling constants analysis. Due to the α-orientation of H-10, the ring junction C-15 methyl group is β-oriented, as no correlation was observed between H-10 and H_3-15. In the NOESY spectrum of **5**, H-10 is correlated to H-2 and OH-8, suggesting that these protons are located on the same face and can be assigned as α protons. H-14 was found to exhibit a response with H_3-15, but not with H-10, showing that this proton is β-oriented. H-9 was found to show correlations with H-10, H-17, H_3-18, and OH-8, and, from molecular models, was found to be reasonably close to H-10, H-17, H_3-18, and OH-8; therefore, H-9 should be placed on the α face in **5**, and H-17 and H_3-18 are β- and α-oriented in the γ-lactone moiety, respectively. H-7 exhibited correlations with H-17 and H-6, suggesting that these protons are on the β face of **5**. The trans geometry of the C-3/4 double bond was indicated by a 16.0 Hz coupling constant between H-3 (δ 5.79) and H-4 (δ 6.63). Furthermore, H-3 showed correlations with H_3-15 and H-16a, but not with H-2; and H-4 showed responses with H-2 and H-10, demonstrating the E configuration of $\Delta^{3,4}$. Therefore, the presence of an *s-cis* diene moiety in **5** was elucidated. A proton of C-20 methylene (δ 2.99, H-20a) was found to exhibit correlations with H-10 and OH-8, but not with H_3-15; and H_3-15 showed a correlation with a proton of C-12 methylene (δ 2.28, H-12β), indicating that the methylenecyclohexane ring of **5** should be presented as boat rather than a chair conformation for **5**, and the configurations of the chiral centers of **5** were assigned as 1R*, 2S*, 6S*, 7R*, 8R*, 9S*, 10S*, 11S*, 14S*, and 17R*.

Robustolide K (**6**) was obtained as a white powder. The HR-ESI-MS of **6** revealed a quasi-molecular ion peak at m/z 563.1663 $(M + Na)^+$ consistent with the molecular formula $C_{26}H_{33}ClO_{10}$ (calcd for $C_{26}H_{33}ClO_{10} + Na$, 563.1660) and 10 degrees of unsaturation. The IR spectrum of **6** showed bands at 3436, 1788, and 1738 cm^{-1}, consistent with the presence of hydroxy, γ-lactone, and ester groups, respectively. From the ^{13}C NMR data of **6** (Table 3), the presence of a disubstituted olefine and a carbon–carbon double bond were deduced from the signals of four carbons resonating at δ 148.0 (s, C-11), 130.6 (d, CH-4), 130.2 (d, CH-3), 111.1 (t, CH_2-20), and were further supported by four olefin proton signals appearing at δ 5.58 (1H, dd, $J = 11.2$, 0.8 Hz, H-4), 5.45 (1H, dd, $J = 11.2$, 8.4 Hz, H-3), 5.05 (1H, s, H-20a), and 4.73 (1H, s, H-20b) in the ^{1}H NMR spectrum of **6** (Table 3). In the ^{13}C NMR spectrum, six ester carbonyl resonances appeared at δ 174.8 (s, C-19), 170.7, 170.2, and 169.4 (ester carbonyls, 3 × s), confirming the presence of a γ-lactone and three other ester groups. In the ^{1}H NMR spectrum of **6**, three acetate methyls (δ 2.14, 2.02, and 1.99, each 3H × s) were observed. Thus, from the NMR data, six degrees of unsaturation were accounted for, and **6** was identified as a tetracyclic compound.

The gross structure of **6** was determined using 2D NMR studies. From the ^{1}H–^{1}H COSY spectrum of **6** (Figure 5), it was possible to establish the separate spin systems between H-2/H-3; H-3/H-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. Based on these data and HMBC correlations (Figure 5 and Table 3), the carbon skeleton of **6** could be established. An exocyclic double bond attached at C-11 was confirmed by the HMBC correlations between H_2-20/C-10, -12 and H-10/C-20. The ring junction C-15 methyl group was positioned at C-1 from the HMBC correlations between H_3-15/C-1, -2, -10, -14 and H-10/C-15. The HMQC and ^{1}H–^{1}H COSY correlations also revealed that the chlorine atom is attached at C-6 methine (δ 4.26; δ 63.8). The presence of an acetate ester positioned at C-9 was established by an HMBC correlation between H-9 (δ 5.73) and an acetate carbonyl (δ 169.4). The remaining two acetoxy groups were positioned at C-2 and C-14, as indicated by analysis of the ^{1}H–^{1}H COSY correlations and characteristic NMR signals analysis,

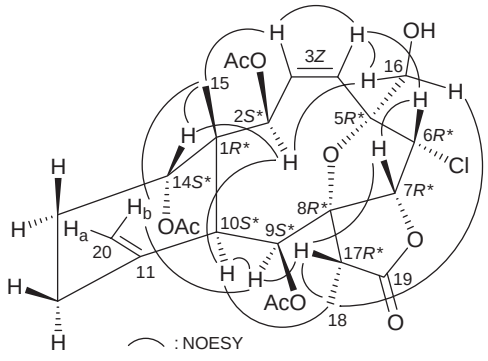

Figure 6. Selective NOESY correlations of **6**.

although no HMBC correlation was observed between the acetate carbonyls and H-2 and H-14. The two gem protons at δ 3.82 together with an oxygenated methylene at δ 66.2 were assigned to a hydroxymethyl group attached to a quaternary carbon. The HMBC correlations between H-7 (δ 4.73) and each of the two oxygenated low-field quaternary carbons appearing at δ 85.1 (C-5) and 90.9 (C-8) suggested the presence of a C-5/8 ether linkage, and the remaining hydroxymethyl group is attached at C-5. These data together with the HMBC correlations between H-17/C-18, -19 and H_3-18/C-8, -17, -19, were used to establish the molecular framework of **6**.

The relative stereochemistry of **6** was elucidated from the NOESY interactions observed in a NOESY experiment (Figure 6). In the NOESY experiment of **6**, H-10 is correlated to H-2, H-9, and H_3-18, but not to H_3-15, indicating that these protons are located on the same face of the molecule and can be assigned as α-protons, as the C-15 methyl group is the β-substituent at C-1. H-14 was found to exhibit correlation with H-2 and H_3-15, showing that this proton is positioned on the equatorial direction and has a β-orientation at C-14. H-7 showed correlations with H-6 and H-17, suggesting that these protons are on the β-face of **6**. The cis geometry of the C-3/C-4 double bond was indicated by an 11.2 Hz coupling constant between H-3 (δ 5.45) and H-4 (δ 5.58) and by a response between H-3 and H-4. Moreover, H-3 showed a correlation with H_3-15, but not with H-2; H-4 showed a response with H-6; and the C-16 methylene protons showed correlations with H-2, H-4, and H_3-18. Based on the above findings, and from consideration of molecular models, the configuration of C-5 was elucidated as of an R* form. In addition, a proton of C-20 methylene (δ 4.73, H-20b) was found to exhibit responses with H-9 and H_3-15, but not with H-10; and H_3-15 did not show response with the protons of C-12 methylene, indicating that the methylenecyclohexane ring in **6** should be presented as a chair rather than a boat conformation for **6**, and the chiral centers of **6** were assigned as 1R*, 2S*, 5R*, 6R*, 7R*, 8R*, 9S*, 10S*, 14S*, and 17R*. By detailed analysis, it was found the NMR data of **6** were similar to those of a known briarane, juncenolide G.[20] However, the ^{1}H and ^{13}C NMR data revealed that the signals corresponding to both the C-3/4 and C-11/20 epoxy groups in juncenolide G were not present and had been replaced by carbon–carbon double bonds in **6**. It is worth noting that the briarane-type natural products possessing a tetrahydrofuran moiety (the ether linkage between C-5/C-8) as is present in **6** are rarely found.[20]

Table 4. Key ^{1}H NMR Data Differences between Diterpenoids **10** and **11**

Position	**10**[a)]	**11**[b)]	$\Delta\delta = \delta(\mathbf{10}) - \delta(\mathbf{11})$
H-2	6.00 d (8.3)	5.97 d (8.4)	+0.03
H-9	5.75 m ($\Delta W_{1/2} = 3$)	5.76 s	−0.01
H-12	4.35 t (3.2)	4.54 dd (3.2, 2.0)	−0.19
H-14	4.85 t (2.8)	4.91 dd (2.8, 2.8)	−0.06

a) Data were reported by Isaacs et al. (see Ref. 21). These data were recorded at 360 MHz in $CDCl_3$. b) Data were reported by Sung et al. (see Ref. 19). These data were recorded at 400 MHz in $CDCl_3$.

Table 5. Inhibitory Effects of Briaranes **5** and **6** on Superoxide Anion Generation by Human Neutrophils in Response to fMet-Leu-Phe/Cytochalastin B

Compound	Superoxide generation inhibition
	IC_{50}/μg mL^{-1} [a)]
5	5.4 ± 0.7
6	6.4 ± 0.4

a) Results are presented as means ± SEM ($n = 3$).

In a previous study, a briarane derivative, juncin F (**10**), was isolated from a gorgonian coral *Junceella juncea*, collected off the Red Sea.[21] However, an accurate structure for this compound, particularly with regards to the positions of the acyloxy groups, was not determined. The structure of **10** was found to be similar with that of a known briarane, robustolide H (**11**), which was isolated from *E. robusta* in a latter study.[19] By comparison of the oxymethine proton chemical shifts of acyloxy groups attached at positions such as C-2, -9, -12, and C-14 of **10** with those of **11**, it was found that only the data of H-12 (δ 4.35, t, $J = 3.2$ Hz), including the chemical shift, coupling pattern, and coupling constant in **10** were different from those of **11** (δ 4.54, dd, $J = 3.2, 2.0$ Hz) (Table 4). Based on the above observations, the isobutyrate ester in **10** was identified as being attached at C-12.

Robustolides J (**5**) and K (**6**) were found to show inhibitory effects on superoxide anion generation by human neutrophils (Table 5), and briaexcavatin S (**3**) exhibited weak cytotoxicity toward CCRF-CEM (human T-cell acute lymphoblastic leukemia) tumor cells ($ED_{50} = 37.8$ μg mL^{-1}).

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