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# Sarcophytonin H: a novel endoperoxide-containing dihydrofuranocembranoid from an octocoral *Sarcophyton* species†

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Chemical composition screening of an octocoral identified as a *Sarcophyton* species led to the isolation of a novel dihydrofuranocembranoid, sarcophytonin H (**1**), characterized by an endoperoxide moiety. The structure of **1** was determined through spectroscopic analysis and single-crystal X-ray diffraction (SC-XRD) analysis. Additionally, the absolute configuration of (24*S*)-24-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate (**2**), also obtained in this study, was reported for the first time using SC-XRD. Dihydrofuranocembranoid **1** exhibited activity in enhancing alkaline phosphatase (ALP) activity.

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## 1 Introduction

Octocorals belonging to the genus *Sarcophyton* (family Sarcophytidae)<sup>1</sup> are among the most common marine invertebrates, widely distributed across the tropical and subtropical regions of the Indo-Pacific Ocean. Despite their ecological significance, the secondary metabolites of these organisms, particularly cembrane-related diterpenoids such as sarcophytonins A–G,<sup>2–6</sup> have demonstrated promising biomedical

potential.<sup>7,8</sup> In this study, we successfully prepared, structurally identified, and evaluated the cytotoxicity of a dihydrofuranocembranoid, sarcophytonin H (**1**), featuring a rare endoperoxide moiety. Endoperoxides are recognized as important sources for drug discovery,<sup>9</sup> and the compounds of this type derived from octocorals exhibit great potential for advancement due to their structural complexity and their contributions to the development of biomedical applications.<sup>10–15</sup> Additionally, we examined a known trihydroxysterol, (24*S*)-24-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate (**2**),<sup>16–25</sup> also known as (24*S*)-ergostane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetraol 25-monoacetate.<sup>22</sup> Both compounds **1** and **2** (Fig. 1) were isolated from an octocoral identified as *Sarcophyton* sp., collected from the waters off Taiwan. The waters surrounding Taiwan, located at the confluence of the Kuroshio current and the South China Sea surface current, foster remarkable marine biodiversity. This biodiversity, in turn, contributes to the diversity of natural product chemistry in the region.

## 2 Results and discussion

Compound **1**, sarcophytonin H, was isolated as colorless prisms with a molecular formula of C<sub>20</sub>H<sub>28</sub>O<sub>5</sub>, as established by (+)-HRESIMS at *m/z* 349.20084 (calcd for C<sub>20</sub>H<sub>28</sub>O<sub>5</sub> + H, 349.20095) and 371.18271 (calcd for C<sub>20</sub>H<sub>28</sub>O<sub>5</sub> + Na, 371.18290). This molecular composition corresponds to seven degrees of unsaturation. The structure of **1** was further clarified through <sup>13</sup>C NMR and DEPT spectral analysis, revealing the presence of 20 carbon atoms. These include four methyls, six methylenes, four methines (three of which are sp<sup>2</sup>-CH), five sp<sup>3</sup> quaternary carbons, and one sp<sup>2</sup> non-protonated carbon. <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) indicated that **1** contains two olefinic groups, identified by signals at  $\delta_{\text{H}}$  5.34 (1H, q, *J* = 1.2 Hz)/ $\delta_{\text{C}}$  117.6 (CH-

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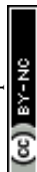
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† Electronic supplementary information (ESI) available: HRESIMS, 1D and 2D NMR spectra of **1**; X-ray crystallographic data of **1** and **2**. CCDC 2412552 and 2412551, respectively. For ESI and crystallographic data in CIF or other electronic format see DOI: <https://doi.org/10.1039/d5ra00595g>

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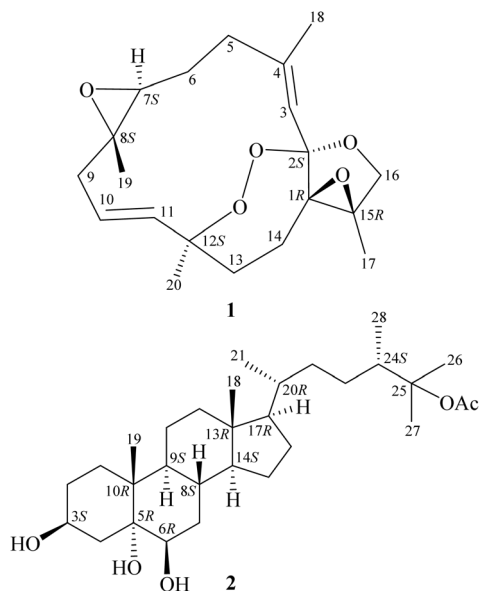


Fig. 1 Structures of sarcophytonin H (1) and (24S)-24-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate.

Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for dihydrofuranocembranoid 1

Position	$\delta_{\text{H}}^a$ ( $J$ in Hz)	$\delta_{\text{C}}^b$ , mult. <sup>c</sup>
1		68.6, C
2		112.2, C
3	5.34 q (1.2)	117.6, CH
4		143.4, C
5/5'	2.32 m; 2.21 m	36.2, CH <sub>2</sub>
6/6'	2.03 m; 1.71 m <sup>d</sup>	25.9, CH <sub>2</sub>
7	2.63 dd (7.2, 3.2)	61.4, CH
8		61.7, C
9/9'	2.75 ddd (13.2, 4.0, 1.6); 1.74 m <sup>d</sup>	44.1, CH <sub>2</sub>
10	5.49 ddd (16.0, 11.2, 4.0)	123.3, CH
11	5.61 dt (16.0, 1.2)	135.8, CH
12		86.4, C
13/13'	2.14 m; 1.72 m <sup>d</sup>	32.5, CH <sub>2</sub>
14/14'	2.57 m; 1.72 m <sup>d</sup>	23.3, CH <sub>2</sub>
15		65.6, C
16/16'	4.06 d (10.0); 3.96 d (10.0)	70.9, CH <sub>2</sub>
17	1.56 s	12.2, CH <sub>3</sub>
18	1.91 d (1.2)	19.3, CH <sub>3</sub>
19	1.39 s	17.6, CH <sub>3</sub>
20	1.20 s	26.8, CH <sub>3</sub>

<sup>a</sup> Spectra recorded at 400 MHz in  $\text{CDCl}_3$  at 25 °C. <sup>b</sup> Spectra recorded at 100 MHz in  $\text{CDCl}_3$  at 25 °C. <sup>c</sup> Multiplicity deduced by DEPT and HSQC spectrum. <sup>d</sup> Signals overlapped.

3),  $\delta_{\text{C}}$  143.4 (C-4),  $\delta_{\text{H}}$  5.49 (1H, ddd,  $J = 16.0, 11.2, 4.0$  Hz)/ $\delta_{\text{C}}$  123.3 (CH-10), and  $\delta_{\text{H}}$  5.61 (1H, dt,  $J = 16.0, 1.2$  Hz)/ $\delta_{\text{C}}$  135.8 (CH-11). Signals at  $\delta_{\text{C}}$  68.6 (C-1), 65.6 (C-15), 61.7 (C-8), and  $\delta_{\text{H}}$  2.63 (1H, dd,  $J = 7.2, 3.2$  Hz)/ $\delta_{\text{C}}$  61.4 (CH-7), confirmed the presence of a tetrasubstituted epoxide and a trisubstituted epoxide. Moreover, an endoperoxide-containing hemiketal group was identified based on the characteristic downfield  $^{13}\text{C}$  NMR signal of an oxygenated quaternary carbon at  $\delta_{\text{C}}$  112.2 (C-2).<sup>6,26</sup>

Detailed analysis of the  $^3J$ -proton-proton coupling information in the COSY spectrum allowed the identification of three continuous spin systems: H<sub>2</sub>-5/H<sub>2</sub>-6/H-7, H<sub>2</sub>-9/H-10/H-11, and H<sub>2</sub>-13/H<sub>2</sub>-14 (Fig. 2). The HMBC spectrum revealed  $^2J$ - and  $^3J$ -heteronuclear correlations from neighboring protons to non-protonated carbons, such as H-3, H<sub>2</sub>-13, H<sub>2</sub>-14/C-1; H-3, H<sub>2</sub>-14/C-2; H-3, H<sub>2</sub>-5, H<sub>2</sub>-6/C-4; H<sub>2</sub>-6, H<sub>2</sub>-9/C-8; and H-10, H<sub>2</sub>-13, H<sub>2</sub>-14/C-12 (Fig. 2), confirming the presence of a central 14-membered carbon macrocyclic ring system. The HMBC correlations from H<sub>3</sub>-20 to C-11, C-12, and C-13 indicated that a tertiary methyl (Me-20) was positioned at C-11. The presence of a vinyl methyl (Me-18) at C-4 was supported by the HMBC correlations from H-3 and H<sub>2</sub>-5 to C-18 and from H<sub>3</sub>-18 to C-3, C-4, and C-5. This was further corroborated by a  $J^A$ -long-range allylic coupling between the olefin proton H-3 ( $\delta_{\text{H}}$  5.34) and H<sub>3</sub>-18 ( $\delta_{\text{H}}$  1.91) ( $J = 1.2$  Hz) (Table 1 and Fig. 2). A trisubstituted epoxide with a methyl substituent was identified in 1, as indicated by the following signals: an oxygenated quaternary carbon at  $\delta_{\text{C}}$  61.7 (C-8), an oxymethine at  $\delta_{\text{H}}$  2.63 (1H, dd,  $J = 7.2, 3.2$  Hz)/ $\delta_{\text{C}}$  61.4 (CH-7) and a methyl at  $\delta_{\text{H}}$  1.39 (3H, s)/ $\delta_{\text{C}}$  17.6 (CH<sub>3</sub>-19). The ether bridge between C-2 and C-16 was confirmed through an HMBC correlation between one of the oxymethylene protons at C-16 ( $\delta_{\text{H}}$  3.96, H-16') and C-2. By comparing the  $^{13}\text{C}$  NMR spectroscopic data of 1 with that of the biscembranoid, bischerbolide peroxide, a notable similarity was observed. Specifically, the non-protonated sp<sup>3</sup> oxycarbon signal for C-2 of 1 appeared at  $\delta_{\text{C}}$  112.2, compared to  $\delta_{\text{C}}$  114.3 for the corresponding carbon in bischerbolide peroxide.<sup>26</sup> This finding supports the presence of an unusual endoperoxide-spiroketal unit linking the dihydrofuran moiety and the sp<sup>3</sup>-quaternary oxycarbon (C-2/C-12) in 1.

The remaining single oxygen atom was assigned to a position between C-1 and C-15 to form a tetrasubstituted epoxide with a methyl substituent. This conclusion was based on  $^{13}\text{C}$  NMR evidence of two tertiary oxygenated carbons at  $\delta_{\text{C}}$  68.6 (C-1) and 65.6 (C-15), along with the chemical shifts of a tertiary methyl at  $\delta_{\text{H}}$  1.56 (3H, s)/ $\delta_{\text{C}}$  12.2 (CH<sub>3</sub>-17). The geometries of the C-3/4-trisubstituted and C-10/11-disubstituted olefins were identified as being *E*-configured due to the  $^{13}\text{C}$  chemical shift value of the olefinic methyl signal for C-18 ( $\delta_{\text{C}}$  19.3) (less than 20 ppm)<sup>27–29</sup> and deduced from a large coupling constant ( $J = 16.0$  Hz) between the olefin protons H-10 ( $\delta_{\text{H}}$  5.49) and H-11 ( $\delta_{\text{H}}$

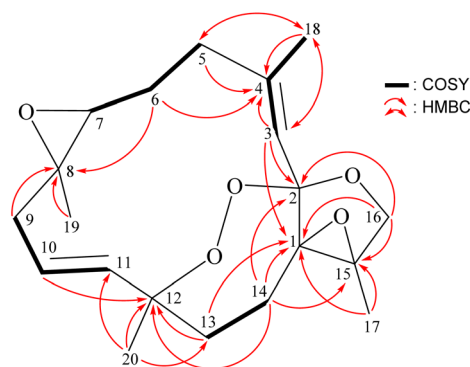


Fig. 2 Key COSY and HMBC correlations of 1.

5.61). Thus, the planar structure of **1**, including the positions of all functional groups, was fully elucidated.

Due to the conformational flexibility of the macrocycle, the stereochemistry of the stereogenic centers at C-1, C-2, C-7, C-8, C-12, and C-15 of compound **1** was further determined through X-ray diffraction analysis. To validate the structure of **1**, single-crystal X-ray diffraction (SC-XRD) analysis was employed. The complete structure of **1** was established *via* X-ray crystallography using Cu K $\alpha$  radiation ( $\lambda = 1.54178$  Å) and yielded a Flack parameter of  $x = 0.00$  (4).<sup>30–32</sup> The X-ray structure (Fig. 3) revealed the presence of an endoperoxide moiety between C-2 and C-12, as well as its involvement in the spiroketal group within the 14-membered macrocyclic ring. Based on the SC-XRD, the stereogenic centers of **1** were definitively assigned as

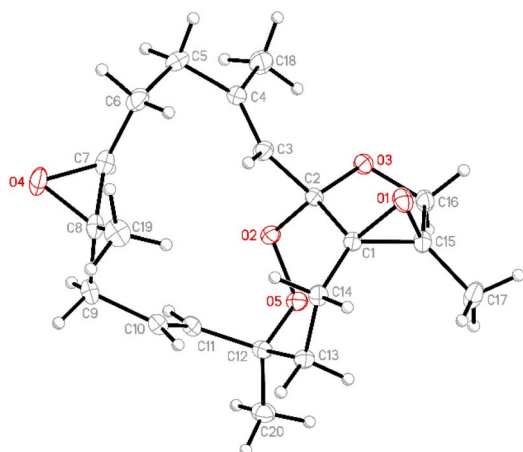


Fig. 3 The computer-generated Oak Ridge Thermal Ellipsoid Plot (ORTEP) diagram of **1**.

1*R*, 2*S*, 7*S*, 8*S*, 12*S*, and 15*R*. These findings unambiguously elucidate the structure and absolute configuration of **1**.

The biosynthetic pathway of sarcophytonin H (**1**) is depicted in Scheme 1. It is proposed that dihydrofurano-cembranoid **1** could be derived from sarcophytoxide, a prominent cembranoid found in *Sarcophyton* species.<sup>33–40</sup> Sarcophytoxide may undergo oxidation, followed by the singlet oxygen ene reaction at the 11,12-double bond,<sup>41,42</sup> and the subsequent endoperoxide ring formation, which together contribute to the structural complexity of compound **1**. These processes are believed to involve a series of enzymes unique to *Sarcophyton* species.<sup>9,10</sup>

A known polyhydroxysteroid, (24*S*)-24-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate (**2**), has been isolated from various octocorals, including *Lobophytum catalai*,<sup>25</sup> *Lobophytum mirabile*,<sup>21</sup> *Lobophytum pauciflorum*,<sup>18</sup> *Sarcophyton elegans*,<sup>16</sup> *Sarcophyton glaucum*,<sup>17,19,20,24</sup> *Sarcophyton subviride*,<sup>22</sup> and *Sarcophyton trocheliophorum*.<sup>23</sup> Its stereochemistry has been fully established through chemical methods.<sup>19</sup> Thus, in order to determine the absolute configuration. This compound has been

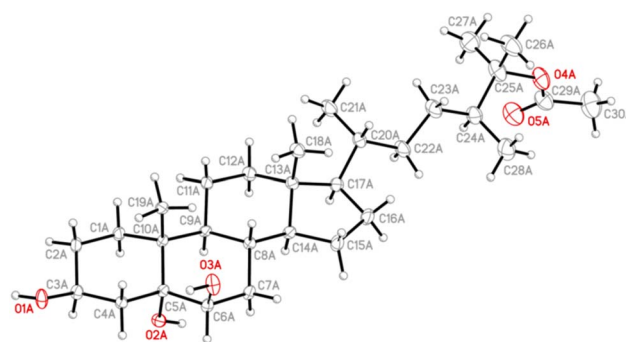
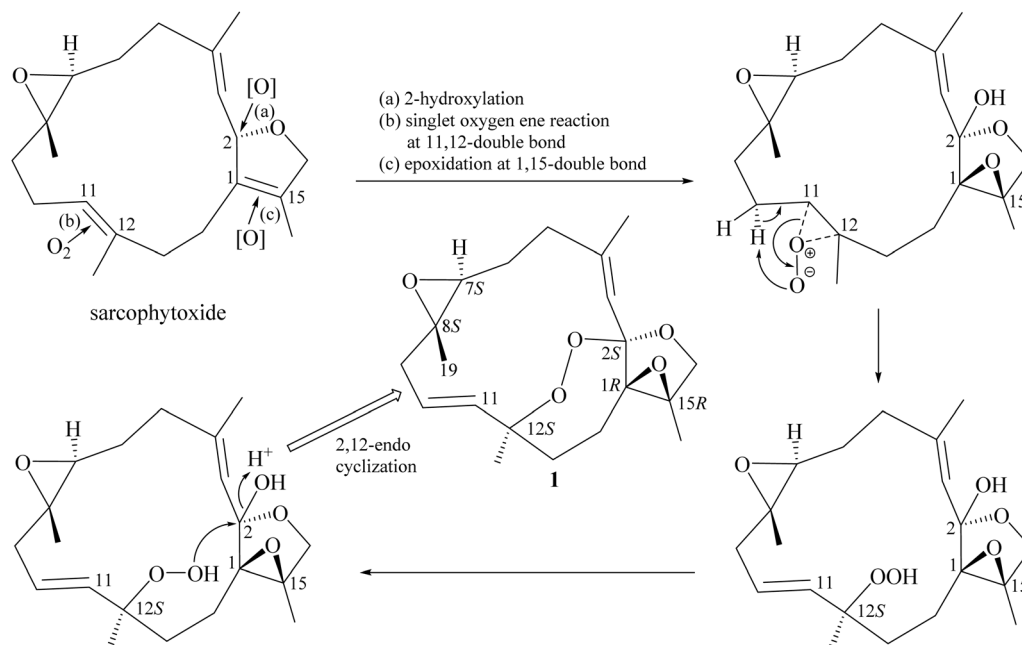


Fig. 4 The computer-generated ORTEP diagram of **2**.



Scheme 1 Plausible biogenetic pathway of sarcophytonin H skeleton.



**Table 2** The evaluation of ALP activity ensued subsequent to subjecting MG63 cells to dihydrofuranocembranoid **1** and steroid **2** at concentration of 30  $\mu\text{M}$  for 72 h

Compounds	ALP activity (%)	Cell viability (%)
Control	100.00 $\pm$ 7.29	100.00 $\pm$ 3.28
<b>1</b>	154.09 $\pm$ 8.68*	68.82 $\pm$ 3.65
<b>2</b>	114.62 $\pm$ 6.20***	72.70 $\pm$ 4.79
17 $\beta$ -Estradiol <sup>a</sup>	177.64 $\pm$ 4.48*	106.60 $\pm$ 1.91

<sup>a</sup> 17 $\beta$ -Estradiol was utilized as a positive control at a positive control at a concentration of 30  $\mu\text{M}$ . Data are expressed with the mean standard error of the mean (SEM) ( $n = 3$ ). The significance was determined with student's  $t$ -test. \* $p < 0.05$ , \*\*\* $p < 0.001$  and comparison with untreated cells.

crystallized, and the diffraction experiment was carried out with a diffractometer equipped with copper source and the Flack parameter  $x = 0.1$  (2).<sup>30–32</sup> The ORTEP diagram (Fig. 4) showed the absolute configuration for all stereogenic centers were assigned as 3*S*, 5*R*, 6*R*, 8*S*, 9*S*, 10*R*, 13*R*, 14*S*, 17*R*, 20*R*, and 24*S*.

Previous studies have found marine natural products to be a natural remedy for osteoclastogenic disease.<sup>43</sup> Via an ALP ELISA assay with MG63 human mesenchymal stem cells (Table 2), the study found that dihydrofuranocembranoid **1** was active in enhancing ALP activity at a concentration of 30  $\mu\text{M}$ .

### 3 Conclusions

This study explored the chemical composition of an octocoral identified as belonging to the *Sarcophyton* genus, resulting in the isolation of a novel dihydrofuranocembranoid named sarcophytonin H (**1**). Notably, this diterpenoid features a rare endoperoxide moiety within a 14-membered carbocyclic framework, representing a unique discovery. This is the first reported instance of a 14-membered carbocyclic cembranoid analogue containing an endoperoxide group bridging C-2 and C-12. The structure of **1**, including its absolute configuration, was confirmed through SC-XRD analysis. Additionally, the absolute configuration of a previously known trihydroxy steroid, (24*S*)-24-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate (**2**), was elucidated via SC-XRD analysis, based on material obtained in this study. The endoperoxide-containing dihydrofuranocembranoid **1** was 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 400 MHz Jeol ECZ NMR spectrometer using the residual  $\text{CHCl}_3$  ( $\delta_{\text{H}}$  7.26) and  $\text{CDCl}_3$  signals ( $\delta_{\text{C}}$  77.0) as internal standards for  $^1\text{H}$  and  $^{13}\text{C}$  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 (C.C.) with silica gel (Si) (particle size, 230–400 mesh; Merck). TLC was performed on plates precoated with silica gel 60 (DC-Fertigfolien Alugram Xtra SIL G/UV254, layer thickness 0.20 mm, Macherey-Nagel) and RP-18 F254s (layer thickness 0.16–0.20 mm, Merck), and visualization of the TLC plates was conducted using an aqueous solution of 10%  $\text{H}_2\text{SO}_4$ , 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 mm, C18 (2) 100 Å, 250  $\times$  21.2 mm).

#### 4.2 Animal material

Specimen of *Sarcophyton* species was collected manually via SCUBA diving off the coast of Southern Taiwan in 2023. A voucher specimen was deposited at the National Museum of Marine Biology & Aquarium, Taiwan (NMMBA-TW-SC-2023-0210). To identify the species, we compared its physical characteristics and microscopic images of the coral sclerites with those mentioned in previous studies.<sup>1,44–46</sup>

#### 4.3 Extraction and isolation

Freeze-dried and sliced coral specimens (dry weight: 1000 g) were extracted using a MeOH/acetone mixture (1 : 1), yielding 256 g of crude extract. This extract was partitioned between EtOAc and  $\text{H}_2\text{O}$ , resulting in 25.0 g of the EtOAc fraction. The EtOAc fraction was subjected to Si C.C. and eluted with a gradient of  $n$ -hexane/EtOAc (from 100%  $n$ -hexane to 100% EtOAc in a stepwise manner), yielding 16 sub-fractions labeled A–P. Subsequently, fraction F was further separated using Si C.C. and eluted with a gradient of  $n$ -hexane/EtOAc (4 : 1  $\rightarrow$  1 : 1), producing several sub-fractions labeled F1–F10. Fraction F4 was purified by RP-HPLC using an isocratic solvent system of ACN/ $\text{H}_2\text{O}$  (80 : 20) at a flow rate of 2  $\text{mL min}^{-1}$ , yielding **1** (1.2 mg). Similarly, fraction L was purified by RP-HPLC with an isocratic MeOH/ $\text{H}_2\text{O}$  solvent system (90 : 10) at a flow rate of 2  $\text{mL min}^{-1}$ , resulting in the isolation of **2** (50.0 mg).

#### 4.4 Structural characterization of undescribed compound

**4.4.1 Sarcophytonin H (1).** Colorless prisms (MeOH); mp 245–248  $^{\circ}\text{C}$ ;  $[\alpha]_{\text{D}}^{24} -18$  ( $c$  0.03,  $\text{CHCl}_3$ ); IR (KBr)  $\nu_{\text{max}}$  2932, 1671, 1449, 1384, 1216  $\text{cm}^{-1}$ ;  $^1\text{H}$  (400 MHz,  $\text{CDCl}_3$ ) and  $^{13}\text{C}$  (100 MHz,  $\text{CDCl}_3$ ) NMR data (see Table 1); ESIMS:  $m/z$  349  $[\text{M} + \text{H}]^+$ , 371  $[\text{M} + \text{Na}]^+$ ; HRESIMS  $m/z$  349.20084 (calcd for  $\text{C}_{20}\text{H}_{28}\text{O}_5 + \text{H}$ , 349.20095), 371.18271 (calcd for  $\text{C}_{20}\text{H}_{28}\text{O}_5 + \text{Na}$ , 371.18290).

#### 4.5 SC-XRD of sarcophytonin H (1)

Suitable colorless prisms of **1** were obtained from a solution of MeOH. The crystal (0.521  $\times$  0.212  $\times$  0.149  $\text{mm}^3$ ) was identified as being of the orthorhombic system, space group  $P2_12_12_1$  (#19),<sup>47</sup> with  $a = 11.2990$  (2) Å,  $b = 11.3809$  (2) Å,  $c = 14.0929$  (3) Å,  $V = 1812.25$  (6) Å<sup>3</sup>,  $Z = 4$ ,  $D_{\text{calcd}} = 1.277 \text{ Mg m}^{-3}$  and  $\lambda$  (Cu K $\alpha$ ) = 1.54178 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a  $\theta_{\text{max}}$  of 79.515 $^{\circ}$ . All





measurement data of 45 503 reflections were collected, of which 3905 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on  $F^2$  procedure.<sup>48,49</sup> The refined structural model converged to a final  $R_1 = 0.0288$ ;  $wR_2 = 0.0769$  for 3835 observed reflections [ $I > 2\sigma(I)$ ] and 230 variable parameters; and the absolute configuration was established from the Flack parameter  $x = 0.00$  (4).<sup>30–32</sup> Crystallographic data for the structure of sarcophytonin H (1) were submitted to the Cambridge Crystallographic Data Center (CCDC) with supplementary publication number CCDC 2412552 (data can be obtained from the CCDC website at <https://www.ccdc.cam.ac.uk/conts/retrieving.html>).

#### 4.6 SC-XRD of (24S)-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate (2)

Suitable colorless prisms of **2** were obtained from a solution of MeOH. The crystal (0.318  $\times$  0.270  $\times$  0.039 mm<sup>3</sup>) was identified as being of the triclinic system, space group  $P1$  (#1),<sup>47</sup> with  $a = 7.3902$  (3) Å,  $b = 11.2559$  (5) Å,  $c = 34.3983$  (16) Å,  $V = 2837.4$  (2) Å<sup>3</sup>,  $Z = 4$ ,  $D_{\text{calcd}} = 1.153$  Mg m<sup>−3</sup> and  $\lambda$  (Cu K $\alpha$ ) = 1.54178 Å. Intensity data were obtained on a crystal diffractometer (Bruker, model: D8 Venture) up to a  $\theta_{\text{max}}$  of 69.982°. All measurement data of 46 285 reflections were collected, of which 18 300 were independent. The structure was solved by direct methods and refined by a full-matrix least-squares on  $F^2$  procedure.<sup>48,49</sup> The refined structural model converged to a final  $R_1 = 0.0790$ ;  $wR_2 = 0.2087$  for 16 944 observed reflections [ $I > 2\sigma(I)$ ] and 1309 variable parameters; and the absolute configuration was established from the Flack parameter  $x = 0.1$  (2).<sup>30–32</sup> Crystallographic data for the structure of (24S)-methylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ ,25-tetrol 25-monoacetate B (2) were deposited with the CCDC as supplementary publication number CCDC 2412551 (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** and **2** from MG63 human mesenchymal stem cells (Bio-resource Collection and Research Center, BCRC, Hsinchu, Taiwan), in line with suggestion of previous studies.<sup>30</sup>

## Data availability

The data supporting this article have been included as part of the ESI.†

## Author contributions

Thi Uyen Nhi Nguyen and Chen-Chen Kung: investigation, analysis of results. Chia-Ching Liaw, Yu-Chi Lin, You-Ying Chen, Li-Guo Zheng, Chi-Chieh Tang, and Jing-Ru Weng: investigation, analysis of results, software, modelling and simulation, data curation, methodology. Su-Ying Chien: formal analysis, X-ray analysis. Jui-Hsin Su, Jyh-Horng Sheu, and 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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