

(-)-11 β ,20 β -Epoxy-4-deacetoxyjunceollolide D, Polyoxygenated Briarane Deriving from Octocoral *Junceella fragilis* in Sea Whip

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ABSTRACT

Through the chemical screening of an octocoral, identified as *Junceella fragilis*, polyoxygenated briarane-type diterpenoid, (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollolide D (**1**) was discovered, which was then subject to single-crystal X-ray diffraction analysis to determine its absolute structure. The structure was verified with 2D NMR experiments, supplemented with a literature review. At the same time, conformation of previously-discovered briarane, (-)-11 α ,20 α -epoxy-4-deacetoxyjunceollolide D was evaluated.

Keywords: *Junceella fragilis*, briarane, junceollolide, absolute configuration, single-crystal X-ray diffraction.

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1 INTRODUCTION

Junceella fragilis (Ridley, 1884), an octocoral, also known as sea whip, belonging to the family Ellisellidae (McFadden et al., 2022), is extensively present in tropical Indo-Pacific Ocean waters and has been found to be a rich source of briarane-type diterpenoids (Chung et al., 2018; Sung et al., 2004; Wu et al., 2011). This study aimed to explore substances from *J. fragilis* collected from Taiwan's coast area, which is well-known for its biodiversity thanks to the convergence of the Kuroshio Current and the surface current of the South China Sea. A highly-oxygenated briarane was isolated and named (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollide D (**1**) (Figure 1). The study employed a diffractometer equipped with a molybdenum ratio source (Mo K α) to analyze the structure of **1** via single-crystal X-ray diffraction, thereby determining its absolute configuration. The analysis also led to the revision of the structure of a known briarane (-)-11 α ,20 α -epoxy-4-deacetoxyjunceollide D (**1**) (García et al., 1999; Sheu et al., 2006).

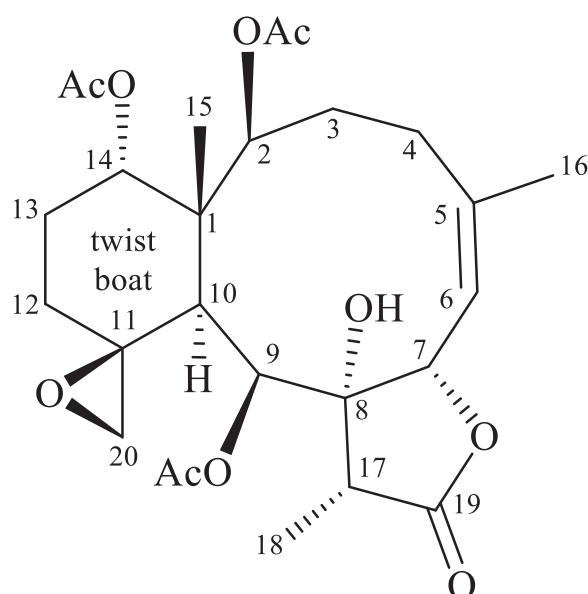


Figure 1. Structure of (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollide D (**1**).

2 RESEARCH METHODS

2.1. Experimental procedures

The optical rotation value and IR spectra of the compound were measured with the JASCO P-2000 digital polarimeter and Thermo Scientific Nicolet iS5 FT-IR spectrophotometer, respectively. The ^1H and ^{13}C spectra were recorded with the Jeol ECZ NMR spectrometer, operating at 400 MHz for ^1H NMR, and 100 MHz for ^{13}C NMR. Chemical shifts were indicated in parts per million (δ), while the coupling constants (J) were expressed in Hertz (Hz). The residual peaks of deuterated solvent, CDCl_3 , served as reference points at δ_{H} 7.26 and δ_{C} 77.0 ppm, respectively. The ESIMS and HRESIMS spectra were gauged with the Thermo Fisher Orbitrap Exploris 120 mass spectrometer equipped with an ESI ion source operating in positive ionization mode. The sample was separated via column chromatography, which utilizes silica gel with a particle size of 230–400 mesh (Merck). To analyze the separated samples, thin-layer chromatography (TLC) was performed on plates coated with silica gel 60 and RP-18 F $_{254}$ S. TLC plates were visualized with a heated solution of 10% H_2SO_4 to identify the spots of interest.



2.2. Animal material

In the study, samples of *Junceella fragilis* (Ridley, 1884), a zooxanthella-containing species, were collected by self-contained underwater breathing apparatus (SCUBA) divers in April 2017, near the coastline of Southern Taiwan, and then identified by Dr. You-Ying Chen at the National Museum of Marine Biology & Aquarium (NMMBA), Taiwan, by comparing the physical characteristics and microscopic images of its coral sclerites with those reported in previous studies (Chang et al., 2007; Dai, 2021, 2022; Dai & Chin, 2019; McFadden et al., 2022). A colony of *J. fragilis*, white or grey in color, grows in a whip-like, unbranched form. A greater density of colorless sclerites, mostly double-heads, was also found in the colony (Chang et al., 2007). A voucher specimen (TWSC-20170413) is stored at the NMMBA.

2.3. Extraction and isolation

The coral specimen was freeze-dried, with the dry version weighing only 313 g, compared to the original 775 g. It was then sliced into pieces and extracted with a solution of methanol (MeOH) and dichloromethane (CH₂Cl₂) (equal volume) at room temperature, producing a crude extract weighing 19.0 g, which was further separated via liquid-liquid partition with ethyl acetate (EtOAc) and H₂O, resulting in two phases. The EtOAc phase (8.0 g) was further subjected to silica gel column chromatography (Si C. C.). A gradient solvent system of *n*-hexane and acetone mixtures (50:1 *n*-hexane/acetone to 1:2 *n*-hexane/acetone) was used to elute the column, producing eight fractions labeled A to H. Fraction F was then purified with Si C. C. A gradient solvent system of *n*-hexane/EtOAc mixtures (2:1→3:2→1:1→1:2→pure EtOAc) was employed to obtain five subfractions, F1–F5, among which fraction F4 was purified with C18 Si C. C. The purification was carried out with a solvent system comprising a mixture of methanol and H₂O in a 65:35 ratio. As a result, compound **1** (50.5 mg) was obtained.

2.3.1. (-)-11 β ,20 β -Epoxy-4-deacetoxyjunceollolide D (**1**)

Colorless prisms; $[\alpha]_D^{23}$ -117 (*c* 0.05, CHCl₃); IR (KBr) ν_{\max} 3305, 1776, 1741 cm⁻¹; ¹H (400 MHz, CDCl₃) and ¹³C NMR (100 MHz, CDCl₃) data, as shown in Table 1; ESIMS *m/z* 531 [M + Na]⁺; HRESIMS *m/z* 531.22028 (calcd. for C₂₆H₃₆O₁₀ + Na 531.22007).

2.4. X-ray crystallography of (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollolide D (**1**)

Colorless prisms of compound **1** were successfully obtained from methanol solution. The crystal was determined to be of the orthorhombic system, space group *P*2₁2₁2₁ (# 19), with lattice parameters of *a* = 9.8573(3) Å, *b* = 18.0398(8) Å, *c* = 30.8017(12) Å, *V* = 5477.3(4) Å³, *Z* = 4, *D*_{calcd} = 1.255 Mg/m³, and λ (Mo *K* α) = 0.71073 Å. Intensity data were collected on a crystal diffractometer (Bruker, model: D8 Venture) up to a maximum angle (θ_{\max}) of 26.246°. Among the total 88,290 sets of reflection data collected, 11,062 were independent. The crystal structure was solved using direct methods and refined with a full-matrix least-squares method via the *F*² procedure (Sheldrick, 2015a; 2015b). The refined structural model had a final *R*₁ = 0.0668, *wR*₂ = 0.1445 for 9613 observed reflections [*I* > 2 σ (*I*)], and 673 variable parameters. The structure of compound **1** has been documented and stored at the Cambridge Crystallographic Data Centre (CCDC) with supplementary publication number CCDC 2113018. This information is available on the CCDC website at <https://www.ccdc.cam.ac.uk/conts/retrieving.html> or via a written request to the CCDC at their address, 12 Union Road, Cambridge CB2 1EZ, UK, or via e-mail at deposit@ccdc.cam.ac.uk.

3 RESULTS AND DISCUSSION

(-)-11 β ,20 β -Epoxy-4-deacetoxyjunceollide D (**1**) was isolated as a colorless prism with [M + Na]⁺ ion peak at *m/z* 531.22028 in HRESIMS embodying molecular formula C₂₆H₃₆O₁₀ (calcd. for C₂₆H₃₆O₁₀ + Na, 531.22007) with nine double-bond equivalents (DBEs). The IR spectrum of **1** showed absorptions at ν_{\max} 3305, 1776, and 1741 cm⁻¹, consistent with the presence of hydroxy, γ -lactone, and ester moieties, respectively. The ¹H and ¹³C NMR data of **1** (Table 1), along with distortionless enhancement by polarization transfer (DEPT) and heteronuclear single quantum coherence (HSQC) spectra, revealed the presence of three acetoxy groups (δ_{H} 2.20, 1.98, 1.94/ δ_{C} 21.6, 20.9, 20.9, 3 \times acetate methyl; δ_{C} 169.5, 170.6, 169.9, 3 \times acetate carbonyl), an exchangeable proton (δ_{H} 4.88, 1H, d, *J* = 1.2 Hz, OH-8), a trisubstituted carbon-carbon double bond (δ_{C} 143.8, C-5; δ_{H} 5.57, 1H, dd, *J* = 10.0, 1.6 Hz/ δ_{C} 120.8, CH-6), and a γ -lactone carbonyl (δ_{C} 176.5, C-19). An exocyclic epoxy group was elucidated from the signals of two oxygenated carbons at δ_{C} 62.3 (C-11) and 59.1 (CH₂-20). The proton chemical shifts at δ_{H} 2.94 (1H, dd, *J* = 4.4, 1.2 Hz, H-20a) and 2.84 (1H, d, *J* = 4.4 Hz, H-20b) confirmed the presence of this group. Thus, from the NMR data, five degrees of DBEs were accounted for, indicating **1** must be tetracyclic.

Table 1. ¹H and ¹³C NMR data for briarane **1**.

C/H	1		(-)-11 β ,20 β -epoxy-4-deacetoxyjunceollide D ^e		(-)-11 α ,20 α -epoxy-4-deacetoxyjunceollide D ^f	
	δ_{H}^a	δ_{C}^b	δ_{H}^a	δ_{C}^b	δ_{H}	δ_{C}
1		46.7, C ^d		47.0, C ^d		47.4, C
2	4.68 d (4.8) ^c	74.6, CH	4.64 d (4.8) ^c	74.9, CH	4.71 br d (4.9) ^c	73.4, CH
3/3'	2.43 m; 1.63 m	32.0, CH ₂	2.43 m; 2.02 m	29.0, CH ₂	2.51 m; 1.71 m	31.9, CH ₂
4/4'	2.49 m; 2.07 m	28.7, CH ₂	2.39 m; 1.56 m	32.3, CH ₂	2.01 m; 1.05 m	28.7, CH ₂
5		143.8, C		144.0, C		135.6, C
6	5.57 dd (10.0, 1.6)	120.8, CH	5.54 dd (10.0, 1.2)	121.1, CH	5.60 br d (10.2)	120.7, CH
7	5.12 dd (10.0, 1.2)	77.8, CH	5.08 d (10.0)	78.3, CH	5.13 d (10.2)	77.9, CH
8		80.2, C		80.5, C		80.1, C
9	5.59 d (5.6)	67.4, CH	5.55 d (5.6)	67.8, CH	4.84 d (4.9)	77.6, CH
10	2.36 dd (5.6, 1.2)	39.6, CH	2.33 d (5.6)	39.9, CH	2.17 s	39.6, CH
11		62.3, C		62.6, C		62.6, C
12/12'	2.27 m; 1.12 m	23.6, CH ₂	2.23 m; 1.08 m	24.0, CH ₂	1.81 m; 1.05 m	23.6, CH ₂
13/13'	2.11 m; 1.74 dt (15.6, 10.0)	24.3, CH ₂	2.07 m; 1.70 m	24.6, CH ₂	2.4 m; 2.1 m	24.3, CH ₂
14	4.80 d (5.2)	73.3, CH	4.76 d (4.8)	73.6, CH	5.63 br d (5.4)	67.4, CH
15	1.07 s	14.5, CH ₃	1.03 s	14.8, CH ₃	1.10 s	14.5, CH ₃
16	1.99 d (1.6)	28.0, CH ₃	1.95 d (1.2)	28.3, CH ₃	2.02 s	28.1, CH ₃
17	2.33 q (7.2)	42.2, CH	2.29 q (7.2)	42.5, CH	2.37 q (7.1)	42.3, CH
18	1.11 d (7.2)	6.5, CH ₃	1.07 d (7.2)	6.9, CH ₃	1.15 d (7.1)	6.5, CH ₃
19		176.5, C		176.7, C		176.8, C
20a	2.94 dd (4.4, 1.2)	59.1, CH ₂	2.90 d (4.0)	59.4, CH ₂	2.98 br d (4.1)	59.1, CH ₂
20b	2.84 d (4.4)		2.79 d (4.0)		2.86 br d (4.1)	
OAc-2		170.6, C		170.9, C		170.6, C
	1.98 s	20.9, CH ₃	1.94 s	74.9, CH ₃	2.02 s	20.8, CH ₃ ^g
OAc-9		169.5, C		169.8, C		169.9, C
	2.20 s	21.6, CH ₃	2.16 s	21.9, CH ₃	2.23 s	21.7, CH ₃
OAc-14		169.9, C		170.2, C		170.2, C
	1.94 s	20.9, CH ₃	1.90 s	21.2, CH ₃	1.97 s	20.9, CH ₃ ^g
OH-8	4.88 d (1.2)		4.83 s			

^a Spectra recorded at 400 MHz in CDCl₃. ^b Spectra recorded at 100 MHz in CDCl₃. ^c *J* values (in Hz) in parentheses. ^d Multiplicity deduced from DEPT and HSQC spectra. ^e Data were reported by Sheu et al., 2006. ^f Data were reported by García et al., 1999. ^g Data exchangeable.



The gross structure of **1** was determined via two-dimensional (2D) NMR studies, including ^1H - ^1H correlation spectroscopy (COSY), HSQC, and heteronuclear multiple bond correlation (HMBC) experiments. The ^1H NMR coupling information deriving from the COSY spectrum of **1** enabled identification of the separate spin systems of H-2/H₂-3/H₂-4, H-6/H-7, H-9/H-10, H₂-12/H₂-13/H-14, and H-17/H₃-18 (Figure 2). These data, together with the key HMBC correlations between protons and non-protonated carbons such as H-2, H-10, H-13', H-14, H₃-15/C-1; H-4', H-7, H₃-16/C-5; H-9, H-10, H₃-18, OH-8/C-8; H-9, H-10, H₂-20/C-11, and H-17, H₃-18/C-19 (Figure 2), permitted the elucidation of the carbon skeleton. The HMBC correlations among H₃-15/C-1, C-2, C-10, C-14 and H₃-18/C-8, C-17, C-19 indicated that Me-15 and Me-18 were placed at C-1 and C-17, respectively. Vinyl methyl Me-16 at C-5 was confirmed by the HMBC correlations among H₃-16/C-4, C-5, C-6, as well as a long-range four-bond ($^4J_{\text{H-H}}$) allylic coupling between H-6/H₃-16 ($J = 1.6$ Hz) (Figure 2). The hydroxy group at C-8 was deduced from the HMBC correlations of a hydroxy proton (δ_{H} 4.88) to C-7, C-8, and C-9. Furthermore, in the HMBC spectrum, H-2 (δ_{H} 4.68), H-9 (δ_{H} 5.59), and H-14 (δ_{H} 4.80) were correlated with the acetate carbonyls at δ_{C} 170.6 (OAc-2), 169.5 (OAc-9), and 169.9 (OAc-14), indicating that these three acetate groups were placed at C-2, C-9, and C-14, respectively. The epoxy group positioned at C-11/20 was confirmed by the HMBC correlations among H₂-20/C-10, C-11 and H-10/C-11, C-20. Interpretation of the ^1H , ^{13}C , DEPT, HSQC, COSY, and HMBC NMR data unequivocally determined the planar structure of **1**.

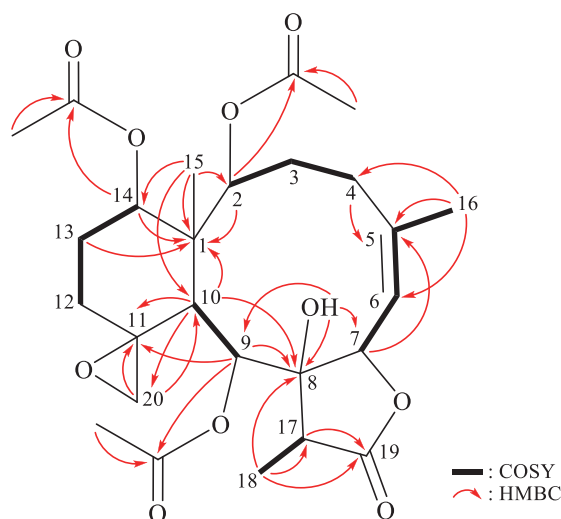


Figure 2. COSY correlations and selective HMBC of **1**.

According to a summary of the chemical shifts of 11,20-epoxy groups in briarane derivatives, with ^{13}C NMR data for C-11 and C-20 at δ_{C} 62–63 and 58–60 ppm, respectively, the epoxy group was β -oriented and the cyclohexane ring existed in a twist-boat conformation (Sheu et al., 2006), suggesting that the configuration of the 11,20-epoxy group in **1** (δ_{C} 62.3, C-11; 59.1, CH₂-20) is β -oriented and the cyclohexane ring is present in a twist-boat conformation. The relative stereochemistry of **1** was based on observations of the nuclear Overhauser effect (NOE) interactions in a nuclear Overhauser effect spectroscopy (NOESY) experiment and vicinal ^1H - ^1H coupling constant analysis. Given the α -orientation of H-10, the ring junction C-15 methyl group should be β -oriented as there was no NOE correlation between H-10 and H₃-15, and different NOE correlations between H-10 and H-2/H-9/OH-8 in the NOESY spectrum, suggesting that H-2, H-9, H-10, and OH-8 were all α -oriented. At the same time, NOE correlation of Me-15 with H-14 indicated that H-14 was β -oriented. H₃-18 demonstrated NOE correlation with OH-8, revealing that Me-18 was α -oriented at C-17. H-7 exhibited NOE correlation with H-17, suggesting that H-7 was positioned on the β -face. The *Z*-configuration of the C-5/6 double bond was confirmed based on the correlation between the C-6 olefin proton (δ_{H} 5.57) and the C-16 vinyl methyl (δ_{H} 1.99). The study identified the structure of **1** via a single crystal diffraction analysis. Suitable

colorless prisms were obtained from a MeOH solution. The Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram (Figure 3) confirmed that the stereogenic centers of **1** have an absolute configuration of 1*S*,2*S*,7*S*,8*R*,9*S*,10*S*,11*S*,14*S* and 17*R*. Crystal data, data collection, structure refinement details, and structural parameters (primary bond distance and angle) for **1** are given in Tables 2 and 3, respectively.

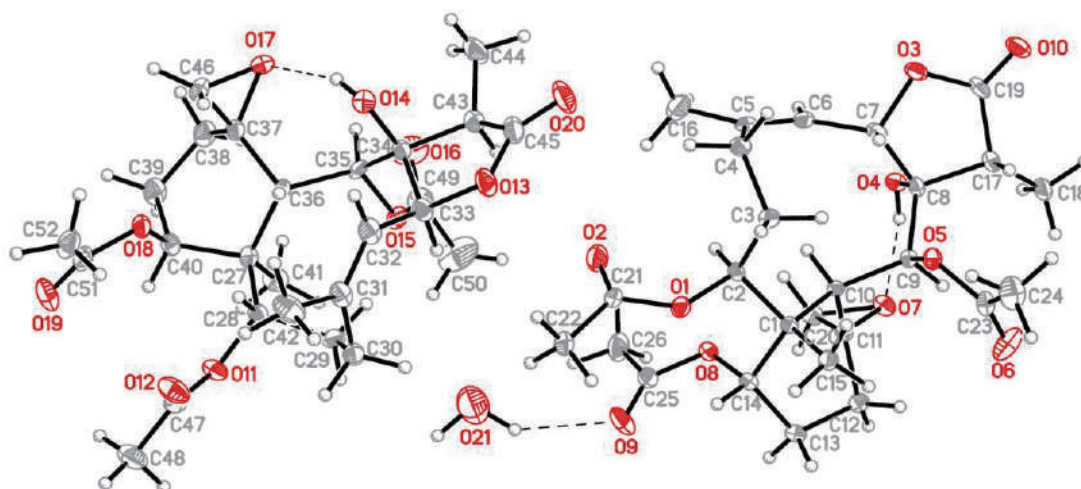


Figure 3. Structure of (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollide D (**1**) revealed using ORTEP.

Table 2. Crystal data and structure refinement for **1**.

Empirical formula	C ₅₂ H ₇₄ O ₂₁
Formula weight	1035.11
Temperature	200(2) K
Wavelength	0.71073 Å
Crystal system	Orthorhombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit cell dimensions	$a = 9.8573(3)$ Å $\alpha = 90^\circ$ $b = 18.0398(8)$ Å $\beta = 90^\circ$ $c = 30.8017(12)$ Å $\gamma = 90^\circ$
Volume	5477.3(4) Å ³
<i>Z</i>	4
Density (calculated)	1.255 Mg/m ³
Absorption coefficient	0.097 mm ⁻¹
<i>F</i> (000)	2216
Crystal size	0.278 × 0.082 × 0.018 mm ³
θ range for data collection	2.169 to 26.246°
Index ranges	-12 ≤ <i>h</i> ≤ 12, -22 ≤ <i>k</i> ≤ 22, -38 ≤ <i>l</i> ≤ 38
Reflections collected	88290
Independent reflections	11062 [<i>R</i> (int) = 0.0814]
Completeness to $\theta = 25.242^\circ$	99.9 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9585 and 0.8790
Refinement method	Full-matrix least-squares on <i>F</i> ²
Data/restraints/parameters	11062/0/673
Goodness-of-fit on <i>F</i> ²	1.120
Final <i>R</i> indices [<i>I</i> > 2 σ (<i>I</i>)]	<i>R</i> ₁ = 0.0668, <i>wR</i> ₂ = 0.1445
<i>R</i> indices (all data)	<i>R</i> ₁ = 0.0778, <i>wR</i> ₂ = 0.1494
Absolute structure parameter	0.2(3)
Extinction coefficient	n/a
Largest diff. peak and hole	0.711 and -0.344 e.Å ⁻³



Table 3. Bond lengths [Å] and angles [°] for **1**.

O(1)-C(21)	1.350(5)	C(3)-C(4)	1.528(6)	O(11)-C(28)	1.470(5)	C(30)-C(31)	1.513(7)
O(1)-C(2)	1.459(5)	C(4)-C(5)	1.520(7)	O(12)-C(47)	1.190(6)	C(31)-C(32)	1.331(7)
O(2)-C(21)	1.196(6)	C(5)-C(6)	1.324(6)	O(13)-C(45)	1.342(6)	C(31)-C(42)	1.509(7)
O(3)-C(19)	1.350(6)	C(5)-C(16)	1.506(7)	O(13)-C(33)	1.472(5)	C(32)-C(33)	1.500(7)
O(3)-C(7)	1.479(5)	C(6)-C(7)	1.491(6)	O(14)-C(34)	1.428(6)	C(33)-C(34)	1.552(6)
O(4)-C(8)	1.416(5)	C(7)-C(8)	1.551(6)	O(15)-C(49)	1.363(6)	C(34)-C(43)	1.535(6)
O(5)-C(23)	1.363(5)	C(8)-C(17)	1.536(6)	O(15)-C(35)	1.437(5)	C(34)-C(35)	1.537(6)
O(5)-C(9)	1.445(5)	C(8)-C(9)	1.538(5)	O(16)-C(49)	1.177(7)	C(35)-C(36)	1.557(6)
O(6)-C(23)	1.194(6)	C(9)-C(10)	1.565(5)	O(17)-C(37)	1.451(6)	C(36)-C(37)	1.534(6)
O(7)-C(20)	1.463(6)	C(10)-C(11)	1.529(6)	O(17)-C(46)	1.453(6)	C(37)-C(46)	1.460(7)
O(7)-C(11)	1.464(5)	C(11)-C(20)	1.453(6)	O(18)-C(51)	1.334(6)	C(37)-C(38)	1.495(7)
O(8)-C(25)	1.340(5)	C(11)-C(12)	1.504(6)	O(18)-C(40)	1.466(5)	C(38)-C(39)	1.537(7)
O(8)-C(14)	1.456(5)	C(12)-C(13)	1.539(6)	O(19)-C(51)	1.200(7)	C(39)-C(40)	1.513(6)
O(9)-C(25)	1.203(6)	C(13)-C(14)	1.531(6)	O(20)-C(45)	1.209(6)	C(43)-C(45)	1.484(7)
O(10)-C(19)	1.204(5)	C(17)-C(18)	1.510(6)	C(27)-C(41)	1.535(6)	C(43)-C(44)	1.516(7)
C(1)-C(14)	1.546(6)	C(17)-C(19)	1.512(6)	C(27)-C(28)	1.539(6)	C(47)-C(48)	1.499(7)
C(1)-C(15)	1.549(6)	C(21)-C(22)	1.486(6)	C(27)-C(40)	1.544(6)	C(49)-C(50)	1.476(9)
C(1)-C(2)	1.554(6)	C(23)-C(24)	1.485(7)	C(27)-C(36)	1.605(6)	C(51)-C(52)	1.502(7)
C(1)-C(10)	1.591(5)	C(25)-C(26)	1.479(7)	C(28)-C(29)	1.532(6)		
C(2)-C(3)	1.528(6)	O(11)-C(47)	1.342(6)	C(29)-C(30)	1.533(7)		
C(21)-O(1)-C(2)	118.7(3)	C(8)-C(9)-C(10)	113.4(3)	C(47)-O(11)-C(28)	117.0(3)	C(34)-C(35)-C(36)	113.1(3)
C(19)-O(3)-C(7)	110.3(3)	C(11)-C(10)-C(9)	107.1(3)	C(45)-O(13)-C(33)	109.8(4)	C(37)-C(36)-C(35)	107.2(3)
C(23)-O(5)-C(9)	116.5(3)	C(11)-C(10)-C(1)	109.7(3)	C(49)-O(15)-C(35)	118.0(4)	C(37)-C(36)-C(27)	110.0(3)
C(20)-O(7)-C(11)	59.5(3)	C(9)-C(10)-C(1)	122.5(3)	C(37)-O(17)-C(46)	60.3(3)	C(35)-C(36)-C(27)	121.6(3)
C(25)-O(8)-C(14)	117.4(3)	C(20)-C(11)-O(7)	60.2(3)	C(51)-O(18)-C(40)	119.1(4)	O(17)-C(37)-C(46)	59.9(3)
C(14)-C(1)-C(15)	104.6(3)	C(20)-C(11)-C(12)	120.1(4)	C(41)-C(27)-C(28)	110.8(4)	O(17)-C(37)-C(38)	114.7(4)
C(14)-C(1)-C(2)	108.6(3)	O(7)-C(11)-C(12)	114.2(3)	C(41)-C(27)-C(40)	106.2(4)	C(46)-C(37)-C(38)	118.6(4)
C(15)-C(1)-C(2)	110.3(3)	C(20)-C(11)-C(10)	120.6(4)	C(28)-C(27)-C(40)	108.0(3)	O(17)-C(37)-C(36)	115.2(4)
C(14)-C(1)-C(10)	109.2(3)	O(7)-C(11)-C(10)	115.2(3)	C(41)-C(27)-C(36)	116.8(4)	C(46)-C(37)-C(36)	120.5(4)
C(15)-C(1)-C(10)	117.6(3)	C(12)-C(11)-C(10)	114.5(3)	C(28)-C(27)-C(36)	107.8(3)	C(38)-C(37)-C(36)	115.6(4)
C(2)-C(1)-C(10)	106.2(3)	C(11)-C(12)-C(13)	108.4(3)	C(40)-C(27)-C(36)	106.8(4)	C(37)-C(38)-C(39)	108.2(4)
O(1)-C(2)-C(3)	107.8(3)	C(14)-C(13)-C(12)	112.6(3)	C(11)-C(28)-C(29)	108.1(3)	C(40)-C(39)-C(38)	112.5(4)
O(1)-C(2)-C(1)	108.2(3)	O(8)-C(14)-C(13)	108.5(3)	O(11)-C(28)-C(27)	107.0(3)	O(18)-C(40)-C(39)	111.0(4)
C(3)-C(2)-C(1)	113.3(3)	O(8)-C(14)-C(1)	107.5(3)	C(29)-C(28)-C(27)	116.0(4)	O(18)-C(40)-C(27)	106.4(3)
C(4)-C(3)-C(2)	113.9(4)	C(13)-C(14)-C(1)	113.0(3)	C(28)-C(29)-C(30)	113.7(4)	C(39)-C(40)-C(27)	114.1(4)
C(5)-C(4)-C(3)	114.3(3)	C(18)-C(17)-C(19)	114.7(4)	C(31)-C(30)-C(29)	114.2(4)	C(45)-C(43)-C(44)	114.3(5)
C(6)-C(5)-C(16)	119.6(4)	C(18)-C(17)-C(8)	118.0(4)	C(32)-C(31)-C(42)	119.3(5)	C(45)-C(43)-C(34)	101.8(4)
C(6)-C(5)-C(4)	124.7(4)	C(19)-C(17)-C(8)	100.5(3)	C(32)-C(31)-C(30)	124.9(5)	C(44)-C(43)-C(34)	116.5(4)
C(16)-C(6)-C(4)	115.7(4)	O(10)-C(19)-O(3)	121.8(4)	C(42)-C(31)-C(30)	115.8(4)	O(20)-C(45)-O(13)	120.5(5)
C(5)-C(6)-C(7)	128.5(4)	O(10)-C(19)-C(17)	128.2(4)	C(31)-C(32)-C(33)	127.1(5)	O(20)-C(45)-C(43)	128.8(5)
O(3)-C(7)-C(6)	106.4(3)	O(3)-C(19)-C(17)	110.1(4)	O(13)-C(33)-C(32)	106.0(4)	O(13)-C(45)-C(43)	110.7(4)
O(3)-C(7)-C(8)	102.5(3)	C(11)-C(20)-O(7)	60.3(3)	O(13)-C(33)-C(34)	103.1(3)	O(17)-C(46)-C(37)	59.8(3)
C(6)-C(7)-C(8)	117.3(3)	O(2)-C(21)-O(1)	123.5(4)	C(32)-C(33)-C(34)	117.5(4)	O(12)-C(47)-O(11)	124.2(4)
O(4)-C(8)-C(17)	108.3(3)	O(2)-C(21)-C(22)	125.7(4)	O(14)-C(34)-C(43)	108.6(4)	O(12)-C(47)-C(48)	124.8(5)
O(4)-C(8)-C(9)	110.6(3)	O(1)-C(21)-C(22)	110.8(4)	O(14)-C(34)-C(35)	110.1(3)	O(11)-C(47)-C(48)	110.9(4)
C(17)-C(8)-C(9)	112.4(3)	O(6)-C(23)-O(5)	123.8(4)	C(43)-C(34)-C(35)	114.8(4)	O(16)-C(49)-O(15)	122.9(5)
O(4)-C(8)-C(7)	106.8(3)	O(6)-C(23)-C(24)	126.1(5)	O(14)-C(34)-C(33)	106.2(4)	O(16)-C(49)-C(50)	126.0(5)
C(17)-C(8)-C(7)	101.3(3)	O(5)-C(23)-C(24)	110.1(4)	C(43)-C(34)-C(33)	100.2(3)	O(15)-C(49)-C(50)	111.1(5)
C(9)-C(8)-C(7)	116.7(3)	O(9)-C(25)-O(8)	123.1(4)	C(35)-C(34)-C(33)	116.1(4)	O(19)-C(51)-O(18)	124.7(5)
O(5)-C(9)-C(8)	105.9(3)	O(9)-C(25)-C(26)	125.9(5)	O(15)-C(35)-C(34)	106.6(3)	O(19)-C(51)-C(52)	124.6(5)
O(5)-C(9)-C(10)	116.6(3)	O(8)-C(25)-C(26)	111.0(4)	O(15)-C(35)-C(36)	115.4(3)	O(18)-C(51)-C(52)	110.7(5)

A previous study reported that briarane **1** has a mild inhibitory effect on human neutrophil elastase release (Sheu et al., 2006). Therefore, in vitro study of anti-inflammatory activity has employed immunoblot analysis to evaluate upregulation of pro-inflammatory inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) protein expression in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophage cells. Unfortunately, briarane **1** was found to be inactive in reducing the level of iNOS and COX-2. Other possible biological activities of briarane **1** will be assayed in the future.

4 CONCLUSIONS

Junceella fragilis has been found to contain a variety of briarane diterpenoids with different structures and pharmacological properties. Our ongoing research on *J. fragilis*, an 11,20-epoxybriarane, looks into (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollolide D (**1**), a compound to be a chemical marker for the octocorals belonging to the family Ellisellidae (Su et al., 2007). Briarane **1** was first isolated as (-)-11 α ,20 α -epoxy-4-deacetoxyjunceollolide D from *J. fragilis*, collected on Halmahera Island, Indonesia (García et al, 1999) and the structure of this compound was revised to (-)-11 β ,20 β -epoxy-4-deacetoxyjunceollolide D in a later study (Sheu et al., 2006) (Table 1). In this study, the NMR data for this compound was updated by extensive 1D and 2D NMR spectroscopic methods, and the structure, including the absolute configuration of **1**, was further confirmed with single-crystal X-ray diffraction analysis for the first time.

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