附件五

國家科學及技術委員會補助專題研究計畫出席國際學術會議

心得報告

日期：113 年 10 月 21 日

計畫編號	NSTC 112 － 2320 － B － 291 － 002 － MY3		
計畫名稱	台灣產海綿及其共生微生物活性天然物之開發與持續藥源之建立		
出國人員姓名	宋秉鈞	服務機構及職稱	國立海洋生物博物館
會議時間	113 年 07 月 13 日 至 113 年 07 月 17 日	會議地點	波蘭-克拉克夫
會議名稱	(中文)2024 國際天然產物研究大會 (英文)2024 International Congress On Natural Products Research		
發表題目	(中文)來自八放珊瑚 *Junceella fragilis* 的含氯多乙酰氧基 briarane 類化合物。 (英文) Chlorine-containing polyacetoxybriaranes from the octocoral *Junceella fragilis.*		

一、 參加會議經過

今年的會議將於 2024 年 7 月 13 日至 17 日，在波蘭克拉克夫市連續舉行五天。此次會議由華沙醫科大學主辦，吸引了來自全球的專家學者及研究人員，參與人數超過一千人。會議內容豐富，包括海洋微生物、對抗新興傳染病的天然產物研究、天然產物的合成技術以及食品和藥品應用的規範等多個尖端領域，共展示了 868 篇關於前沿技術的壁報。

在這次大會中，我有榮幸發表了一篇關於八放珊瑚 *Junceella fragilis* 的研究論文。這篇論文主要介紹從八放珊瑚分離出的天然產物，並通過光譜技術及其數據解析進行了詳細的化學結構鑑定。更重要的是，我們的研究利用人類成骨細胞的模型，證實這些天然產物具有顯著的治療骨質疏鬆症的潛力。此外，本次大會也設有每日上下午共五場的專家演講和座談會，進一步促進了學術交流和知識分享，為參與者提供了一個卓越的學習平台。

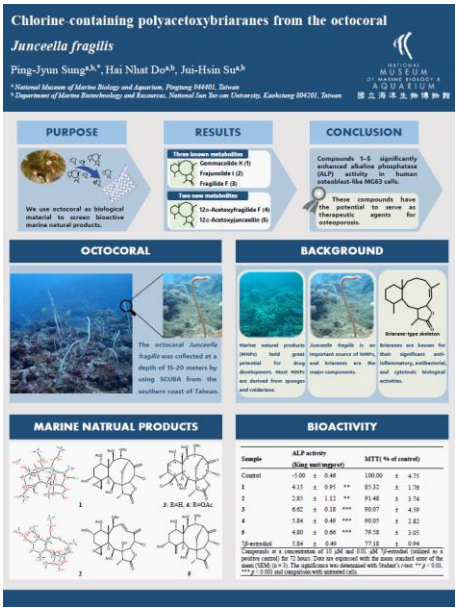
二、 與會心得

在會議中可明顯看出國際間在天然產物領域的合作與交流研究上有很多值得學習的地方。不僅涵蓋天然產物化學研究，也涉及二次代謝物在生物體內的生合成路徑，顯示新藥的需求愈發重要。因此，跨領域合作對天然物學家來說是必要的。在我國，參與此次會議的有國立成功大學的吳天賞教授、高雄醫學大學張芳榮教授和中國醫藥大學吳永昌教授等，他們主要發表了關於天然產物的不同領域研究。我參加此次研討會的目的是關注國際間天然產物研究的發展趨勢，比較我國在醫藥生技研發方面的優缺點。

值得注意的是，各國學者都在利用其獨特的生物多樣性來發展具有特色的研究方向。建議台灣應該加強在海洋天然物研究上的投資，特別是因為台灣地處熱帶及亞熱帶海域交匯處，具有非常高的生物多樣性和歧異度，有潛力在海洋天然物化學研究領域建立獨特的研究學門。這不僅符合國家的海洋政策，也能推動相關的科學和技術發展。

此外，美國的學者如 Prof. Guido F. Pauli 和 Dr. Valerie Paul 也在會議中分享了他們的研究成果。Prof. Pauli 專注於利用複雜奈米顆粒作為保健品和新藥的來源，並開發了包括定量核磁共振波譜和反流分離在內的分析和天然產物技術。Dr. Valerie Paul 則專注於海洋化學生態學和珊瑚礁研究，其工作在國際海洋科學社區中具有重要影響。這些研究突顯了海洋天然物研究的多面性和應用潛力，為未來的研究方向提供了重要的參考。

三、 發表論文全文或摘要

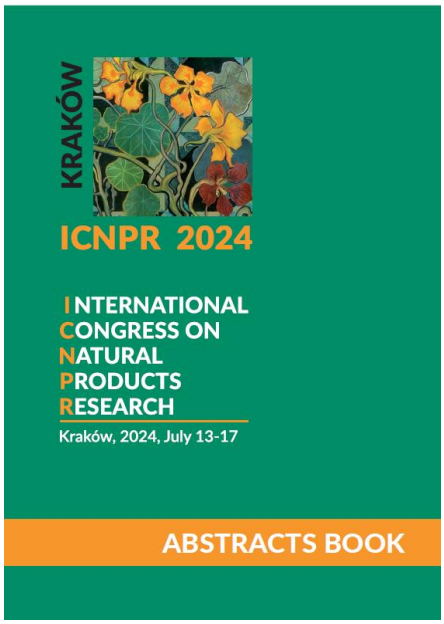


四、 建議

建議應多鼓勵並提供充足資源給國內的博士後研究員和博士班學生，以便他們能積極參與國際學術研討會，從而擴展其視野並增進專業知識。通過這些學術活動，研究人員不僅能夠與全球的專家學者交流思想，還可以掌握行業最新動態，促進學術合作與專業發展。這種國際交流經驗對於他們未來的研究工作及職業生涯規劃將具有不可估量的價值。

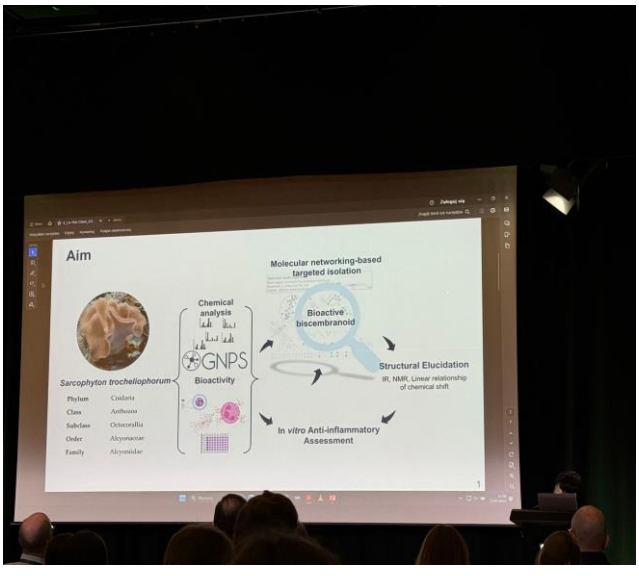
五、 攜回資料名稱及內容

大會會議手冊一份。



歐洲藥學會專家學者分享

臺灣優秀博士生報告



六、 其他

研討會發表海報相關 SCI 論文。

RSC Advances

PAPER

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Chlorine-containing polyacetoxybriarane diterpenoids from the octocoral *Junceella fragilis*†

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The chemical screening of an octocoral identifed as *Junceella fragilis* has led to the isolation of five chlorinated briarane-type diterpenoids, including three known metabolites, gemmacolide X (**1**), frajunolide I (**2**), and fragilide F (**3**), along with two new analogs, 12α-acetoxyfragilide F (**4**) and 12α-acetoxyjunceellin (**5**). Single-crystal X-ray diffraction analysis was carried out to determine the absolute configurations of **1** and **2**, while the structures of new compounds **4** and **5** were ascertained with 2D NMR experiments. Briaranes **1** and **3**–**5** were active in enhancing alkaline phosphatase (ALP) activity.

1 Introduction

The octocorals belonging to genus *Junceella* (phylum Cnidaria, sub-phylum Anthozoa, class Octocorallia, order Scleralcyonacea, family Ellisellidae),[1] distributed in the shallow waters of the tropical Indo-Pacific Ocean, have been proven to be a rich source of briarane-type diterpenoids with uncommon structures.[2] This study explored further new substances from *Junceella fragilis* (Ridley 1884), collected from waters off the coast of Taiwan, an area with high biodiversity at the intersection of Kuroshio and South China Sea surface currents. The study successfully isolated five chlorinated briaranes, including three known metabolites, gemmacolide X (**1**),[3] frajunolide I (**2**),[4] and fragilide F (**3**),[5] as well as two new analogs, 12α-acetoxyfragilide F (**4**) and 12α-acetoxyjunceellin (**5**) (Fig. 1) and ascertained their structures and ALP activity. The absolute configurations of **1** and **2** were further determined *via* single-

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† Electronic supplementary information (ESI) available: HRESIMS, 1D and 2D NMR spectra of **4** and **5**; X-ray crystallographic data of **1** and **2**. CCDC 2323829 and 2326820, respectively. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d4ra03062a

‡ These authors have contributed equally to this work.

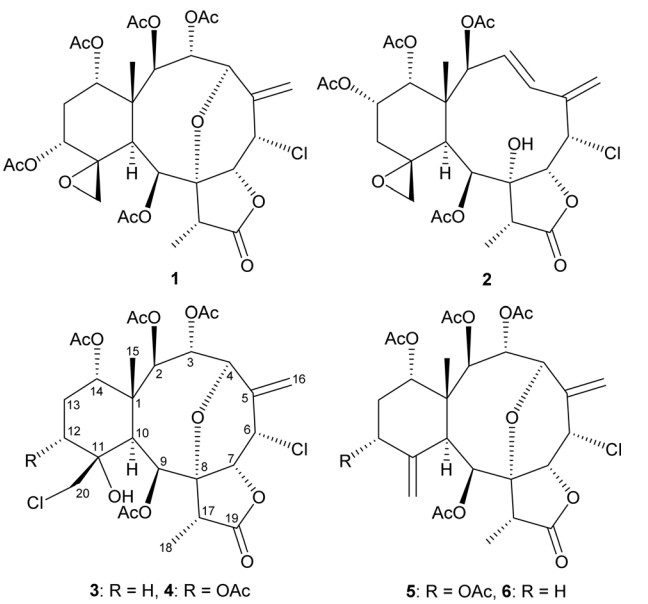

Fig. 1 Structures of gemmacolide X (**1**), frajunolide I (**2**), fragilide F (**3**), 12α-acetoxyfragilide F (**4**), 12α-acetoxyjunceellin (**5**), and junceellin (**6**).

crystal X-ray diffraction analysis with a diffractometer equipped with a copper (Cu Kα) source.

2 Results and discussion

Gemmacolide X (**1**) and frajunolide I (**2**) were originally isolated from the octocorals *Dichotella gemmacea* and *Junceella fragilis*, respectively; and the structures of these two briaranes, including the relative configuration, were elucidated by spectroscopic analysis.[3,4] The absolute configuration of these two compounds was supported in this study by single-crystal X-ray diffraction analysis (Flack parameter $x = 0.000(5)$ for **1** and $-0.002(13)$ for **2**).[6] The ORTEP diagram (Fig. 2) showed that the absolute configuration of stereogenic carbons of **1** and **2** are assigned as 1*R*, 2*R*, 3*R*, 4*R*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*R*, 12*R*, 14*S*, 17*R* and 1*S*, 2*S*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*R*, 13*S*, 14*R*, 17*R*, respectively.

The (+)-ESIMS of **3** showed sodiated peaks at *m*/*z* 657/659/661 ($[M + Na]^+/[M + 2 + Na]^+/[M + 4 + Na]^+$) (9 : 6 : 1) with a relative intensity suggestive of two chlorine atoms. Strong bands at 3478, 1790, and 1742 cm^{-1} observed in the IR spectrum confirmed the presence of hydroxy, γ-lactone, and ester groups in **3**. It was found that the spectroscopic data of **3** were identical to those of a known briarane, fragilide F, and these two compounds possessed negative optical values ([α] −15 for **3** and [α] −19 for fragilide F);[5] thus, compound **3** was identified as fragilide F.

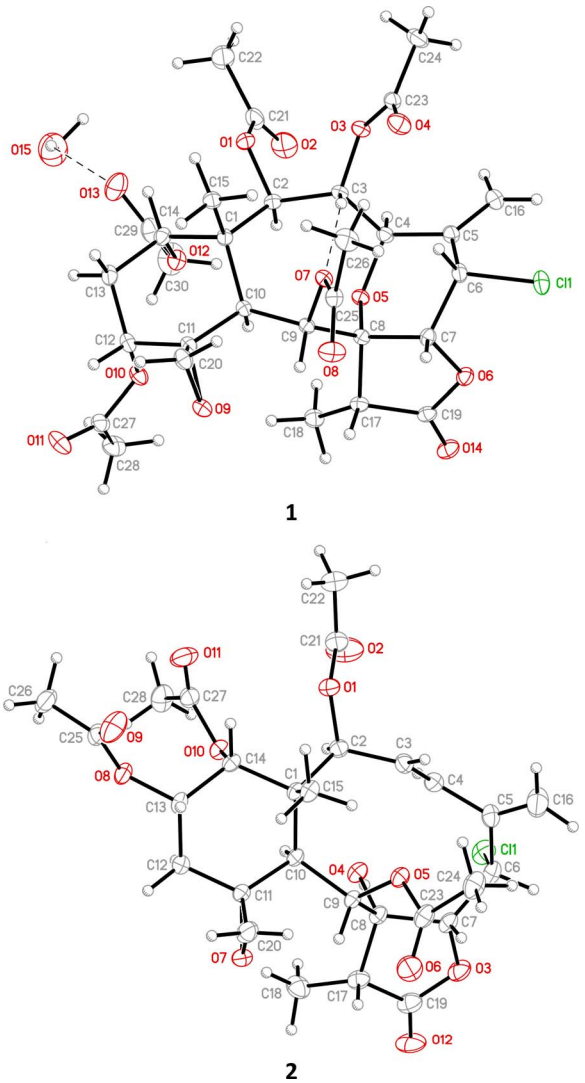

Fig. 2 ORTEP demonstrates the structures of gemmacolide X (**1**) and frajunolide I (**2**).

12α-Acetoxyfragilide F (**4**) was isolated as an amorphous powder and its molecular formula was determined to be $C_{30}H_{38}Cl_2O_{14}$ ($\Omega = 11$) by (+)-HRESIMS at *m*/*z* 715.15302 (calcd for $C_{30}H_{38}Cl_2O_{14}$ + Na, 715.15308). Comparison of the ^{1}H NMR, HSQC, and HMBC data with the molecular formula indicated that there must be an exchangeable proton, requiring the presence of a hydroxy group, and this deduction was supported by a broad absorption in the IR spectrum at 3466 cm^{-1}. The IR spectrum of **4** also showed strong bands at 1791 and 1740 cm^{-1}, consistent with the presence of γ-lactone and ester groups. The presence of an exocyclic olefin was deduced from the signals of an sp^2 methylene carbon at δ_C 119.6 (CH_2-16). Six carbonyl resonances at δ_C 175.2 (C-19), 171.2, 170.2, 170.2, 169.8, and 169.6, confirmed the presence of a γ-lactone and five ester groups; five acetate methyls (δ_H 2.36, 2.07, 2.04, 2.03, and 2.01, each 3H × s) were also observed. From the above NMR data (Table 1), seven degrees of unsaturation were accounted for, and **4** must be tetracyclic.

In addition, a tertiary methyl singlet, a methyl doublet, a pair of aliphatic methylene protons, two aliphatic methine protons, seven oxymethine protons, a downfield methine proton (δ_H 5.04, 1H, ddd, J = 2.4, 2.4, 2.4 Hz, H-6), a pair of low field methylene protons (δ_H 3.52, 1H, d, J = 12.0 Hz; 3.84, 1H, d, J = 12.0 Hz, H-20a/b), and a hydroxy proton (δ_H 3.11, 1*H*, s, OH-11) were observed in the ^{1}H NMR spectrum of **4** (Table 1).

The gross structure of **4** was verified by 2D NMR studies. ^{1}H NMR coupling information in the ^{1}H–^{1}H COSY spectrum of **4** enabled identification of C2–C3–C4, C6–C7, C12–C13–C14, and C17–C18 units, which were assembled with the assistance of an HMBC experiment (Fig. 3). The HMBC between protons and non-protonated carbons of **4**, such as H-2, H-9, H-10, H_3-15/C-1; H-4, H-10, H_3-18/C-8; H-9, H-10, H-20a/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 and by the HMBC between H-16a/C-4, C-6; and H-16b/C-6. The ring junction C-15 methyl group was positioned at C-1 from the HMBC between H_3-15/C-1, C-2, C-10, C-14. The presence of a hydroxy group at C-11 was deduced from the HMBC between a hydroxy proton (δ_H 3.11) with C-10 methine (δ_C 41.6). The acetate ester at C-9 was established by a correlation between H-9 (δ_H 6.40) and the acetate carbonyl (δ_C 169.6) observed in the HMBC spectrum. Thus, the remaining four acetoxy groups should be positioned at C-2, C-3, C-12, and C-14, as indicated by the characteristic NMR signal analysis of the oxymethines CH-2 (δ_H 5.48/δ_C 73.1), CH-3 (δ_H 6.19/δ_C 63.8), CH-12 (δ_H 5.52/δ_C 68.6), and CH-14 (δ_H 4.83/δ_C 72.6), respectively, although no HMBC was observed between the oxymethine protons H-2, H-3, H-12, and H-14 and those acetate carbonyls.

Table 1 ^{1}H and ^{13}C NMR data for briarane **4**

Position	δ_H[a] (J in Hz)	δ_C[b], Mult.[c]
1		45.1, C
2	5.48 d (6.6)	73.1, CH
3	6.19 dd (10.8, 6.6)	63.8, CH
4	4.47 d (10.8)	78.5, CH
5		N. o.[d]
6	5.04 ddd (2.4, 2.4, 2.4)	53.9, CH
7	4.37 d (2.4)	78.9, CH
8		83.9, C
9	6.40 s	73.2, CH
10	2.99 s	41.6, CH
11		74.6, C
12	5.52 dd (3.0, 3.0)	68.6, CH
13α/β	2.20 ddd (16.8, 3.0, 3.0); 1.97 ddd (16.8, 3.0, 3.0)	26.2, CH_2
14	4.83 dd (3.0, 3.0)	72.6, CH
15	1.30 s	16.4, CH_3
16a/b	5.36 d (2.4); 5.57 d (2.4)	119.6, CH_2
17	2.82 q (7.2)	49.5, CH
18	1.39 d (7.2)	7.2, CH_3
19		175.2, C
20a/b	3.52 d (12.0); 3.84 d (12.0)	48.3, CH_2
OH-11	3.11 s	
Acetoxy groups	2.36 s	21.0, CH_3
		169.6, C
	2.07 s	21.2, CH_3
		171.2, C
	2.04 s	21.0, CH_3
		170.2, C
	2.03 s	20.4, CH_3
		170.2, C
	2.01 s	21.0, CH_3
		169.8, C

[a] Spectra recorded at 600 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 150 MHz in $CDCl_3$ at 25 °C. [c] Data assigned with the assistance of HSQC and HMBC spectra. [d] N. o. = not observed.

The intensity of sodiated molecules $(M + 2 + Na)^+$ and $(M + 4 + Na)^+$ isotope peaks observed in (+)-ESIMS spectrum $[(M + Na)^+ : (M + 2 + Na)^+ : (M + 4 + Na)^+ = 9 : 6 : 1]$ were strong evidence of the presence of two chlorine atoms in **4**. The methine unit at δ_C 53.9 was more shielded than that expected for an oxygenated C-atom and was correlated to the methine proton at δ_H 5.04 in the HSQC spectrum and this proton signal was ^{3}J-correlated with H-7 (δ_H 4.37) (J = 2.4 Hz), proving the attachment of a chlorine atom at C-6. In addition, the methylene unit at δ_C 48.3 was also more shielded than that expected for an oxygenated C-atom and was correlated to the methylene protons at δ_H 3.52 and 3.84 in the HSQC spectrum and one of the methylene proton signals (δ_H 3.52, H-20a) exhibited HMBC with C-11 and C-12, proving the attachment of a chloromethyl group at C-11. Furthermore, an HMBC between H-4 (δ_H 4.47) and an oxygenated quaternary carbon at δ_C 83.9 (C-8) suggested the presence of a C-4/8 ether linkage.

The relative stereochemistry of **4** was elucidated by analysis of NOESY correlations and by vicinal ^{1}H–^{1}H proton coupling constants analysis. In the NOESY experiment (Fig. 4), H-10 correlated with H-2, H-9, and H_3-18 indicated that these

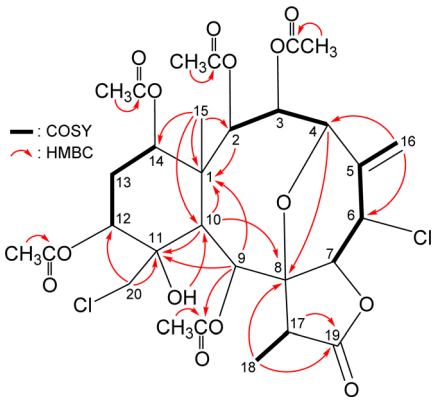

Fig. 3 Key HMBC and COSY correlations of **4**.

protons 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. Also, no coupling was found between H-9 and H-10, indicating that the dihedral angle between these two protons was approximately 90°, further confirmed that H-9 had an α-orientation. Due to H-14 proton being correlated with H_3-15, this proton was of a β-orientation at C-14. The C-13 methylene protons displayed identical coupling constants with H-14 (J = 3.0, 3.0 Hz) and H-12 (J = 3.0, 3.0 Hz), respectively, indicating that both H-14 and H-12 should be positioned on the β-equatorial direction in the six-membered ring of **4**.

The oxymethine proton H-3 and one of the chlorinated C-20 methylene protons (δ_H 3.84, H-20b) were found to exhibit responses with H_3-15 but not with H-10, revealing H-3 and C-20 methylene were β-oriented at C-3 and C-11, respectively. H-9 was found to show correlations with H-7, H-17, and one proton of C-20 methylene protons (δ_H 3.52, H-20a). From modeling analysis, H-9 was found to be reasonably close with H-7, H-17, and H-20a and can therefore be placed on the α face in the 10-membered

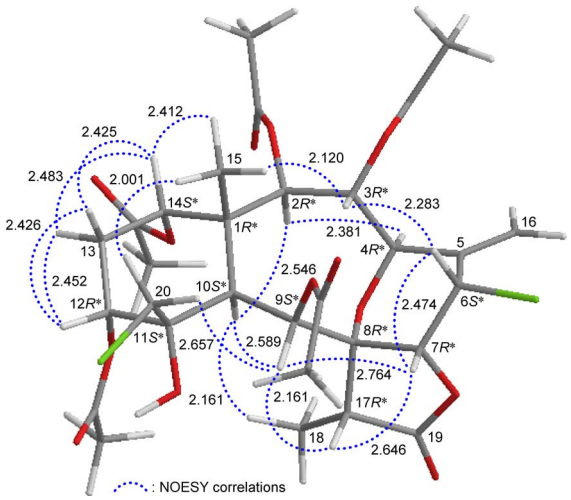

Fig. 4 Stereo-view of **4** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

Table 2 ^{1}H and ^{13}C NMR data for briarane **5**

Position	δ_H[a] (J in Hz)	δ_C[b], Mult.[c]
1		47.1, C
2	5.55 d (6.6)	72.6, CH
3	6.16 dd (10.8, 6.6)	63.6, CH
4	4.51 d (10.8)	79.0, CH[g]
5		134.1, C
6	5.01 ddd (3.0, 1.8, 1.8)	53.8, CH
7	4.52 d (3.0)	79.0, CH[g]
8		82.8, C
9	5.89 s	77.5, CH
10	3.56 s	39.7, CH
11		144.5, C
12	5.38 dd (3.6, 3.6)[de]	74.8, CH
13α/β	2.19 ddd (16.2, 3.6, 3.0); 1.90 ddd (16.2, 3.6, 2.4)	30.6, CH_2
14	4.93 dd (3.0, 2.4)	73.6, CH
15	1.14 s	14.5, CH_3
16a/b	5.38 d (1.8)[df]; 5.59 d (1.8)[f]	119.7, CH_2
17	2.78 q (7.2)	49.9, CH
18	1.35 d (7.2)	6.9, CH_3
19		173.9, C
20a/b	5.44 s; 5.03 br s	117.5, CH_2
Acetoxy groups	2.34 s	21.2, CH_3
		169.8, C
	2.05 s	21.0, CH_3
		170.7, C
	2.05 s	21.0, CH_3
		169.4, C
	2.01 s	21.0, CH_3
		170.4, C
	2.01 s	21.0, CH_3
		169.6, C

[a] Spectra recorded at 600 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 150 MHz in $CDCl_3$ at 25 °C. [c] Data assigned with the assistance of HSQC and HMBC spectra. [d] Signals overlapped. [e] The coupling pattern and coupling constant for H-12 were assigned by its vicinal couplings with H-13α/β. [f] The coupling pattern and coupling constant for H-16a/b were assigned by their allylic long-range ^{4}J-coupling with H-6. [g] Signals overlapped.

ring and both H-7 and H-17 are 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 H-7 and H-6 are on the β face. 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. The above interpretation enables the identification of the relative configuration of all stereogenic centers of **4** as 1*R**, 2*R**, 3*R**, 4*R**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 11*S**, 12*R**, 14*S**, 17*R**. According to the above and comparing the NMR data of **4** with those of the literature, the structure of **4** was similar to that of fragilide F (**3**) (Fig. 1),[5] except for the 12α-proton in **3** was instead of an acetoxy group in **4**. Hence, **4** was found to be the 12α-acetoxy derivative of **3** and named 12α-acetoxyfragilide F.

12α-Acetoxyjunceellin (**5**) was isolated as an amorphous powder. Its (+)-HRESIMS peak was at m/z 663.18121, consistent with the molecular formula $C_{30}H_{37}ClO_{13}$ (calcd for $C_{30}H_{37}ClO_{13}$ + Na, 663.18149) with 12 degrees of unsaturation. The IR spectrum of **5** contained signals of γ-lactone (ν_{max} 1791 cm^{-1}) and ester (ν_{max} 1740 cm^{-1}) functionalities. Analyzing the ^{1}H NMR (Table 2), HSQC, and HMBC spectra of **5** led to the assignment of five acetoxy groups; as well as two exocyclic carbon–carbon double bonds, a γ-lactone moiety, and other 15 carbon signals (Table 2).

The carbon skeleton of **5** was fully established by following correlations observed in the ^{1}H–^{1}H COSY and HMBC spectra (Fig. 5). The oxymethine protons H-3 (δ_H 6.16), H-9 (δ_H 5.89), and H-2 (δ_H 5.55) showed HMBC to the acetate carbonyls at δ_C 169.6, 169.8, and 170.4, confirmed the position of acetoxy groups at C-3, C-9, and C-2, respectively. Evaluated on the NMR chemical shifts of oxymethines CH-12 (δ_H 5.38/δ_C 74.8) and CH-14 (δ_H 4.93/δ_C 73.6), the remaining acetoxy groups should be positioned at C-12 and C-14, respectively.

The relative stereochemistry of **5** was established by analyzing the NOESY information in combination with the computer-generated model structure. We have noticed that all naturally-occurring briaranes possess a β-Me-15 placed at C-1 and have an α-orientation of H-10. In the NOESY spectrum (Fig. 6), H-10 showed correlations with H-2, H-9, and H_3-18; H_3-15 was correlated with H-3, H-14, and one of the C-13 methylene protons (δ_H 1.90, H-13β); and H-13β was correlated with H-12, proving the α-orientation of OAc-3, OAc-12, and OAc-14; and β-orientation of OAc-2 and OAc-9. H-3 exhibited an interaction with H-6; and H-6 correlated with H-7, indicating that H-6 and H-7 are on the β face. 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. Additionally, there was a correlation between H-7 and H-17, suggesting that H-17 is situated on the β face in the γ-lactone moiety. The above interpretation enables the identification of the relative configuration of all stereogenic centers of **5** as 1*R**, 2*R**, 3*R**, 4*R**, 6*S**, 7*R**, 8*R**, 9*S**, 10*S**, 12*R**, 14*S**, 17*R**. It was found that the NMR signals of **5** were similar to those of a known briarane, junceellin (**6**),[7,8] except that the signals corresponding to the α-proton at C-12 in **6** were replaced by signals for an acetoxy group in **5**. Thus, **5** was found to be the 12α-acetoxy derivative of **6** and named 12α-acetoxyjunceellin.

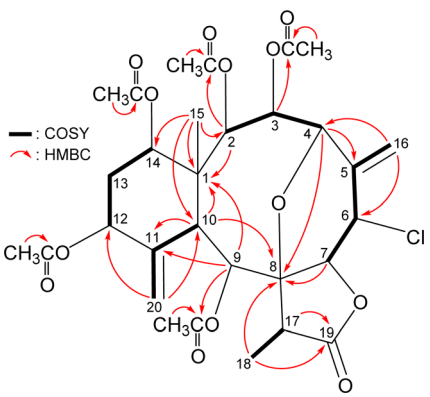

Fig. 5 Key HMBC and COSY correlations of **5**.

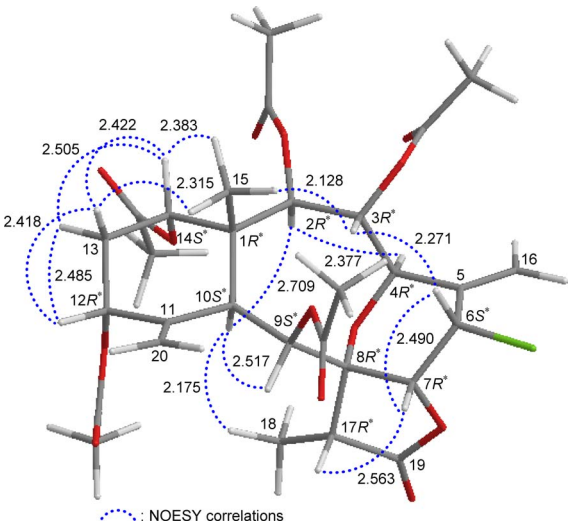

Fig. 6 Stereo-view of **5** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

Table 3 The evaluation of ALP activity ensued subsequent to subjecting MG63 cells to briaranes **1**–**5** at concentration of 10 μM or 100 μM rutin (utilized as a positive control) for[a] 72 h

Compounds	ALP activity (king unit per mg prot.)
1	4.2 ± 1.0**
2	2.9 ± 1.1**
3	6.6 ± 0.2***
4	5.8 ± 0.5***
5	4.8 ± 0.7***
Rutin	3.1 ± 0.2
Control	−5.0 ± 0.5

[a] Data are expressed with the mean standard error of the mean (SEM) (n = 3). The significance was determined with Student's t-test. **p < 0.01, ***p < 0.001 and comparison with untreated cells.

As briaranes **4** and **5**, in addition to **1** and **2**, were isolated from the same target organism, *J. fragilis*, it is reasonable to assume on biogenetic grounds that briaranes **4** and **5** have the same absolute configuration as **1** and **2**. Therefore, the absolute configurations of **4** and **5** were suggested to be (1*R*, 2*R*, 3*R*, 4*R*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 11*S*, 12*R*, 14*S*, 17*R*) and (1*R*, 2*R*, 3*R*, 4*R*, 6*S*, 7*R*, 8*R*, 9*S*, 10*S*, 12*R*, 14*S*, 17*R*), respectively.

Previous studies have found briarane-type natural products to be a natural remedy for osteoclastogenic disease.[9,10] *Via* an ALP ELISA assay with MG63 human mesenchymal stem cells (Table 3), the study found that briaranes **1** and **3**–**5** were active in enhancing ALP activity at a concentration of 10 μM.

3 Conclusions

The octocorals belonging to the genus *Junceella* have demonstrated a wide structural diversity of briarane diterpenoids with various pharmacological properties.[2] In our ongoing research on *J. fragilis*, we isolated five chlorinated briaranes, including two previously undiscovered briaranes: 12α-acetoxyfragilide F (**4**) and 12α-acetoxyjunceellin (**5**). Three known analogs were also identified: gemmacolidex (**1**),[3] frajunolide I (**2**),[4] and fragilide F (**3**).[5] The structures, including the absolute configurations, of **1** and **2** were further established through single-crystal X-ray diffraction analysis. For compounds **4** and **5**, their structures were confirmed using various spectroscopic techniques, particularly 2D NMR experiments and comparison with existing literature data. Briaranes **1** and **3**–**5** were active in enhancing ALP activity.

4 Experimental

4.1 General experimental procedures

Optical rotation values were measured using a JASCO P-1010 digital polarimeter. IR spectra were obtained with a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. NMR spectra were recorded on a 600 MHz Jeol ECZ NMR spectrometer using the residual $CHCl_3$ (δ_H 7.26 ppm) and $CDCl_3$ (δ_C 77.0 ppm) as internal standards for ^1H and ^{13}C NMR, respectively; coupling constants (J) are presented in Hertz (Hz). The ESIMS and HRESIMS spectra were ascertained with Thermo Fisher orbitrap Exploris 120 mass spectrometer equipped with an ESI ion source in positive ionization mode. The extracted samples were separated *via* column chromatography with silica gel (particle size, 230–400 mesh; Merck). TLC was performed on plates precoated with silica gel 60 (DC-Fertigfolien Alugram Xtra SIL G/UV_{254}, layer thickness 0.20 mm, Macherey-Nagel) and RP-18 F_{254}S (layer thickness 0.16–0.20 mm, Merck), and visualization of the TLC plates was conducted using an aqueous solution of 10% H_2SO_4, subsequently to be heated to show the spots of signals. Reverse-phase HPLC (RP-HPLC) separation was carried out with a system containing a pump (Hitachi, model L-7110) with a photo-diode array detector (Hitachi, model L-2400), equipped with a reverse-phase column (Luna, 5 μm, C18(2) 100 Å, 250 × 21.2 mm). Normal-phase HPLC (NP-HPLC) separation was carried out with a system containing a pump (Hitachi, model L-5110), equipped with a normal-phase column (Galaksil, EF-SiO_2, 5 μm 120 Å, 250 × 10 mm).

4.2 Animal material

Specimen of *J. fragilis* was collected manually *via* SCUBA diving off the coast of Southern Taiwan in 2012. A voucher specimen was deposited at the National Museum of Marine Biology & Aquarium, Taiwan. To identify the species, we compared its physical characteristics and microscopic images of the coral sclerites with those mentioned in previous studies.[1,11–13]

4.3 Extraction and isolation

The freeze-dried specimen (wet/dry weight = 6.01/2.39 kg) was sliced and treated with a 1 : 1 mixture solvent of MeOH and CH_2Cl_2 at room temperature to produce crude extract weighing 140.1 g, which was then subjected to liquid–liquid partition between EtOAc and H_2O. The EtOAc phase (19.2 g) was applied to a silica gel column chromatography (Si. C. C.). Elution was

carried out with a gradient solvent system containing *n*-hexane, followed by increasing polarity mixtures of *n*-hexane and EtOAc, pure acetone, and pure methanol for use as eluting solvents. The process yielded 13 fractions A–M. Fraction D was chromatographed with NP-HPLC *via* an isocratic solvent system, *n*-hexane/acetone mixture (6 : 1). The process yielded 7 fractions D1–D7. Fraction D6 was separated with NP-HPLC *via* an isocratic solvent system, DCM/acetone mixture (18 : 1). The process yielded 3 fractions D6A–D6C. Fraction D6A was purified with RP-HPLC *via* an isocratic solvent system, ACN/H_2O mixture (60 : 40; flow rate = 3 mL min^{-1}), to afford **3** (0.4 mg). Fraction E was chromatographed with Si. C.C. and eluted with an isocratic solvent system, DCM/acetone mixture (10 : 1). The process yielded 8 fractions E1–E8. Fraction E2 was separated by NP-HPLC *via* an isocratic solvent system, *n*-hexane/EtOAC mixture (2 : 1) to obtain 5 fractions E2A–E2E. Fraction E2E was purified by RP-HPLC with an isocratic solvent system, ACN/H_2O mixture (50 : 50; flow rate = 3 mL min^{-1}), to obtain **2** (0.3 mg). Fraction E2D was purified by RP-HPLC with an isocratic solvent system, ACN/H_2O mixture (60 : 40; flow rate = 3 mL min^{-1}), to obtain **5** (0.4 mg). Fraction F was separated on Si. C. C. with an isocratic solvent system, DCM/acetone mixture (15 : 1). The process yielded 8 fractions F1–F8. Fraction F4 was separated Si C. C. and eluted with DCM/EtOAc mixture (20 : 1) to yield 6 fraction F4A–F4F. Fraction F4E was purified by RP-HPLC with an isocratic solvent system, MeOH/H_2O mixture (70 : 30; flow rate = 3 mL min^{-1}) to obtain **1** (0.8 mg) and **4** (0.4 mg), respectively.

4.4 Structural characterization of undescribed compounds

4.4.1 12α-Acetoxyfragilide F (4). Amorphous powder; [α] −39 (*c* 0.02, $CHCl_3$); IR (KBr) ν_{max} 3466, 1791, 1740 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data (see Table 1); ESIMS: *m*/*z* 715 $[M + Na]^+$, 717 $[M + 2 + Na]^+$, 719 $[M + 4 + Na]^+$; HRESIMS: *m*/*z* 715.15302 (calcd for $C_{30}H_{38}Cl_2O_{14}$ + Na, 715.15308).

4.4.2 12α-Acetoxyjunceellin (5). Amorphous powder; [α] +275 (*c* 0.02, $CHCl_3$); IR (KBr) ν_{max} 1791, 1740 cm^{-1}; ^1H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data (see Table 2); ESIMS: *m*/*z* 663 $[M + Na]^+$, 665 $[M + 2 + Na]^+$; HRESIMS: *m*/*z* 663.18121 (calcd for $C_{30}H_{37}ClO_{13}$ + Na, 663.18149).

4.5 Single-crystal X-ray crystallography of gemmacolide X (1)

Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.363 × 0.235 × 0.198 mm^3) was identified as being of the orthorhombic system, space group $P2_12_12_1$ (#19),[14] with a = 12.2945(2) Å, b = 12.4454(2) Å, c = 22.2997(4) Å, V = 3412.08(10) $Å^3$, Z = 4, D_{calcd} = 1.297 Mg m^{-3} and λ (Cu Kα) = 1.54178 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a θ_{max} of 68.349°. All measurement data of 34 394 reflections were collected, of which 6232 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[15,16] The refined structural model converged to a final R_1 = 0.0292; wR_2 = 0.0758 for 5969 observed reflections [$I > 2\sigma(I)$] and 425 variable parameters; and the absolute configuration was established from the Flack parameter x = 0.000(5).[6,17,18] Crystallographic data for the structure of gemmacolide X (**1**) were submitted to the Cambridge Crystallographic Data Center (CCDC) with ESI publication number CCDC 2323829 (data can be obtained from the CCDC website at https://www.ccdc.cam.ac.uk/conts/retrieving.html).

4.6 Single-crystal X-ray crystallography of frajunolide I (2)

Suitable colorless prisms of **2** were obtained from a solution of MeOH. The crystal (0.191 × 0.102 × 0.049 mm^3) was identified as being of the hexagonal system, space group $P6_1$ (#169),[14] with $a = b$ = 22.8317(3) Å, c = 10.2937(2) Å, V = 4647.06(15) $Å^3$, Z = 6, D_{calcd} = 1.284 Mg m^{-3} and λ (Cu Kα) = 1.54178 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a θ_{max} of 74.402°. All measurement data of 47 636 reflections were collected, of which 6221 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[15,16] The refined structural model converged to a final R_1 = 0.0358; wR_2 = 0.0960 for 5789 observed reflections [$I > 2\sigma(I)$] and 377 variable parameters; and the absolute configuration was established from the Flack parameter x = −0.002(13).[6,17,18] Crystallographic data for the structure of frajunolide I (**2**) were submitted to CCDC with ESI publication number CCDC 2326820 (data can be obtained from the CCDC website at https://www.ccdc.cam.ac.uk/conts/retrieving.html).

4.7 Alkaline phosphatase (ALP) activity assay

The ALP assay was released to assess the activity of compounds **1–5** from MG63 human mesenchymal stem cells, in line with suggestion of previous studies.[19]

Author contributions

Hai Nhat Do and Yu-Ta Chen: methodology, software, analysis, investigation, data curation, original draft writing. Su-Ying Chien: analysis, investigation. You-Ying Chen: investigation. Mingzi M. Zhang, Lun Kelvin Tsou, Jih-Jung Chen, Zhi-Hong Wen, and Yi-Hao Lo: methodology, software, analysis. Li-Guo Zheng and Ping-Jyun Sung: conceptualization, resources, draft review & editing, visualization, supervision, project administration, fundraising.

Conflicts of interest

There are no conflicts to declare.

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