出國報告：其他--參加國際會議

Fragilide F, Discovery of a New Chlorinated Briarane from the Formosan Gorgonian Coral *Junceella fragilis*

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出席國際學術會議心得報告

計畫編號	NSC 98-2323-B-291-001
計畫名稱	臺灣海域產四屬柳珊瑚 *Bebryce*, *Rumphella*, *Ellisella* 及 *Pinnigorgia* 及養殖型 *Briareum* 所含化學成份之與研究
出國人員姓名 服務機關及職稱	宋秉鈞 國立海洋生物博物館企劃研究組研究員
會議時間地點	Aug. 2th-7th , **2009**, SECC, Glasgow (格拉斯哥), Scotland (蘇格蘭), UK (英國)
會議名稱	42nd IUPAC CONGRESS-Chemistry Solutions 第四十二屆國際純粹暨應用化學聯盟年會
發表論文題目	Fragilide F, Discovery of a New Chlorinated Briarane from the Formosan Gorgonian Coral *Junceella fragilis*

一、會議目的

國際純粹暨應用化學聯盟為全球最大化學學門聯盟組織，每年並定期舉辦重要之例行國際會議， 2009 年八月二日至七日於英國蘇格蘭格拉斯哥的 SECC 世貿中心舉行，會議由英國皇家化學會(Royal Society of Chemistry-RSC)主辦，與會人士達近貳仟人，且均為全球各國化學界之重要研究學者。會議主要針對全球化學學門各方面的最新研究成果進行交流與探討。

二、會議過程

有關天然物化學之研究在會中亦受到相當之重視，各國研究學者在在生技製藥、化學生態、生物多樣性與天然物多樣性等議題上進行廣泛的討論，會中並邀請全球多名國際知名學者進行精闢的邀請演講。個人亦於會議中發表論文壹篇”Fragilide F, Discovery of a New Chlorinated Briarane from the Formosan Gorgonian Coral *Junceella fragilis* ”發表一個帶二個氯原子的稀有 briarane 類化合物，並以 X-ray 繞射方法定出該化合物之絕對立體構型，並針對 briarane 類化合物的結構分析作了詳盡的解釋與探討，並與參加會議的各國學者進行詳細的討論，並交換研究心得 (論文結果即將已發表於 *Bull. Chem. Soc. Jpn.* 2009, 82, in press)。

二、心得及建議事項

本次會議為一有關全球化學研究的極重要交流會議，與會學者之研究背景廣泛，背景歧異度高，故常能在討論時有相當特殊之意見提出，對各領域之研究人員時有耳目一新之感，且邀請演講之學者均為全球各國知名化學界之各學門主持人，其熱烈參與程度相當引人注意。惟在在會議中可明顯看出歐、美等國在化學研究上在全球仍執牛耳地位，日本及中國則

表現出其極大之影響力，其他國家如臺灣、韓國則緊跟於後，中國大陸在論文數量上有驚人的成長，但在論文的精準度上則有進步空間。本次會議個人對美國及日本在天然物方面之研究成果尤感特殊，其善盡利用國家之科技優勢發展天然物之研究，而其他亞洲國家在此方面之研究水準顯然有程度上的差異，而學者們再次建議台灣因正處於熱帶及亞熱帶海域的交會處，生物的多樣性與歧異度極高，如能在此方面加強投入研究資源則應可能在一定時間內在海洋天然物化學的研究上建立起相對具有特色的研究學門。亦符合國家之海洋政策與發展方向。

此外，建議應加強鼓勵國內博士後研究人員及博士班學生能積極的參與此一類型之國際學術活動以增廣見聞。

附錄：會議後續相關發表 SCI 論文壹篇全文；

Sung, P.-J.*; Wang, S.-H.; Chiang, M. Y.; Su, Y.-D.; Chang, Y.-C.; Hu, W.-P.; Tai, C.-Y.; Liu, C.-Y. Discovery of New Chlorinated Briaranes from *Junceella fragilis*. *Bull. Chem. Soc. Jpn.* **2009**, *82*, in press.

Discovery of New Chlorinated Briaranes from *Junceella fragilis*

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Four briarane-type diterpenoids, including two new chlorinated metabolites, fragilides F (**1**) and G (**2**), along with two known compounds, junceellonoid D (**3**) and juncin Z (**4**), were isolated from the gorgonian coral *Junceella fragilis*. The structures of new briaranes **1** and **2** were elucidated by spectral data analysis and the absolute configuration of **1** was directly determined by a single-crystal X-ray diffraction analysis. The relationships between proton chemical shifts and conformations of the methylenecyclohexane ring in briaranes possessing a C-11/20 carbon–carbon double bond are described. Briarane **4** was found to exhibit significant cytotoxicity toward the CCRF-CEM tumor cells.

In our continuing research on bioactive substances from the invertebrates collected in Taiwanese waters as part of the NSTPBP, Taiwan,[1] we analyzed the chemical constituents from the organic extracts of gorgonian coral *Junceella fragilis* in the hope of identifying extracts that exhibit bioactivity. A series of interesting briarane derivatives, including fragilides A–E,[2–6] had been isolated from *J. fragilis*.[7] In this paper, we report the isolation, structure determination, and bioactivity of four highly functionalized diterpenoids with briarane carbon skeleton (3,8-cyclized cembranoid), including two new chlorinated metabolites, fragilides F (**1**) and G (**2**), and two known briaranes, junceellonoid D (**3**) and juncin Z (**4**),[8,9] from the further studies on *J. fragilis* (Chart 1).

Results and Discussion

Fragilide F (**1**) was isolated from the male *Junceella fragilis* and obtained as a white powder. The molecular formula for **1** was determined to be $C_{28}H_{36}Cl_2O_{12}$ (ten degrees of unsaturation) by HR-ESI-MS ($C_{28}H_{36}{}^{35}Cl_2O_{12}$ + Na: found 657.1485, calcd 657.1481). Comparison of the ^{1}H NMR and DEPT data with the molecular formula indicated that there must be an exchangeable proton (Table 1), requiring the presence of a hydroxy group, and this deduction was supported by a broad absorption in the IR spectrum at 3437 cm^{-1}. The IR spectrum of **1** also showed strong bands at 1786 and 1741 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 an exocyclic olefin was deduced from the signals of carbons at δ 134.3 (s, C-5) and 119.4 (t, CH_2-16), and supported by two olefin proton signals at δ 5.55 (1H, d, J = 2.4 Hz, H-16a) and 5.33 (1H, d, J = 2.4 Hz, H-16b) in the ^{1}H NMR spectrum of **1**. Moreover, five carbonyl resonances at δ 175.1 (s, C-19), 170.2, 169.9, 169.8, and 169.7 (4 × s, ester carbonyls), confirmed the presence of a γ-lactone and four ester groups in **1**; four acetate methyls (δ 2.34, 2.04, 2.03, and 1.99, each 3H × s) were also observed. From the above NMR data, six degrees of unsaturation were accounted for, and **1** must be tetracyclic. In addition, a methyl singlet (δ 1.23, 3H, s, H_3-15), a methyl doublet (δ 1.40, 3H, d, J = 7.2 Hz, H_3-18), two pairs of aliphatic methylene protons (δ 1.69, 1H, m; 2.21, 1H, m, H-12α/β; 1.78, 1H, m; and 1.64, 1H, m, H-13α/β), two aliphatic methine

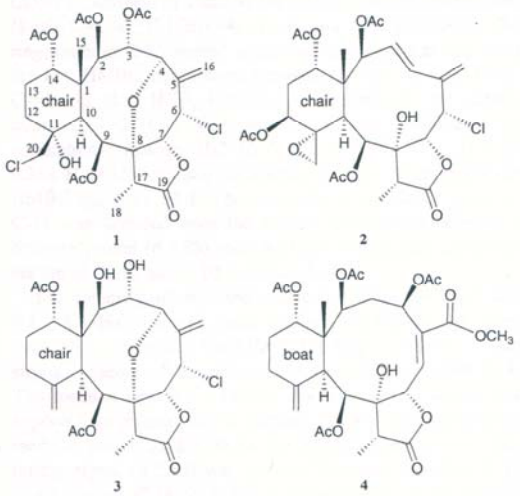

Chart 1.

Table 1. ^{1}H and ^{13}C NMR Data (δ), ^{1}H–^{1}H COSY, and HMBC (H → C) Correlations for Diterpenoid **1**

Position	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC
1		45.4 (s)[b]		
2	5.33 d (6.0)[a]	73.2 (d)	H-3	C-1, -3, -10, -14, -15, acetate carbonyl
3	6.13 dd (10.8, 6.0)	64.0 (d)	H-2, H-4	C-1, -2, -4, -5, acetate carbonyl
4	4.44 d (10.8)	78.3 (d)	H-3	C-2, -3, -5, -6, -8, -16
5		134.3 (s)		
6	5.04 ddd (2.8, 2.4, 2.4)	54.0 (d)	H-7, H_2-16	C-5, -16
7	4.37 d (2.8)	79.0 (d)	H-6	C-5, -6, -8
8		83.1 (s)		
9	6.36 s	73.7 (d)	H-10	C-1, -8, -10, -11, -17, acetate carbonyl
10	2.70 s	46.1 (d)	H-9	C-1, -2, -8, -9, -11, -12, -15, -20
11		73.8 (s)		
12α	1.69 m	29.6 (t)	H-12β, H_2-13, H-20b	C-11, -13, -14, -20
β	2.21 m		H-12α, H_2-13	C-10, -11, -14, -20
13α	1.78 m	22.9 (t)	H_2-12, H-13β, H-14	C-1, -11, -14
β	1.64 m		H_2-12, H-13α, H-14	C-11, -14
14	4.90 d (3.2)	73.3 (d)	H_2-13	C-10, -12, -15, acetate carbonyl
15	1.23 s	16.5 (q)		C-1, -2, -10, -14
16a	5.55 d (2.4)	119.4 (t)	H-6	C-4, -6
b	5.33 d (2.4)		H-6	C-4, -5, -6
17	2.89 q (7.2)	49.7 (d)	H_3-18	C-9, -18, -19
18	1.40 d (7.2)	7.5 (q)	H-17	C-8, -17, -19
19		175.1 (s)		
20a	3.89 d (11.2)	48.9 (t)	H-20b	C-11
b	3.46 dd (11.2, 1.2)		H-12α, H-20a	C-10, -11, -12
OH-11	2.88 s			C-10, -11
Acetate methyls	2.34 s	21.2 (q)		acetate carbonyl
	2.04 s	20.9 (q)		acetate carbonyl
	2.03 s	20.4 (q)		acetate carbonyl
	1.99 s	20.4 (q)		acetate carbonyl
Acetate carbonyls		170.2 (s)		
		169.9 (s)		
		169.8 (s)		
		169.7 (s)		

a) J values (in Hz) in parentheses. b) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols.

protons (δ 2.89, 1H, q, $J = 7.2$ Hz, H-17 and 2.70, 1H, s, H-10), six oxymethine protons (δ 6.36, 1H, s, H-9; 6.13, 1H, dd, $J = 10.8$, 6.0 Hz, H-3; 5.33, 1H, d, $J = 6.0$ Hz, H-2; 4.90, 1H, d, $J = 3.2$ Hz, H-14; 4.44, 1H, d, $J = 10.8$ Hz, H-4; and 4.37, 1H, d, $J = 2.8$ Hz, H-7), a downfield methine proton (δ 5.04, 1H, ddd, $J = 2.8$, 2.4, 2.4 Hz, H-6), a pair of low field methylene protons (δ 3.89, 1H, d, $J = 11.2$ Hz and 3.46, 1H, dd, $J = 11.2$, 1.2 Hz, H-20a/b), and a hydroxy proton (δ 2.88, 1H, s, OH-11) were observed in the ^{1}H NMR spectrum of **1**.

The gross structure of **1** was verified by 2D NMR studies. ^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **1** enabled identification of C2–C3–C4, C6–C7, C6–C16 (by allylic coupling), C9–C10, C12–C13–C14, C12–C20 (by w-coupling), and C17–C18 units (Table 1), which were assembled with the assistance of an HMBC experiment (Table 1). The HMBC correlations between protons and quaternary carbons of **1**, such as H-2, H-3, H-9, H-10, H-13α, H_3-15/C-1; H-3, H-4, H-6, H-7, H-16b/C-5; H-4, H-7, H-9, H-10, H_3-18/C-8; H-9, H-10, H_2-12, H_2-13, H_2-20/C-11; and H-17, H_3-18/C-19, permitted elucidation of the carbon skeleton. 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-16a/C-4, -6; H-16b/C-4, -5, -6; and H-4, H-6/C-16. The ring junction C-15 methyl group was positioned at C-1 from the key HMBC correlations between H_3-15/C-1, C-2, C-10, C-14 and H-2, H-10, H-14/C-15. Furthermore, the acetate esters at C-2, C-3, C-9, and C-14 were established by correlations between H-2 (δ 5.33), H-3 (δ 6.13), H-9 (δ 6.36), H-14 (δ 4.90) and the acetate carbonyls observed in the HMBC spectrum of **1**. The presence of a hydroxy group at C-11 was deduced from the HMBC correlations between a hydroxy proton (δ 2.88) with the C-11 oxygenated quaternary carbon (δ 73.8) and C-10 methine (δ 46.1).

The intensity of sodiated molecules $(M + 2 + Na)^+$ and $(M + 4 + Na)^+$ isotope peaks observed in ESI-MS spectrum $[(M + Na)^+:(M + 2 + Na)^+:(M + 4 + Na)^+ = 9:6:1]$ were strong evidence of the presence of two chlorine atoms in **1**. The methine unit at δ 54.0 (d) was more shielded than that expected for an oxygenated C-atom, and was correlated to the methine proton at δ 5.04 in the HMQC spectrum and this proton signal (δ 5.04) was ^2J- and ^3J-correlated with C-5 (δ 134.3, s) and C-16 (δ 119.4, t), respectively, in the HMBC spectrum of **1**, proving the attachment of a chlorine atom at

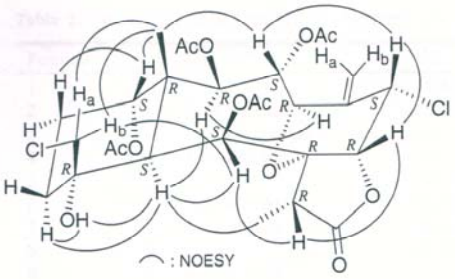

Figure 1. Selective NOESY correlations of **1**.

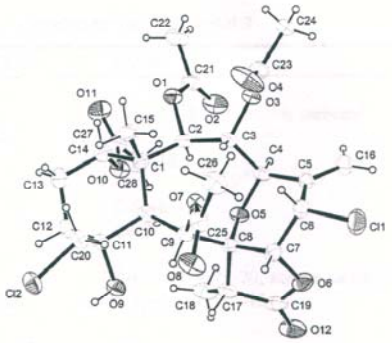

Figure 2. Computer-generated ORTEP plot of **1** showing the absolute configuration.

C-6. In addition, the methylene unit at δ 48.9 (t) was also more shielded than that expected for an oxygenated C-atom, and was correlated to the methylene protons at δ 3.89 and 3.46 in the HMQC spectrum and one of the methylene proton signals (δ 3.46) exhibited HMBC correlations with C-10, C-11, and C-12, proving the attachment of a chlorinated methylene group at C-11. Furthermore, an HMBC correlation between H-4 (δ 4.44) and an oxygenated quaternary carbon at δ 83.1 (s, C-8) suggested the presence of a C-4/8 ether linkage. These data, together with the HMBC correlations between H-17/C-9, C-18, C-19 and H_3-18/C-8, C-17, C-19, unambiguously established the molecular framework of **1**.

The relative stereochemistry of **1** was elucidated by analysis of NOESY correlations (Figure 1) and by vicinal ^{1}H–^{1}H proton coupling constants analysis. In the NOESY experiment of **1**, H-10 correlated with H-2, H-9, H_3-18, OH-11, and one proton of C-12 methylene (δ 1.69), indicated that these protons (H-2, H-9, H-10, H-12α, H_3-18, and OH-11) were situated on the same face; they were assigned as α protons, as C-15 methyl was β-oriented at C-1 and H_3-15 did not show correlation with H-10. The oxymethine protons H-3, H-14, and the chlorinated C-20 methylene protons (H-20a/b) were found to exhibit responses with H_3-15 but not with H-10, revealing H-3, H-4, and C-20 methylene were β-oriented at C-3, C-4, and C-11, respectively. H-9 was found to show correlations with H-17 and one proton of C-20 methylene (δ 3.46, H-20b). From modeling analysis, H-9 was found to be reasonably close with H-17 and H-20b and can therefore be placed on the α face in the 10-membered ring of **1** and H-17 is β-oriented in the γ-lactone moiety. H-7 exhibited interactions with H-6 and H-17; and H-6 correlated with H-3, indicating that these protons are on the β face of **1**. Furthermore, H-4 showed a correlation with H-2; and a large coupling constant was found between H-4 and H-3 (J = 10.8 Hz), indicating the dihedral angle between H-4 and H-3 is approximately 180° and H-4 has an α-orientation at C-4.

A single-crystal X-ray diffraction analysis was carried out in order to determine the structure of **1**. On the basis of X-ray structure (Figure 2), the chiral centers in **1** were assigned as 1R, 2R, 3S, 4R, 6S, 7R, 8R, 9S, 10S, 11R, 14S, and 17R. From the above findings, the structure of **1** was elucidated. It is worth noting that fragilide F (**1**) is the second sample of briarane metabolites which possesses two halogen atoms.[10]

The briaranes **2**–**4** were isolated from the female *J. fragilis*. Fragilide G (**2**) was obtained as a white powder that gave an $(M + Na)^+$ ion with m/z 621.1711 in the HR-ESI-MS analysis, appropriate for the molecular formula $C_{28}H_{35}ClO_{12}$ (calcd for $C_{28}H_{35}{}^{35}ClO_{12}$ + Na, 621.1715). Inspection of the IR spectrum revealed absorptions indicative of hydroxy (3450 cm^{-1}), γ-lactone (1781 cm^{-1}), and ester (1738 cm^{-1}) groups. From the ^{13}C and ^{1}H NMR data of **2** (Table 2), a disubstituted olefin and an exocyclic carbon–carbon double bond were deduced from the signals of four carbons at δ 141.5 (s, C-5), 133.5 (d, CH-4), 129.3 (d, CH-3), and 116.2 (t, CH_2-16); and further supported by four olefin proton signals at δ 6.83 (1H, d, J = 16.0 Hz, H-4), 6.01 (1H, dd, J = 16.0, 9.6 Hz, H-3), 5.36 (1H, s, H-16a), and 5.32 (1H, s, H-16b). Moreover, five carbonyl resonances at δ 174.7 (s, C-19) and 170.2 (4 × s), confirmed the presence of a γ-lactone and four other ester groups in **2**. In the ^{1}H NMR spectrum of **2**, four acetate methyls (δ 2.18, 2.11, 2.02, and 1.98, each 3H × s) were observed. An exocyclic epoxy group was confirmed from the signals of two oxygenated carbons at δ 55.4 (s, C-11) and 50.2 (t, CH_2-20). The chemical shifts of C-20 methylene protons (δ 2.68, 1H, dd, J = 3.2, 1.2 Hz, H-20a and 2.57, 1H, dd, J = 3.2, 2.8 Hz, H-20b) confirmed the presence of this group. From the ^{1}H–^{1}H COSY experiment of **2** (Table 2), it was possible to establish the spin systems that established the proton sequences from H-2/H-3/H-4, H-6/H-7, H-9/H-10, H-10/H-20a (by w-coupling), H-12/H-20b (by w-coupling), H-12/H_2-13/H-14, and H-17/H_3-18. Based on these data and the HMBC correlations (Table 2), the carbon skeleton of **2** could be established.

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 (11R*), and the cyclohexane ring is of a chair conformation. Moreover, if the epoxy group was found to exist in the 11S* configuration, the ^{13}C NMR data for C-11 and C-12 were shifted downfield and appeared at δ 62–63 and 58–60, and the cyclohexane rings were found to exist in the twist boat conformation.[11] Based on the above observations, the configuration of 11,20-epoxy group in **2** (δ 55.4, s, C-11 and 50.2, t, CH_2-20) should be α-oriented and the cyclohexane ring in **2** should be in a chair conformation. The relative stereochemistry of **2** was elucidated by a NOESY experiment (Figure 3) and by vicinal ^{1}H–^{1}H

Table 2. ^{1}H and ^{13}C NMR Data (δ), ^{1}H–^{1}H COSY, and HMBC (H → C) Correlations for Diterpenoid **2**

Position	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC
1		48.4 (s)[b]		
2	5.48 d (9.6)[a]	75.4 (d)	H-3	C-1, -3, -4, -15, acetate carbonyl
3	6.01 dd (16.0, 9.6)	129.3 (d)	H-2, H-4	C-5
4	6.83 d (16.0)	133.5 (d)	H-3	C-2, -16
5		141.5 (s)		
6	5.07 d (3.6)	64.0 (d)	H-7	C-5, -8, -16
7	4.12 d (3.6)	81.6 (d)	H-6	n.o.[c]
8		82.6 (s)		
9	5.12 d (2.0)	72.7 (d)	H-10	C-10, -11, -17, -20, acetate carbonyl
10	3.21 br s	38.6 (d)	H-9, H-20a	C-1, -8, -9, -11, -15
11		55.4 (s)		
12	5.13 ddd (12.8, 4.8, 2.8)	67.4 (d)	H_2-13, H-20b	C-11
13α	2.41 ddd (12.8, 12.0, 1.2)	35.3 (t)	H-12, H-13β, H-14	C-11, -12, -14
β	1.44 ddd (12.0, 4.8, 1.2)		H-12, H-13α, H-14	C-11
14	5.30 dd (1.2, 1.2)	73.0 (d)	H_2-13	n.o.
15	1.25 s	15.0 (q)		C-1, -2, -10, -14
16a	5.36 s	116.2 (t)		C-4, -5, -6
b	5.32 s			C-4
17	2.89 q (7.2)	50.5 (d)	H_3-18	C-8, -9, -18, -19
18	1.27 d (7.2)	6.6 (q)	H-17	C-8, -17, -19
19		174.7 (s)		
20a	2.68 dd (3.2, 1.2)	50.2 (t)	H-10, H-20b	n.o.
b	2.57 dd (3.2, 2.8)		H-12, H-20a	n.o.
OH-8	2.98 s			C-8, -9
Acetate methyls	2.18 s	20.8 (q)		acetate carbonyl
	2.11 s	21.3 (q)		acetate carbonyl
	2.02 s	21.2 (q)		acetate carbonyl
	1.98 s	20.9 (q)		acetate carbonyl
Acetate carbonyls		170.2 (s)		
		170.2 (s)		
		170.2 (s)		
		170.2 (s)		

a) J values (in Hz) in parentheses. b) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. c) n.o. = not observed.

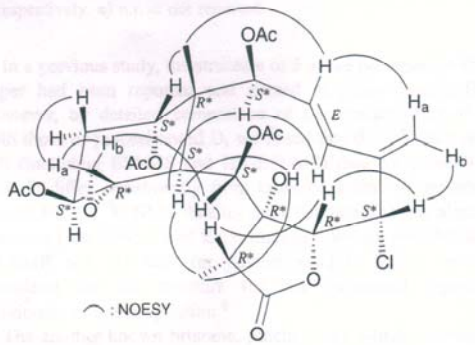

Figure 3. Selective NOESY correlations of **2**.

coupling constant analysis. Due to the α orientation of H-10, the 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 correlated with H-2, H-9, H_3-18, and OH-8; and H_3-18 showed a correlation with OH-8, suggesting that these protons (H-2, H-9, H-10, H_3-18, and OH-8) are located on the same face and can be assigned as α protons, as the C-15 methyl group was β-oriented at C-1. H-14 was found to exhibit a response with H_3-15, but not with H-10, showing that this proton is of β-orientation. H-12 exhibited ^{1}H–^{1}H correlations with C-13 methylene protons and one proton of C-20 methylene (δ 2.57, H-20b); and a triple doublet (ddd) coupling was found between this proton (H-12) and protons of C-13 methylene ($J = 12.8$, 4.8 Hz) and H-20b ($J = 2.8$ Hz, by w-coupling) indicating that H-12 has an α-orientation by modeling analysis. H-7 exhibited correlations with H-6 and H-17, suggesting that these protons are on the β face of **1**. The trans geometry of C-3/C-4 double bond is indicated by a large coupling constant ($J = 16.0$ Hz) between H-3 (δ 6.01) and H-4 (δ 6.83). Moreover, the olefin proton H-3 showed a correlation with H_3-15, but not with H-2; and H-4 showed responses with H-2 and OH-8, demonstrating the E configuration of C-3/C-4 double bond. Therefore, an *s-cis*-diene moiety in **2** was elucidated. Based on the above findings, the configurations of all chiral centers of **2** were assigned to be 1R*, 2S*, 6S*, 7R*, 8R*, 9S*, 10S*, 11R*, 12S*, 14S*, and 17R*.

Table 3. ^{1}H and ^{13}C NMR Data (δ), and ^{1}H–^{1}H COSY, and HMBC (H → C) Correlations for Diterpenoid **3** and the ^{1}H and ^{13}C NMR Data (δ) for Junceellonoid D

	3				Junceellonoid D[d]	
Position	^{1}H	^{13}C	^{1}H–^{1}H COSY	HMBC	^{1}H	^{13}C
1		49.0 (s)[b]				49.1 (s)
2	3.71 d (6.8)[a]	71.9 (d)	H-3	C-15	3.91 d (7.1)	72.0 (d)
3	4.33 dd (10.0, 6.8)	64.5 (d)	H-2, H-4	C-1, -2	4.33 dd (7.2, 9.8)	64.6 (d)
4	4.06 d (10.0)	82.4 (d)	H-3	C-2, -3, -5, -6, -8, -16	4.06 d (9.8)	82.2 (d)
5		135.0 (s)				135.2 (s)
6	4.96 ddd (3.2, 2.0, 2.0)	54.6 (d)	H-7, H_2-16	n.o.[c]	5.21 d (2.7)	54.7 (d)
7	4.50 d (3.2)	79.3 (d)	H-6	C-5	4.39 d (3.1)	79.3 (d)
8		82.2 (s)				82.5 (s)
9	5.86 s	78.0 (d)	H-10	C-1, -8, -10, -11, -17, acetate carbonyl	5.50 br s	78.2 (d)
10	2.93 s	43.5 (d)	H-9	C-1, -8, -11, -15, -20	3.08 br s	43.6 (d)
11		147.7 (s)				147.8 (s)
12α	2.43 m	32.6 (t)	H-12β, H_2-13	n.o.	2.31 m	28.0 (t)
β	2.27 ddd (12.4, 3.6, 3.2)		H-12α, H_2-13	n.o.	1.22 m	
13α	1.79 m	27.9 (t)	H_2-12, H-13β	C-14	2.12 m	32.7 (t)
β	1.81 m		H_2-12, H-13α	C-14	1.82 m	
14	5.23 dd (2.8, 2.8)	77.2 (d)	H_2-13	n.o.	5.23 br s	76.7 (d)
15	1.03 s	14.3 (q)		C-1, -2, -10, -14	1.23 s	14.3 (q)
16a	5.47 d (2.0)	118.9 (t)	H-6	C-4, -6	5.60 br s	118.8 (t)
b	5.63 d (2.0)		H-6	C-4, -5, -6	5.45 br s	
17	2.72 q (6.8)	49.6 (d)	H_3-18	C-9, -19	3.20 q (7.0)	49.6 (d)
18	1.23 d (6.8)	7.3 (q)	H-17	C-8, -17, -19	1.47 d (7.0)	7.4 (q)
19		174.5 (s)				174.4 (s)
20a	5.06 s	111.5 (t)		C-10, -12	5.05 br s	111.5 (t)
b	4.69 s			C-10, -12	4.69 br s	
OH-2	4.15 br s		H-2	n.o.	n.r.[e]	
OH-3	3.67 br s		H-3	C-4	n.r.	
Acetate methyls	2.21 s	21.4 (q)		acetate carbonyl	2.10 s	21.4 (q)
	2.19 s	21.2 (q)		acetate carbonyl	1.88 s	21.2 (q)
Acetate carbonyls		172.5 (s)				172.4 (s)
		169.8 (s)				169.7 (s)

a) J values (in Hz) in parentheses. b) Multiplicity deduced by DEPT and HMQC spectra and indicated by usual symbols. c) n.o. = not observed. d) Data were reported by Qi et al. (see Ref. 8). These data were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$, respectively. e) n.r. = not reported.

In a previous study, the structure of **3** as we presented in this paper had been reported and named as junceellonoid D.[8] However, by detailed comparison of the spectral data of **3** with those of junceellonoid D, we found that the ^{1}H NMR and MS (including ESI-MS and HR-ESI-MS) data for junceellonoid D differ significantly from those of **3** that we reported herein, but the ^{13}C NMR data for these two briaranes are almost identical (see Table 3 and Experimental). We suggest that the ^{1}H NMR and MS data for junceellonoid D should be re-examined but the structure for this compound reported previously is not in question.[8]

The another known briarane, juncin Z (**4**), which possesses an unprecedented α,β-unsaturated conjugated ester group in structure, was first isolated from the gorgonian coral *Junceella juncea*, collected off the South China Sea.[9] From the characteristics of chemical shifts it was known that the briarane derivatives contained an exocyclic carbon–carbon double bond between C-11/20. We summed up the chemical shifts for the olefin protons H_2-20; these appear at δ 4.95–5.30 and 4.85–5.15, respectively, while the cyclohexane rings to show a twist boat conformation (Figure 4 and Table 4).[5,12–14] Furthermore, the ^{1}H NMR data for H_2-20 appeared at δ 4.95–5.10 and 4.40–4.75, the cyclohexane rings were found to exist in the chair conformation (Figure 4 and Table 4).[8,13,15]

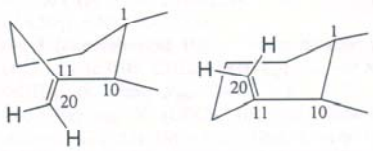

Figure 4. The twist boat (left) and chair (right) conformations for the methylenecyclohexane ring in briarane analogs possess an exocyclic carbon–carbon double bond between C-11 and C-20.

The cytotoxicity of briaranes **1**–**4** toward the CCRF-CEM (human T cell acute lymphoblastic leukemia) and DLD-1 (human colon adenocarcinoma) tumor cells was assayed, and it

Table 4. ^{1}H NMR Chemical Shifts for C-20 Olefin Protons and Conformations of Methylenecyclohexane Ring in Briaranes Possessing a C-11/20 Double Bond

Compound	H-20a (δ_a)	H-20b (δ_b)	$\delta_a - \delta_b$	Conformation	Ref.
4	5.02	4.95	0.07	twist boat	
Juncin Z[a)]	5.0	4.96	0.04		9
Junceellolide E	5.04	4.92	0.12	twist boat	12
Robustolide B	4.96	4.86	0.10	twist boat	13
Robustolide C	5.14	5.02	0.12	twist boat	13
Junceol A	5.03	4.88	0.15	twist boat	5
Junceol D	5.26	5.11	0.15	twist boat	14
Junceol E	5.02	4.87	0.15	twist boat	14
Junceol F	5.02	4.87	0.15	twist boat	14
Junceol G	5.20	5.11	0.09	twist boat	14
Junceol H	5.20	5.11	0.09	twist boat	14
3	5.06	4.69	0.37	chair	
Junceellonoid D[a)]	5.05	4.69	0.36		8
Robustolide A	4.96	4.45	0.51	chair	13
Robustolide K	5.05	4.73	0.32	chair	15

a) The conformations for the methylenecyclohexane rings in these two briaranes were not described in the literature.[8,9]

was found that compound **4** showed significant cytotoxicity toward the CCRF-CEM cells ($ED_{50} = 1.6\,\mu g\,mL^{-1}$), but not active toward the DLD-1 cells ($ED_{50} > 40\,\mu g\,mL^{-1}$) and briaranes **1–3** were inactive ($ED_{50} > 40\,\mu g\,mL^{-1}$) toward the above two cell lines.[16]

The reproductive physiology for the corals is also a major research topic in the NMMBA. Therefore, we investigate the chemical constituents of male and female *J. fragilis* separately. We hope that our research results could be coordinated with those of the other research groups focused on ecology and physiology studies in NMMBA. However, it is difficult to discuss the differences of chemical constituents from male and female *J. fragilis* on limited natural products at this stage.

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 spectrometer. 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 $CDCl_3$, respectively. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (δ 7.26); ^{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 silica gel (230–400 mesh, Merck, Darmstadt, Germany), and 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 composed of a HITACHI L-7100 pump, a HITACHI photo diode array detector L-7455, a RHEODYNE 7725 injection port, a normal phase semi-preparative column (Hibar $250 \times 25\,mm^2$, LiChrospher Si 60, 5 μm), and a reverse phase semi-preparative column (Hibar $250 \times 10\,mm^2$, Purospher STAR RP-18e, 5 μm) were used for HPLC. Preparative TLC was carried out on precoated Kieselgel 60 F_{254} (layer thickness 210–270 μm, Merck).

Animal Material. Specimens of the gorgonian coral *Junceella fragilis* were collected by divers equipped with SCUBA off the coast of southern Taiwan in August 2006, at a depth of −20 m. Living reference specimens are being maintained in the authors' marine organisms cultivating tank and a voucher specimen was deposited in the NMMBA, Taiwan.

Extraction and Isolation. Male *J. fragilis*: The freeze-dried male *J. fragilis* (dry weight 74 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 19 fractions A–S. Fraction Q was separated by gravity column with silica gel and eluted using hexane/acetone to afford 14 fractions. Fraction Q10 was repurified by preparative TLC and eluted using a mixture of hexane/EtOAc to afford **1** (12.6 mg, 2:1).

Fragilide F (1); White powder; mp 302–303 °C; $[\alpha]_D^{23}$ −19 (*c* 0.51, $CHCl_3$); IR (neat) ν_{max} 3437, 1786, 1741 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 1; ESI-MS *m/z* 657 $(M + Na)^+$; HR-ESI-MS *m/z* 657.1485 (calcd for $C_{28}H_{36}{}^{35}Cl_2O_{12}$ + Na, 657.1481).

Female *J. fragilis*: The freeze-dried female *J. fragilis* (dry weight 270 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 the mixtures of hexane/EtOAc (stepwise, 50:1–pure EtOAc) to yield fractions A–Q. Fraction K was further separated with silica gel and eluted using CH_2Cl_2/EtOAc to afford fractions K1–K23. Fraction K13 was further repurified by reverse phase HPLC, using a mixture of CH_3CN and H_2O to afford **3** (0.9 mg, 60:40). Fraction K14 was repurified by reverse phase HPLC, using a mixture of MeOH/H_2O to afford **2** (2.2 mg, 70:30). Fraction K16 was purified by normal phase HPLC, using a mixture of hexane and acetone to afford **4** (0.6 mg, 5:2).

Fragilide G (2); White powder; mp 214–215 °C; $[\alpha]_D^{25}$ −6 (*c* 0.10, $CHCl_3$); IR (neat) ν_{max} 3450, 1781, 1738 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR data, see Table 2; ESI-MS *m/z* 621 $(M + Na)^+$; HR-ESI-MS *m/z* 621.1711 (calcd for $C_{28}H_{35}{}^{35}ClO_{12}$ + Na, 621.1715).

Briarane 3 (Junceellonoid D); White powder; mp 303–304 °C; $[\alpha]_D^{23}$ −31 (*c* 0.05, $CHCl_3$) [Ref. 8, $[\alpha]_D$ −44.8 (*c* 0.10, $CHCl_3$/MeOH)]; IR (neat) ν_{max} 3450, 1781, 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* 521 $(M + Na)^+$ [Ref. 8, *m/z* 517 $(M + H)^+$]; HR-ESI-MS *m/z* 521.1552 (calcd for $C_{24}H_{31}{}^{35}ClO_9$ + Na, 521.1554) [Ref. 8, *m/z* 517.1836 $(M + H)^+$, $C_{24}H_{33}ClO_{10}$ + H, 517.1840].

Juncin Z (4); White powder; mp 140–142 °C; $[\alpha]_D^{23}$ +22 (*c* 0.03, $CHCl_3$) [Ref. 9, $[\alpha]_D$ +31.57 (*c* 0.95, $CHCl_3$)]; IR (neat) ν_{max} 3443, 1782, 1732, 1642 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.07 (1H, dd, J = 10.0, 1.2, Hz, H-6), 5.94 (1H, ddd, J = 12.4, 6.0, 1.2 Hz, H-4), 5.63 (1H, d, J = 10.0 Hz, H-7), 5.54 (1H, d, J = 3.2 Hz, H-9), 5.02 (1H, s, H-20a), 4.95 (1H, s, H-20b), 4.85 (1H, d, J = 6.8 Hz, H-2), 4.69 (1H, ddd, J = 2.0, 2.0, 2.0 Hz, H-14), 3.84 (3H, s, $-OCH_3$), 3.25 (1H, d, J = 3.2 Hz, H-10), 2.71 (1H, dd, J = 14.8, 12.4 Hz, H-3β), 2.62 (1H, q, J = 7.2 Hz, H-17), 2.22 (3H, s, acetate methyl), 2.14 (1H, m, H-13), 2.13 (1H, ddd, J = 14.8, 6.8, 6.0 Hz, H-3α), 2.06 (3H, s, acetate methyl), 1.97 (3H, s, acetate methyl), 1.90 (3H, s, acetate methyl), 1.79 (2H, m,

H_2-12), 1.30 (1H, m, H-13′), 1.19 (3H, d, $J = 7.2$ Hz, H_3-18), 1.04 (3H, s, H_3-15); ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 175.2 (s, C-19), 170.5, 169.9, 169.7, 169.2 (4 × s, acetate carbonyls), 166.5 (s, C-16), 150.6 (s, C-11), 139.0 (d, CH-6), 137.0 (s, C-5), 112.5 (t, CH_2-20), 83.1 (s, C-8), 76.7 (d, CH-7), 74.0 (d, CH-14), 72.6 (d, CH-9), 72.1 (d, CH-2), 67.5 (d, CH-4), 52.8 (q, $-OCH_3$), 47.9 (s, C-1), 43.3 (d, CH-17), 42.7 (d, CH-10), 37.4 (t, CH_2-3), 31.9 (t, CH_2-13), 27.2 (t, CH_2-12), 21.7, 21.2, 21.1, 20.8 (4 × q, acetate methyls), 14.5 (q, CH_3-15), 6.4 (q, CH_3-18); ESI-MS m/z 617 $(M + Na)^+$; HR-ESI-MS m/z 617.2206 (calcd for $C_{29}H_{38}O_{13}$ + Na, 617.2210).

Single-Crystal X-ray Crystallography of Fragilide F (1). Suitable colorless prisms of **1** were obtained from a solution of MeOH/acetone (2:1). The crystal ($0.50 \times 0.30 \times 0.30\,mm^3$) belongs to the monoclinic system, space group $P2_1$ (#4), with $a = 9.587(1)$ Å, $b = 15.556(2)$ Å, $c = 10.537(2)$ Å, $\beta = 98.22(3)°$, $V = 1555.4(4)$ Å^3, $Z = 2$, $D_{calcd} = 1.357\,g\,cm^{-3}$, λ(Mo Kα) = 0.71073 Å. Intensity data were measured on a Rigaku AFC7S diffractometer up to $2\theta_{max}$ of 52°. All 4257 reflections were collected. The structure was solved by direct methods and refined by a full-matrix least-square procedure. The refined structural model converged to a final $R1 = 0.0349$; $wR2 = 0.0883$ for 2809 observed reflections [$I > 2\sigma(I)$] and 388 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's parameter was determined to be −0.06(7).[17]

Crystallographic data for the structure of fragilide F (**1**) has been deposited with the Cambridge Crystallographic Data Center as supplementary publication number CCDC-733916. Copies of the data can be obtained, free of charge, on application to CCDC, 12, Union Road, Cambridge, CB2 1EZ, U.K [fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk].

Cytotoxicity Assays. The cytotoxicity of tested compounds **1–4** were assayed with a modification of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method. Cytotxicity assays were carried out according to the procedures described previously.[18]

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