國科會補助專題研究計畫出席國際學術會議心得報告

日期：112 年 9 月 18 日

計畫編號	MOST 109-2320-B-291-001-MY3		
計畫名稱	台灣南部海域海綿及其共伴微生物所含生理活性物質之研究		
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研究員
會議時間	112 年 7 月 2 日至 5 日	會議地點	愛爾蘭都柏林三一學院
會議名稱	(中文)第 71 屆國際藥用植物暨天然物研究學會年會 (英文) 71 st International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA2023)		
發表題目	(中文)指形軟珊瑚 *Sinularia* sp.所含之十三環新穎双萜化合物-Sinularianone A (英文) Sinulariaone A: A novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species		

一、參加會議經過

原每年舉辦之全球最盛大之天然物藥學國際會議-第 71 屆國際藥用植物暨天然物研究學會年(71 rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research) 於 2023 年 7 月 2 日至 5 日於愛爾蘭都柏林三一學院 (Trinity College)舉行。自 6 月 29 日啟程前往愛爾蘭都柏林並於 7 月 2 日抵達都柏林三一學院。會議議題涵蓋範圍廣泛，自年輕學者的 workshop 和動物保健之植物療法(Animal Healthcare and Veterinary Phytotherap)。乃至大會演講(plenary speech)以及論文海報的發表。會議統計約有 1 千多名來自世界各國的與會學者前來共襄盛舉，但以歐洲地區的學者佔較高之比例，亞洲地區參與度有很大的進步空間，相信有一部分是受到 Covid-19 疫情之影響，台灣學者與會比重在亞洲區域而言相對積極，中央研究院、台灣大學、陽明交通大學、中國醫藥大學、台北醫學大學、成功大學、中山大學、中山醫學大學、海洋生物博物館等各相關教研單位均有人員與會，積極的參與會議並與國際研究學者們廣泛的進行專業學術交流。

大會學術議程

二、 與會心得

本次主辦單位邀請各個領域有關天然藥物相關之研究學者進行研究成果發表，在研究之領域與應用上能跳脫較為傳統之研究領域，能同時在傳統上創新，激發出不同領域間的互相激勵，如獸醫-植物化學、植物藥理學、植物療法、綠色化學、大麻和大麻素(在台灣仍屬違法使用)等各項專題演講及壁報論文發表。惟在海洋天然物化學方面之涉獵性較低，但也相對地了解台灣在海洋天然物方面之研究有一定之優勢存在，應更好好得把握。個人於會議發表"Sinulariaone A: A novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species"論文一篇。與各國與會學者討論 sinulariaone A 在化學動力學與構型上的可能性，獲益匪淺，並依此研究成果後續發表論文於英國皇家化學會 *RSC Advances*。

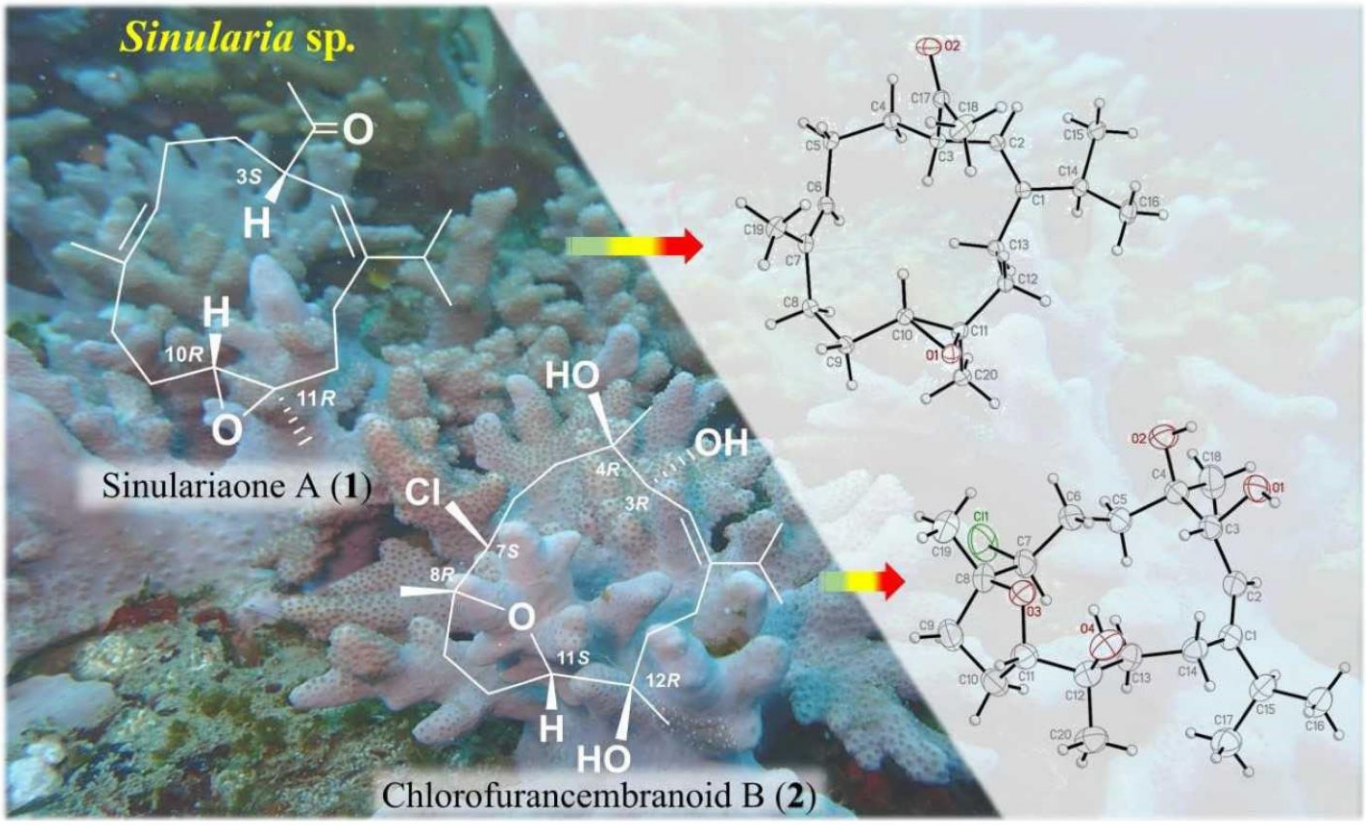
P-054

Sinulariaone A: A novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species

Ping-Jyun Sung[1], Hsuan-Jung Tseng[2], Jui-Hsin Su[1]

[1]National Museum of Marine Biology & Aquarium, Checheng, Taiwan, [2]National Dong Hwa University, Checheng, Taiwan

Chemical composition screening of an octocoral identified as *Sinularia* species led to the isolation of a novel diterpenoid, sinulariaone A (**1**), featuring an unprecedented 13-membered carbocyclic skeleton. The structure of **1** was established by spectroscopic elucidation, computed calculation and X-ray diffraction analysis. It is to note that diterpenoid **1**, involving an uncommon 13-membered carbocyclic carbon system, with suggested biosynthesis from the common 14-membered carbocyclic cembrane analogues by ring contraction, is one of a kind. This is the first time to obtain a 13-membered carbocyclic cembranolide analogue featuring an acetyl group at C-3. Moreover, a single-crystal X-ray diffraction analysis of chlorofurancembranoid B (**2**), obtained in our previous study from the same octocoral species, was reported for the first time to demonstrate the absolute configuration. Diterpenoid **1** showed cytotoxicity towards human promyelocytic leukemia HL-60 cells, with an IC_{50} value of 38.01 μM.



三、建議

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天然藥物及植物藥學研究領域之翹楚，其熱烈參與程度相當引人注意。惟在會議中可明顯看出歐、美等國在天然藥物研究上仍執全球牛耳地位，而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，如能在海洋天然物方面加強投入研究資源則應可在一定時間

內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碩、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；

Tseng, H.-J.; Kuo, L.-M.; Tsai, Y.-C.; Hu, H.-C.; Chen, P.-J.; Chien, S.-Y.; Sheu, J.-H.*; **Sung, P.-J.*** Sinulariaone A: A novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species. *RSC Adv.* **2023**, *13*, 10408–10413.

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Sinulariaone A: a novel diterpenoid with a 13-membered carbocyclic skeleton from an octocoral *Sinularia* species†

Hsuan-Jung Tseng,‡[ab] Liang-Mou Kuo,‡[c] Yu-Chi Tsai,[d] Hao-Chun Hu,[e] Po-Jen Chen,[f] Su-Ying Chien,[g] Jyh-Horng Sheu*[h] and Ping-Jyun Sung *[behij]

Chemical composition screening of an octocoral identified as *Sinularia* species led to the isolation of a novel diterpenoid, sinulariaone A (**1**), featuring a 13-membered carbocyclic skeleton. The structure of **1** was established by spectroscopic elucidation, computed calculation, and X-ray diffraction analysis. Moreover, a single-crystal X-ray diffraction analysis of chlorofurancembranoid B (**2**), obtained in our previous study from the same octocoral species, was reported for the first time to demonstrate the absolute configuration. Diterpenoid **1** showed cytotoxicity towards human promyelocytic leukemia HL-60 cells, with an IC_{50} value of 38.01 μM.

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1 Introduction

Octocorals of the genus *Sinularia* (phylum Cnidaria, subphylum Anthozoa, class Octocorallia, order Malacalcyonacea, family Sinulariidae)[1] are one of the most common marine invertebrates natively distributed throughout tropical and subtropical regions of the Indo-Pacific Ocean. Despite their ecological importance, the secondary metabolites, in particular terpenoid derivatives from these organisms were proven to have potential for biomedical uses.[2–4] In this research, we completed the preparation, structural identification, and cytotoxicity assessment of sinulariaone A (**1**), a diterpenoid featuring with a rare 13-membered carbocyclic skeleton and chlorofurancembranoid B (**2**)[5] (Fig. 1), from an octocoral identified as *Sinularia* sp., collected from the waters of Taiwan, an area with high biodiversity at the intersection of the Kuroshio current, South China Sea surface current, and Mainland Coastal current.

2 Results and discussion

Sinulariaone A (**1**) was obtained as colorless prisms with the molecular formula determined to be $C_{20}H_{32}O_2$ by (+)-HRESIMS at *m*/*z* 327.22928 (calcd for $C_{20}H_{32}O_2$ + Na, 327.22945), corresponding to five double-bond equivalents (DBEs). The IR spectrum of **1** showed a strong absorption at ν_{max} 1716 cm^{-1}, consistent with a ketone moiety in the structure. The ^{13}C spectrum (Table 1), in combination with the DEPT and HSQC spectrum, showed signals of 20 carbons, including a ketonic carbonyl (δ_C 210.0, C-17), four olefinic carbons (δ_C 149.8, C-1;

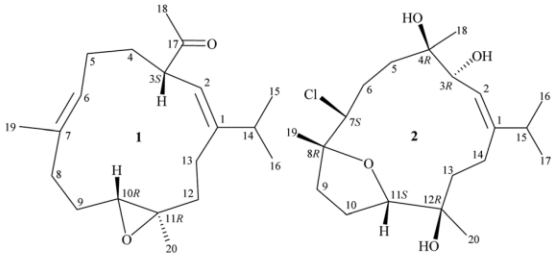

Fig. 1 Structures of sinulariaone A (**1**) and chlorofurancembranoid B (**2**).

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† Electronic supplementary information (ESI) available: HRESI-MS, 1D and 2D NMR spectra of **1**; experimental and calculated SOR values of **1**; X-ray crystallo-graphic data of **1** and **2**. CCDC 2226689 and 2208811. For ESI and crystallographic data in CIF or other electronic format see DOI: https://doi.org/10.1039/d3ra01589k

‡ These authors have contributed equally to this work.

Table 1 ^{1}H and ^{13}C NMR spectroscopic data of sinulariaone A (**1**)

Position	δ_H[a] (J in Hz)	δ_C[b], Mult.[c]
1		149.8, C
2	5.06 d (10.0)	121.6, CH
3	3.16 ddd (10.0, 10.0, 2.0)	49.9, CH
4	2.23 m	31.5, CH_2
4′	1.04 m	
5	1.97–2.05 m[d]	25.6, CH_2
6	5.13 dd (6.8, 6.8)	127.4, CH
7		134.8, C
8	2.36 ddd (12.4, 5.2, 4.4)	36.7, CH_2
8′	2.18 dd (12.4, 4.0)	
9	2.24 ddd (13.2, 5.2, 4.0)[e,h]	24.4, CH_2
9′	1.37 dddd (13.2, 10.0, 4.4, 4.0)	
10	2.74 dd (10.0, 4.0)	62.6, CH
11		61.3, C
12α	2.26 m[e]	40.7, CH_2
β	1.07 m	
13	1.97–2.05 m[d]	25.2, CH_2
14	2.25 m[e]	35.4, CH
15	0.99 d (6.8)[f]	21.9, CH_3[g]
16	0.99 d (6.8)[f]	21.9, CH_3[g]
17		210.0, C
18	2.06 s	29.4, CH_3
19	1.62 br s	14.5, CH_3
20	1.30 s	16.0, CH_3

[a] Spectra recorded at 400 MHz in $CDCl_3$ at 25 °C. [b] Spectra recorded at 100 MHz in $CDCl_3$ at 25 °C. [c] Multiplicity deduced by DEPT and HSQC spectrum and indicated by usual symbols. [d] Signals overlapped. [e] Signals overlapped. [f] Signals overlapped. [g] Signals overlapped. [h] The coupling constants for H-9 were assigned by its geminal coupling with H-9′ and vicinal couplings with H-8 and H-10, respectively.

121.6, CH-2; 127.4, CH-6; 134.8, C-7), and two oxygenated carbons (δ_C 62.6, CH-10; 61.3, C-11), as well as five methyls, six aliphatic sp^3 methylenes, and two aliphatic sp^3 methines.

Analysis of ^{1}H (Table 1), ^{13}C, and HSQC spectra illustrated that **1** contained two trisubstituted carbon–carbon double bonds (δ_H 5.13, 1H, dd, J = 6.8, 6.8 Hz/δ_C 127.4, CH-6; δ_C 134.8, C-7; δ_H 5.06, 1H, d, J = 10.0 Hz/δ_C 121.6, CH-2; δ_C 149.8, C-1) and an acetyl group (δ_H 2.06, 3H, s/δ_C 29.4, CH_3-18; δ_C 210.0, C-17). The ^{3}J-proton-proton coupling information in the COSY spectrum led to the assignment of four continuous spin systems from H-2/H-3/H_2-4/H_2-5/H-6, H_2-8/H_2-9/H-10, H_2-12/H_2-13, and H-14/H_3-15 (H_3-16) (Fig. 2). The HMBC spectrum showed ^{2}J- and ^{3}J-heteronuclear correlations from neighbor protons to the non-protonated carbons such as H-3, H_2-12, H_2-13, H-14, H_3-15, H_3-16/C-1; H_2-5, H_2-8, H_2-9, H_3-19/C-7; H_2-12, H_2-13, H_3-20/C-11; and H-2, H-3, H_2-4, H_3-18/C-17 (Fig. 2), confirming the presence of central 13-membered carbon macrocyclic ring system.[6–10] The HMBC correlations from H_3-20/C-10, C-11, and C-12 indicated that Me-20 was placed at C-11. The presence of a vinyl methyl (Me-19) at C-7 was substantiated by the HMBC correlations from H-6, H-8′ (δ_H 2.18)/C-19 and H_3-19/C-6, C-7, C-8, and further confirmed by a long-range allylic coupling between H-6/H_3-19 (Fig. 2). The presence of an isopropyl group at C-1 was substantiated by the HMBC correlations from H-14, H_3-15, H_3-16 to C-1. The above analysis enabled the establishment of the carbon skeleton of **1**. A trisubstituted epoxide

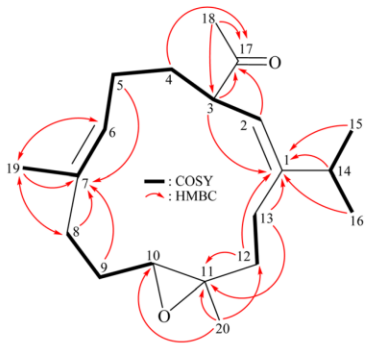


Fig. 2 Key COSY and HMBC correlations of **1**.

containing a methyl substituent in **1** was established from the signals of an oxygenated quaternary carbon at δ_C 61.3 (C-11) and an oxymethine (δ_H 2.74, 1H, dd, J = 10.0, 4.0 Hz/δ_C 62.6, CH-10), and from the proton signal of a methyl at δ_H 1.30 (3H, s, H_3-20). An acetyl group at C-3 was confirmed by the HMBC correlations from the methine proton at δ_H 3.16 (H-3) to the ketonic carbonyl at δ_C 210.0 (C-17); the other HMBC correlations from the methyl protons resonating at δ_H 2.06 (H_3-18) to C-17 ketonic carbonyl (δ_C 210.0) and C-3 methine (δ_C 49.9), further supporting that this group was positioned at C-3.

The relative stereochemistry of **1** was determined based on correlations obtained from NOESY experiments. In the NOESY spectrum (Fig. 3), H-10 exhibited cross-peaks with H-3 and one of the diastereotopic methylene protons at C-12 (δ_H 1.07, H-12β); and H-12β was correlated with H-3 but not with H_3-20, which illustrated the β-orientations of H-3 and H-10, and the α-orientation of Me-20. The Z-form of Δ^1 and Δ^6 was confirmed by

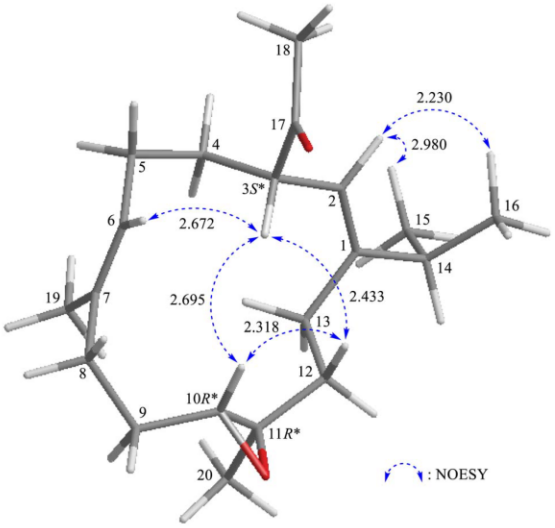
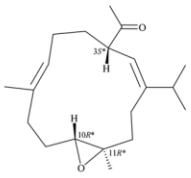
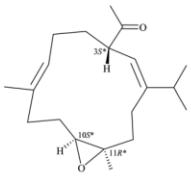
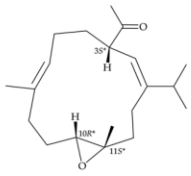
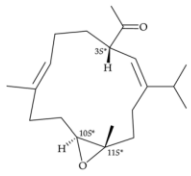


Fig. 3 Stereo-view of **1** (generated by computer modeling) and calculated distances (Å) between selected protons with key NOESY correlations.

NOESY correlations between H-2 (olefin proton)/H_3-15 (H_3-16); and H-6 (olefin proton)/H-3, respectively, and there were no NOESY correlations were found between H-6/H_3-19 (vinyl methyl) and H-2/H_2-13.

After the program of the above analysis, the gross structure of **1** displayed four possible relative configurations, including **1**-3*S**, 10*R**, 11*R**; **1**-3*S**, 10*S**, 11*R**; **1**-3*S**, 10*R**, 11*S**; and **1**-3*S**, 10*S**,11*S**. The four possible relative configurations were inputted into Spartan'16 and optimized at the MMFF94 level.[11–13] The predicted distance of key NOESY of possible configurations is shown in Table 2. The **1**-3*S**, 10*R**, 11*R** displayed the best result matching the experimental key NOESY correlations, and the calculated single optical rotation (SOR) value of **1**-3*S*, 10*R*, 11*R* and **1**-3*R*, 10*S*, 11*S* were +226 and −226, respectively. Comparing the calculated with the experimental SOR value of **1** (+236), the absolute configuration of **1** could be assigned as 3*S*, 10*R*, 11*R*.

Table 2 The predicted distance (Å) of key NOESY of optimized top 3 possible relative configurations of **1**

	3*S**, 10*R**, 11*R**	3*S**, 10*S**, 11*R**
Structures		
	Top 1	
H-3/H-6	2.775	2.688
H-3/H-10	2.404	4.931
H-3/H-12	2.468	2.479
	Top 2	
H-3/H-6	2.742	2.740
H-3/H-10	2.430	4.857
H-3/H-12	2.565	2.308
	Top 3	
H-3/H-6	2.636	2.517
H-3/H-10	2.409	5.071
H-3/H-12	2.411	2.479
	3*S**, 10*R**, 11*S**	3*S**, 10*S**, 11*S**
Structures		
	Top 1	
H-3/H-6	2.597	3.197
H-3/H-10	3.235	3.896
H-3/H-12	4.123	2.346
	Top 2	
H-3/H-6	2.636	3.199
H-3/H-10	3.138	3.839
H-3/H-12	4.161	3.829
	Top 3	
H-3/H-6	2.604	3.269
H-3/H-10	3.237	3.935
H-3/H-12	4.229	3.220

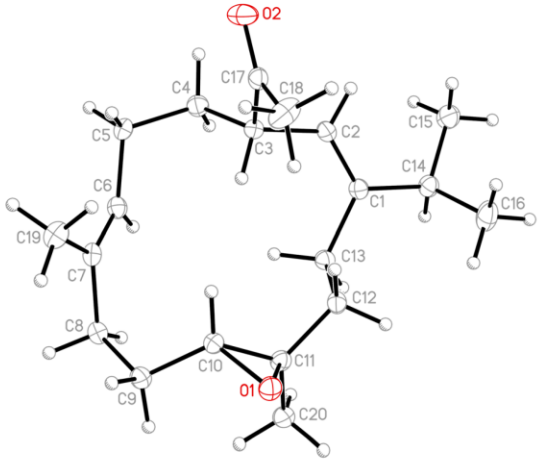


Fig. 4 The computer-generated ORTEP diagram of **1**.

Due to the conformational mobility of the macrocycle, the stereochemistry of the stereogenic centers C-3, C-10, and C-11 of **1** would be further determined from an X-ray diffraction analysis. Regarding validation of the structure of **1**, a single-crystal X-ray diffraction analysis was employed. The structure of **1** was fully established by X-ray crystallography, as observed by Cu Kα radiation (λ = 1.54178 Å) and the Flack parameter x = 0.0(3).[14,15] The X-ray structure (Fig. 4) demonstrates the location of an acetyl group at C-3 and an epoxy group between C-10/11 in the 13-membered macrocycle ring. Based on the X-ray diffraction analysis, the stereogenic centers in **1** were assigned as 3*S*, 10*R*, 11*R*. From the above findings, the structure, including the absolute configuration, of **1** was therefore elucidated unambiguously.

Chlorofurancembranoid B (**2**), a cytotoxic cembranoid toward human promyelocytic leukemia HL-60 cells, was reported in our previous publication, and its stereochemistry was established by combination of a NOESY experiment.[5] Thus, in order to determine the absolute configuration. This compound has been crystallized, and the diffraction experiment was carried out with a diffractometer equipped with molybdenum radiation (Mo Kα, λ = 0.71073 Å) source. The ORTEP diagram (Fig. 5) showed the absolute configuration for all stereogenic centers were assigned as 3*R*, 4*R*, 7*S*, 8*R*, 11*S*, 12*R*.

The cytotoxicity of **1** against cancer cells HL-60 and HepG2 (human hepatoma cell line) were investigated. The assay used in this study was performed as described in previous

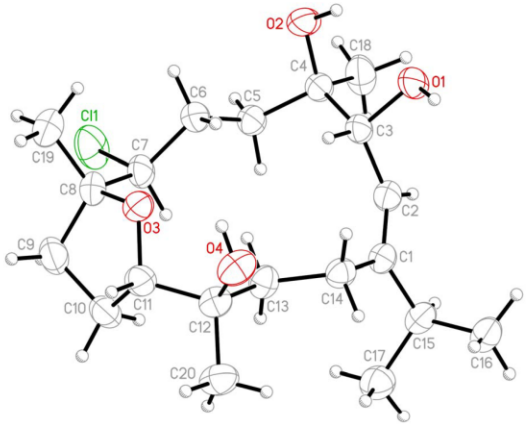


Fig. 5 The computer-generated ORTEP diagram of **2**.

Table 3 Effects of compound **1** on cell viability in HL-60 and HepG2 tumor cells

	HL-60		HepG2	
Compound	Cell viability[a] (%)	IC_{50}[b] (μM)	Cell viability[a] (%)	IC_{50}[b] (μM)
1	42.42 ± 0.70***	38.01 ± 1.21	71.76 ± 2.90	> 50
DMSO	100.00 ± 2.16		100.00 ± 0.72	

[a] Cell viability at 50 μM for 48 h. Results are expressed as mean ± SEM (n = 3). ***p < 0.001 compared with DMSO alone. [b] Concentration necessary for 50% inhibition (IC_{50}).

publications.[16,17] The results are shown in Table 3. According to the outcomes of cytotoxic assays, diterpenoid **1** showed cytotoxicity towards human promyelocytic leukemia HL-60 cells, with an IC_{50} value of 38.01 μM.

3 Conclusions

In this study, the chemical composition of an octocoral identified as *Sinularia* sp. was screened, resulted in the isolation of a novel diterpenoid, sinulariaone A (**1**). It is to note that diterpenoid **1**, involving an uncommon 13-membered carbocyclic carbon system, which was suggested biosynthesized from the common 14-membered carbocyclic cembrane analogues by ring contraction,[6,7] however, to be one of a kind, this is the first time to obtain a 13-membered carbocyclic cembranolide analogue featuring with an acetyl group at C-3. The structure of **1**, including the absolute configuration, was determined by spectroscopic methods and further confirmed by a single-crystal X-ray diffraction analysis and this compound showed cytotoxicity toward the HL-60 tumor cells. In addition, the absolute configuration of a known cytotoxic cembranoid, chlorofurancembranoid B (**2**), was determined using a single-crystal X-ray diffraction analysis with the molybdenum radiation source, with the material obtained in previous study.[5]

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 400 MHz Jeol ECZ NMR spectrometer using the residual $CHCl_3$ (δ_H 7.26 ppm) and $CDCl_3$ signals (δ_C 77.0 ppm) as internal standards for ^1H and ^{13}C NMR, respectively; coupling constants (J) are presented in Hz. ESIMS and HRESIMS were recorded using a Bruker 7 Tesla solariX FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck). TLC was performed on plates precoated with silica gel 60 F_{254} (Merck) and RP-18W/UV_{254} (0.15 mm-thick, Macherey-Nagel), then sprayed with 10% H_2SO_4 solution followed by heating to visualize the spots.

4.2 Animal material

Specimens of *Sinularia* sp. were collected on Turtle Island, Yilan County, Taiwan. The samples were stored in a freezer at −20 °C until extraction. A voucher specimen was deposited in the National Museum of Marine Biology & Aquarium, Taiwan (NMMBA-TW–SC–2018-0619). Identification of this organism was performed by comparison with previous descriptions.[1,18]

4.3 Extraction and isolation

Freeze-dried and sliced bodies (wet/dry weight = 510/172 g) of the coral specimens were extracted with a mixture of MeOH/CH_2Cl_2 (1 : 1) to give 17.8 g of crude extract, which was partitioned between EtOAc and H_2O. The EtOAc extract (6.8 g) was subjected to silica gel column chromatography (Si C. C.) and eluted with gradients of *n*-hexane/EtOAc (100% *n*-hexane–100% EtOAc, stepwise) to furnish 14 sub-fractions A–N. Fraction D was chromatographed by Si C. C. and eluted with a mixture of CH_2Cl_2/EtOAc (20 : 1) to obtain 18 sub-fractions D1–D18. Fraction D13 was further separated by Si C. C. and eluted with CH_2Cl_2 to afford **1** (3.5 mg).

4.4 Structural characterization of undescribed compound

4.4.1 Sinulariaone A (1). Colorless prisms (MeOH); mp 102–104 °C; [α] + 236 (c 0.05, $CHCl_3$); IR (KBr) ν_{max} 1716 cm^{-1}; ^1H (400 MHz, $CDCl_3$) and ^{13}C (100 MHz, $CDCl_3$) NMR data (see Table 1); ESIMS: m/z 327 $[M + Na]^+$; HRESIMS m/z 327.22928 (calcd for $C_{20}H_{32}O_2$ + Na, 327.22945).

4.5 Single-crystal X-ray crystallography of sinulariaone A (1)

Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.600 × 0.484 × 0.138 mm^3) was identified as being of the orthorhombic system, space group $P2_12_12_1$ (#19), with a = 9.0173(3) Å, b = 10.8659(3) Å, c = 18.6542(6) Å, V = 1827.76(10) $Å^3$, Z = 4, D_{calcd} = 1.106 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 69.999°. All measurement data of 18 147 reflections were collected, of which

3472 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[19,20] The refined structural model converged to a final $R_1 = 0.0601$; $wR_2 = 0.1637$ for 3095 observed reflections [$I > 2\sigma(I)$] and 199 variable parameters; and the absolute configuration was established from the Flack parameter $x = 0.0(3)$.[14,15] Crystallographic data for the structure of sinulariaone A (**1**) were submitted to the Cambridge Crystallographic Data Center (CCDC) with supplementary publication number CCDC 2226689 (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 chlorofurancembranoid B (2)

Suitable colorless prisms of **2** were obtained from a solution of MeOH. The crystal (0.388 × 0.145 × 0.028 mm^3) was identified as being of the triclinic system, space group $P1$ (#1), with a = 10.1046(4) Å, b = 10.4180(3) Å, c = 10.9661(4) Å, V = 1066.93(7) $Å^3$, $Z = 2$, D_{calcd} = 1.167 Mg m^{-3} and λ (Mo Kα) = 0.71073 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a θ_{max} of 29.998°. All measurement data of 38 323 reflections were collected, of which 12 414 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on F^2 procedure.[19,20] The refined structural model converged to a final $R_1 = 0.0561$; wR_2 = 0.1188 for 8865 observed reflections [$I > 2\sigma(I)$] and 468 variable parameters; and the absolute configuration was established from the Flack parameter $x = -0.01(3)$.[14,15] Crystallographic data for the structure of chlorofurancembranoid B (**2**) were deposited with the Cambridge Crystallographic Data Center (CCDC) as supplementary publication number CCDC 2208811 (data can be obtained from the CCDC website at **https://www.ccdc.cam.ac.uk/conts/retrieving.html**).

4.7 *In silico* calculations

The conformational search and calculated SOR results were carried out with the same method published as ref. 11–13. The brief procedure was described as follows. First, we optimized the minimized energy of the structure in the MM2 level and outputted an xyz file. Then, we submitted the file into spartan'16 software (Wavefunction Inc.; Irvine, CA, USA) at MMFF94 to generate conformational search results. The output data were imported into the Gaussian 09 software (Gaussian Inc.; Wallingford, CT, USA) and optimized using the time-dependent density functional theory (TDDFT) methodology at the B3LYP/6-31G* level in the gas phase and the B3LYP/6-31(d) levels in the solvent phase for SOR calculation, and the GIAO-DFT at the PCM/mpw1pw91/6-311 + g(d,p) level in the solvent phase for GIAO-NMR DP4+ analysis. The results were averaged by the proportion of each conformer.

4.8 *In vitro* cytotoxic assay

The cytotoxicity assay used in this study was performed as described in previous publications.[16,17]

Author contributions

Hsuan-Jung Tseng: investigation, analysis of results. Liang-Mou Kuo: investigation, analysis of results. Yu-Chi Tsai: investigation, software, modelling and simulation. Hao-Chun Hu: software, modelling and simulation. Po-Jen Chen: data curation, methodology. Su-Ying Chien: formal analysis, X-ray analysis. Jyh-Horng Sheu: conceptualization, supervision, visualization. Ping-Jyun Sung: analysis of results, conceptualization, visualization, supervision, writing-original draft, writing-reviewing and editing.

Conflicts of interest

There are no conflicts to declare.

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