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Unprecedented Diterpenoids as a PTP1B Inhibitor from the Hainan Soft Coral *Sarcophyton trocheliophorum* Marenzeller

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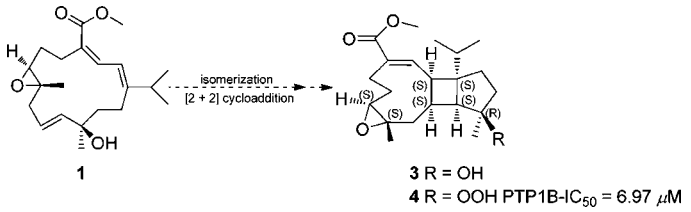
ABSTRACT



Methyl sarcotroates A and B (3 and 4), two unprecedented diterpenoids possessing a tetradecahydrocyclopenta[3′,4′]cyclobuta[1′,2′:4,5]-cyclonona[1,2-b]oxirene ring system, along with their probable biogenetic precursor, sarcophytonolide M (1), were isolated from the Hainan soft coral *Sarcophyton trocheliophorum*. Their structures were elucidated by detailed spectroscopic analysis, and the absolute configuration of compound 3 was determined by TDDFT ECD calculations. Compound 4 exhibited significant inhibitory activity against protein tyrosine phosphatase 1B (PTP1B), being similar to that of positive control oleanolic acid.

Soft corals of the genus *Sarcophyton* (order Alcyonacea, family Alcyoniidae) are known to be a rich source of diterpenes with intriguing structural features. These metabolites can be roughtly casssified into three large groups due to their chemical correlations: diterpenoids derived from cembrane, lobane, and perhydrophenanthrene, by dimerization, cyclic addition, ring clevage, or ring arrangement. These diterpenes exhibited a wide spectrum of biological activities, including neuroprotective, ichthyotoxic, cytotoxic, antiviral, antifouling, and anti-inflammatory properties.[1]

In the course of our researching for biologically active substances from marine sources,[2] we had chemically investigated four specices of the soft coral of genus *Sarcophyton*, namely *S. trocheliophorum*,[2d] *S. glaucum*,[3]

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S. latum,[4] and *S. tortuosum*,[5] leading to the isolation and structural elucidation of a series of cembrane diterpenes and biscembranes with an unprecedented carbon skeleton. Our reinvestigation on the extract of the title animal has now resulted in the discovery of two unprecedented diterpenes, methyl sarcotroates A and B (**3** and **4**), together with a new cembrane, sarcophytonolide M (**1**) (Figure 1). The structures of the new compounds were elucidated on the basis of detailed spectroscopic analysis. The absolute configuration of compound **3** was determined by TDDFT ECD calculations, leading to the absolute configurations of **1** and **4** to be determined by a biogenetic correlation and ECD comparison, respectively. In a bioassay in vitro, compound **4** exhibited significant inhibitory activity against PTP1B, a key target for the treatment of Type-II diabetes and obesity,[6] being similar to that of positive control oleanolic acid. This paper describes the isolation, structure elucidation, and bioactivity of the new compounds. A biogenetic relationship of these compounds was proposed, suggesting sarcophytonolide M (**1**) to be a precusor to methyl sarcotroates A and B (**3** and **4**).

The frozen animals of *S. trocheliophorum* were cut into pieces and extracted exhaustively with acetone at room temperature. The Et_2O-soluble portion of acetone extract was subjected to repeated column chromatography on silica gel, Sephadex LH-20, and RP-HPLC to yield pure diterpenes **1**, **3**, and **4**.

Sarcophytonolide M (**1**)[7] was obtained as an optically active, colorless oil. The molecular formula of $C_{21}H_{32}O_4$ was deduced from HRESIMS. The ^1H and ^{13}C NMR spectra of **1** were closely resembled to those of sarcophytonolide A (**2**) (Table S1, Supporting Information),[5a] a cembranolide obtained from the Hainan soft coral *S. latum* by our group. A major difference was recognized for signals assigned to C-9–C-13 (Figure 1). The presence of a *trans*-disubstituted double bond in **1** was clearly indicated by the diagnostic downfield ^1H NMR signals and coupling patterns (δ 5.41, ddd, J = 15.5, 10.4, 3.2 Hz and 5.30, dd, J = 15.5, 2.0 Hz). The location of the double bond at Δ^{10} was deduced from the proton sequence of H_2-9/H-10/H-11 as established by $^1H-^1H$ COSY experiment. The HMBC correlations from H_3-20 to C-11, C-12, and C-13 (Figure S22, Supporting Information) confirmed the NMR assignment and consequently led the oxgenated tertiary carbon (δ_C 72.5) at C-12. An α configuration of Me-20 was deduced from the NOE effects between H_3-20 and H-10, and between H_3-19 and H-11, leading the determination of structure **1**.

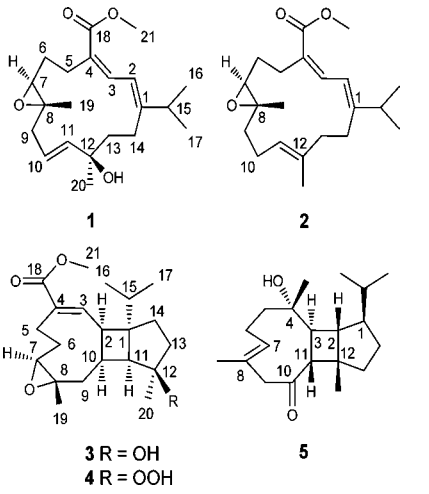

Figure 1. Structures of compounds **1**–**5**.

Methyl sarcotroate A (**3**)[8] was isolated as an optically active, colorless oil. Its molecular formula was established as $C_{21}H_{32}O_4$ by HRESIMS, indicating six degrees of unsaturation in the molecule. The IR spectrum showed the presence of hydroxyl (3528 cm^{-1}), epoxy (1252 and 850 cm^{-1}), and α,β-unsaturated ester (1715 cm^{-1}) groups.[9] This observation was in agreement with the presence of signals for two tertiary oxygenated carbon atoms (δ 59.5 and 79.4), a secondary oxygenated carbon atom (δ 65.6), a trisubstituent double bond (δ 143.6, CH; 128.6, C), and a ester carbonyl atom (δ 168.4, C) in the ^{13}C NMR spectrum (Table S1, Supporting Information), accounting for three degrees of unsaturation. The remaining three degrees of unsaturation were due to the presence of three rings in the molecule.

Analysis of the $^1H-^1H$ COSY spectrum of **3** readily revealed four proton connectivities as shown in Figure 2 for **a** from H-3 to H-11 and H_2-9, **b** from H_2-5 to H-7, **c** from H_2-13 to H_2-14, and **d** from H_3-16 to H_3-17. On the basis of an HMBC experiment, the four fragments could be fully connected by inserting the "loose ends" of the tertiary and quaternary carbon atoms of C-1, C-4, C-8, C-12, and C-18. The diagnostic HMBC correlation from H-15 to C-1, C-2, C-11, and C-14 was in agreement with those from H_2-14 to C-1, C-2, C-11, and C-15, leading the fromation of the four-membered ring by the lingkage of fragment **d** to partial structures **a** and **c** through the quaternary carbon C-1. The distinct HMBC correlation from H_3-20 to C-11, C-12, and C-13 resulted in the formation of the five-membered ring by connection of partial structures **a**

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(7) Sarcophytonolide M (**1**): colorless oil; $[\alpha]^{24}_D$ +253 (*c* 0.10, $CHCl_3$); ECD (CH_3CN, $c = 1.7 \times 10^{-3}$) λ_{max} ($\Delta\varepsilon$) 284 (0.47), 216 (−0.13); UV (MeOH) λ_{max} = 282 nm (log ε 3.86); IR (KBr) ν_{max} cm^{-1} 3509, 2950, 1693, 1642, 1445, 1260, 1063; ^1H and ^{13}C NMR data, see Table S1 (Supporting Information); ESIMS *m*/*z* 371.3 $[M + Na]^+$; HRESIMS *m*/*z* 371.2202 ($[M + Na]^+$, calcd for $C_{21}H_{32}O_4Na$ 371.2198).

(8) Methyl sarcotroate A (**3**): colorless oil; $[\alpha]^{24}_D$ +54 (*c* 0.12, $CHCl_3$); ECD (CH_3CN, $c = 3.8 \times 10^{-4}$) λ_{max} ($\Delta\varepsilon$) 282 (−0.16), 232 (4.36); IR (KBr) ν_{max} cm^{-1} 3528, 2952, 1715, 1640, 1437, 1266, 1252, 1070, 850; ^1H and ^{13}C NMR data, see Table S1 (Supporting Information); ESIMS *m*/*z* 371.3 $[M + Na]^+$; HRESIMS *m*/*z* 371.2229 ($[M + Na]^+$, calcd for $C_{21}H_{32}O_4Na$ 371.2198).

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and **c** with carbon C-12 bound by a tertiary hydroxyl group. Partial fragment **a** was found to be linked to **b** by the tertiary-oxygenated carbon C-8 and the quaternary carbon C-4 as deduced from the significant HMBC correlations of H_3-19 with C-7, C-8, and C-9, and of both H-3 and H_2-5 with C-4 and C-18, respectively. Finally, the clear HMBC correlation between H_3-21 and C-18 established the planar structure of **3** to be a diterpenoic acid methyl ester with a tetradecahydrocyclopenta[3′,4′]cyclobuta-[1′,2′:4,5]cyclonona[1,2-b]oxirene ring system.

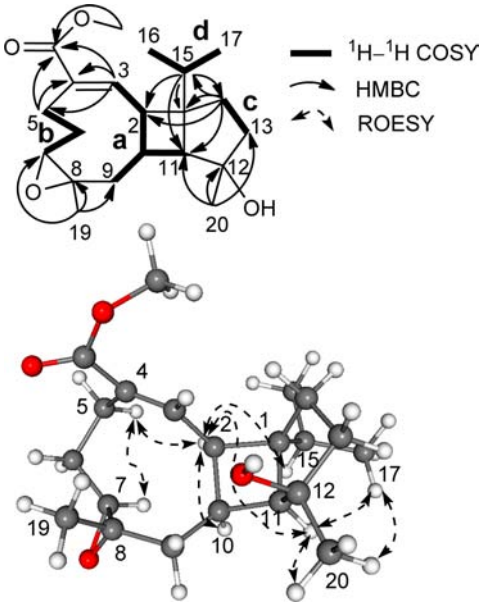

Figure 2. ^{1}H–^{1}H COSY and selected key HMBC and ROESY correlations of compound **3**.

The relative configuration of **3** was determined by a NOE experiment in combination with the analysis of the ^{1}H–^{1}H coupling constant, aided by conformational analysis (Figure 2). As shown on the lowest energy conformer (48.9%) of **3**, clear NOE effects between H-10 and H-2 and H-11, and between H-15 and H-2, H-11, and H_3-20 observed in the NOEDIFF spectra indicated a α configuration of all the protons. The *cis* orientation of H-10 and H-11 in the cyclobutane ring was further confirmed by their coupling constant (^3J = 5.9 Hz) with respect to that of providencin,[10] showing ^3J values of 6.1 and 10.0 Hz for *cis* and *trans* lingkage, respectively. A *trans* configuration for H-7 and H_3-19 in the trisubstituted epoxide was deduced from the ^{13}C NMR shift value of C-19 (δ_C 18.1, < 20 ppm)[4d,5a,9b] and supported by the lack of NOE effect in ROESY experiment. The diagnostic NOE effects between H-5α and H-2 and H-7 not only indicated a *E* geometry of Δ^3 double bond, but also correlated the α configuration of H-2 to H-7. The relative structure of **3** was thus determined.

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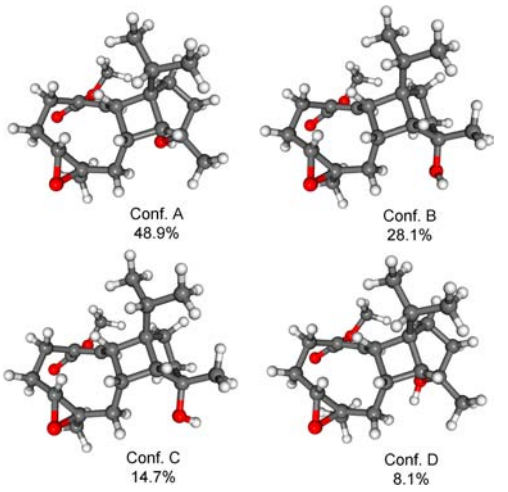


Figure 3. DFT-optimized solution conformers of (1*R*,2*R*,7*R*,-8*R*,10*R*,11*R*,12*S*)-**3** and their populations.

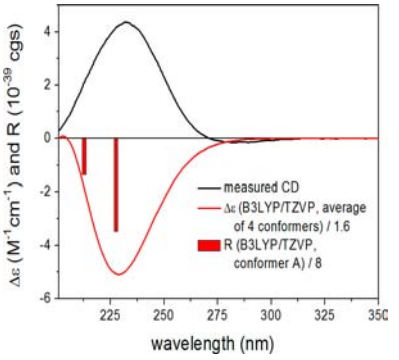


Figure 4. Experimental CD spectrum of **3** in methanol compared with the B3LYP/TZVP spectrum calculated for the (1*R*,2*R*,7*R*,-8*R*,10*R*,11*R*,12*S*) enantiomer.

The absolute configuration of **3** was determined by TDDFT ECD calculations of its solution conformers. The ECD spectrum of **3** showed an intense positive CE at 232 nm ($\Delta\varepsilon$ = 4.36) governed by the inherently chiral α,β-unsaturated ester chromophore. The initial MMFF conformational search of the arbitrarily chosen (1*R*,2*R*,7*R*,8*R*,10*R*,11*R*,12*S*)-**3** resulted in 11 conformers within the 21 kJ/mol energy window, the DFT reoptimization of which afforded four conformers above 0.3% population (Figure 3). Interestingly, the conformation of the cyclononane ring was found the same in all the four conformers and they only differred in the conformation of the cyclopentane ring. The 12-OH had pseudo-*axial* orientation in conformers A and D, and pseudo-*equatorial* one in B and C. The interatomic distances observed in conformer A corroborated well the measured NOE effects. The $\omega_{C\text{-}3,C\text{-}4,C\text{-}18,O}$ dihedral angle, the determinant geometrical parameter of ECD spectrum, was found to be similar (in the range of +133−136°) in all four conformers.

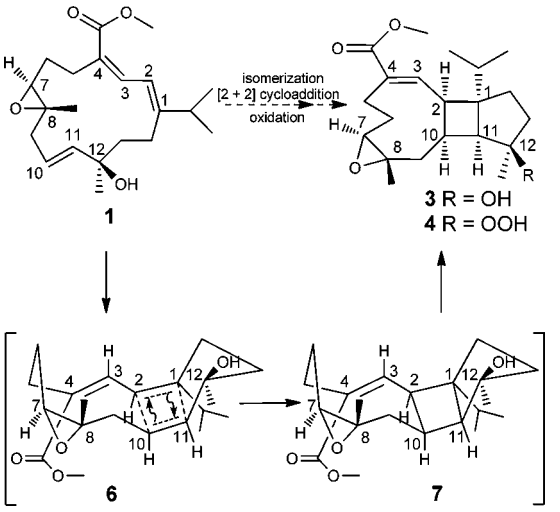
The Boltzmann-averaged B3LYP/TZVP ECD spectrum calculated for the (1*R*,2*R*,7*R*,8*R*,10*R*,11*R*,12*S*) absolute configuration of **3** was found to be the near-mirror image of the experimental curve (Figure 4), which determined the absolute configuration of (+)-**3** as (1*S*,2*S*,7*S*,8*S*,10*S*,11*S*,12*R*).

Methyl sarcotroate B (**4**)[11] was also obtained as a optically active, colorless oil. The molecular formula of $C_{21}H_{32}O_5$, as deduced from HRESIMS, indicated that it contains one more oxygen atom compared to **3**. A positive reaction in the starch test (KI/AcOH) corroborated **4** to be a peroxide.[12] ^{1}H and ^{13}C NMR data of **4** were almost identical to those of **3** (Table S1, Supporting Information) except the ^{13}C NMR for C-12 was downfield shifted from δ 79.4 in **3** to δ 81.5 in **4**, indicating the replacement of the 12-OH in **3** by an OOH group in **4**. This assignment was supported by the observation of obvious upfield-shifted carbon value for C-20 (δ 28.3 in **3**, 22.3 in **4**) and further confirmed by the distinct long-range correlations from H_3-20 to C-11, C-12, and C-13 in the HMBC spectrum. The relative configuration at all of the chiral centers in **4** were proven to be the same as that of **3** due to the same NOE patterns in both compounds. The absolute configuration of **4** was also the same as that of **3** by comparing its ECD spectrum with that of **3** (Figure S23, Supporting Information).

The isolation of sarcophytonolide M (**1**) and methyl sarcotroates A and B (**3** and **4**) demonstrates the productivity of the soft coral, extending the family of marine deterpenoids by a new carbon skeleton. The core ring system of **3** and **4** is correlated to that of sarcoglane (**5**),[9a] a diterpene isolated from the soft coral *S. glaucum* with the absolute configuration being not determined yet. However, there is no easy way to explain the biogenetic origin of **3** and **4** by analogy with that of **5**. In fact, methyl sarcotroates A and B (**3** and **4**) are structurally related to the coisolated sarcophytonolide M (**1**) which may act as a biogenetic precursor as depicted in the proposed hypothetical pathway in Scheme 1.

The isomerization of Δ^{10} in **1** will give the intermediate structure **6**. The following formal coupling of Δ^1 and Δ^{10} in **6** through a Diels–Alder *endo*-cycloaddition may generate the tricyclic terpenoid **7** which will produce the derivative **3** by isomerization of Δ^3. A subsequent oxidation of **3** may give the analog **4**. Obviously, methyl sarcotroates A (**3**) and B (**4**) may be formally derived from sarcophytonolide M

Scheme 1. Possible Biosynthetic Pathway of Compounds **3** and **4**



(**1**) by a intramolecular [2 + 2] cycloaddition. Therefore, it is reasonable to propose the same absolute configurations for the corresponding chirality centers of **1** based on the biogenetic consideration. To the best of our knowledge, until now, only one marine diterpenoid, namely plumisclerin A,[13] was once reported to be originated by involving an intramolecular [2 + 2] cycloaddition, and the viability of thermal [2 + 2] processes for formation of plumisclerin A was theoretically assessed by Tantillo and co-workers.[14]

The isolates were tested in vitro for inhibitory activity against PTP1B. In the bioassay, only compound **4** exhibited potent inhibitory activity (IC_{50} = 6.97 μM), being similar to that of positive control oleanolic acid (IC_{50} = 2.56 μM), whereas **1** and **3** were inactive. This is the first report of a natural PTP1B inhibitor containing a hydroperoxide group. The result is in agreement with that reported by Davies and co-workers,[15] that PTP1B may be inactivated by the reaction of the hydroperoxide with its conserved active-site Cys residue.

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Supporting Information Available. Experimental procedures and full spetral data of sarcophytonolide M (**1**) and methyl sarcotroates A and B (**3** and **4**). This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(11) Methyl sarcotroate B (**4**): colorless oil; $[\alpha]^{24}_D$ +224 (*c* 0.12, $CHCl_3$); ECD (CH_3CN, $c = 7.22 \times 10^{-4}$) λ_{max} ($\Delta\varepsilon$) 334 (−0.27), 289 (1.17), 233 (11.26); IR (KBr) ν_{max} cm^{-1} 3403, 2948, 1713, 1638, 1439, 1262, 1058, 852; ^{1}H and ^{13}C NMR data, see Table S1 (Supporting Information); ESIMS *m*/*z* 387.2 [M + Na]$^+$; HRESIMS *m*/*z* 387.2165 ([M + Na]$^+$, calcd for $C_{21}H_{32}O_5Na$ 387.2147).

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