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A NEW NATURAL BUTENOLIDE, (5*R*)-3-TETRADECYL-5-METHYL-2(5*H*)-FURANONE, FROM OCTOCORAL *CLADIELLA CONIFERA*

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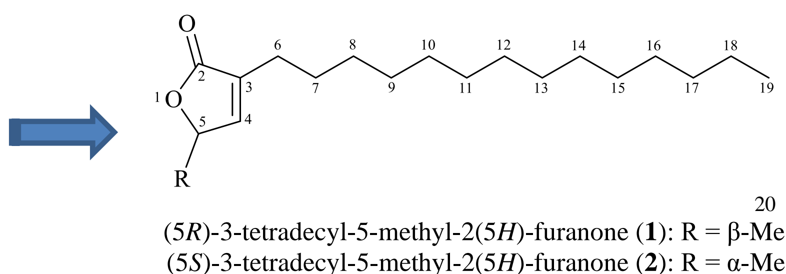
Abstract – A chemical examination of *Cladiella conifera*, octocoral collected in the waters of Taiwan, resulted in isolation of a new natural butenolide, (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**). The structure, including the absolute

configuration, of **1** was established by spectroscopic analysis and **1** was found to inhibit the generation of COX-2 from RAW 264.7 stimulated by LPS.

Octocorals belonging to genus *Cladiella* were found to be rich sources of interesting natural products, of which many were proved to exhibit extensive biomedical proficiency through bioactivities.[1] In our continuing studies of new natural substances from marine invertebrates distributed in Taiwanese reef systems locating in a highly biodiverse environment provided by convergence of the Kuroshio current and South China Sea surface current, an octocoral identified as *Cladiella conifera* (Tixier-Durivault, 1943) (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonacea, suborder Alcyoniina, family Alcyoniidae)[2] was collected off the Penghu Archipelago. Chemical examination of the EtOAc soluble fraction of this specimen resulted in the isolation of a new natural butenolide derivative (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**) (Chart 1). Butenolide derivatives are occasionally encountered among various marine invertebrates, such as sponges,[3,4] brittle stars,[5] octocorals,[6–8] and marine microorganisms, including Gram-positive bacterium *Streptomyces* spp.,[9,10] fungus *Aspergillus terreus*,[11,12] *Paecilomyces variotii*,[13] and *Paradendryphiella salina*.[14] These butenolides of marine origin were found to possess potential bioactivities in cytotoxicity,[5] antifouling activity,[8] PPARα agonistic activity,[9] α-glucosidase inhibitory activity,[11,12] anti-inflammatory activity,[12] antiradical activity,[12,13] and antibacterial effects.[12,14] As follows is the description of isolation, structural characterization, and bioactivity of butenolide **1**.

Cladiella conifera



(5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**): R = β-Me
(5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**2**): R = α-Me

Chart 1. A picture of *Cladiella conifera* and the structures of (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**) and (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**2**)

Compound **1** was obtained as an amorphous solid and had a molecular formula $C_{19}H_{34}O_2$ from (+)-HRESIMS at *m/z* 317.24534 $[M + Na]^+$ (Calcd for $C_{19}H_{34}O_2$ + Na, 317.24510) (unsaturation degrees = 3). The IR spectrum pointed out an absorption at 1743 cm^{-1}, suggesting the presence of an α,β-unsaturated γ-lactone carbonyl functional group. The ^{13}C NMR spectrum (Table 1), in combination with DEPT and HSQC spectra, revealed the presence of one γ-lactone moiety (δ_C 173.9, C-2) and one trisubstituted olefin (δ_C 134.3, C-3; 148.8, CH-4). As one double bond and one carbonyl accounted for two of the three double-bond equivalents, **1** must be a monocyclic compound. Based on the ^{13}C NMR data and numbers of unsaturation, **1** established as a butenolide analogue.

The ^{1}H NMR and HSQC spectra of **1** showed the presence of one olefinic proton signal at δ_H 6.98 (1H, q, J = 1.2 Hz), that was attributed to H-4 of a butenolide. Additionally, a signal at δ_H 4.99 (1H, qq, J = 6.8, 1.6 Hz) was characteristic of the H-5 oxymethine resonance of a butenolide. In addition, a methyl doublet was observed at δ_H 1.40 (3H, br d, J = 6.8 Hz, H_3-20). The ^{13}C NMR resonances at δ_C 173.9 (C-2), 134.3 (C-3), 148.8 (CH-4), 77.4 (CH-5), and 19.2 (CH_3-20) were characteristic of the α,β-unsaturated γ-methyl-γ-lactone of a butenolide. Analysis of ^{1}H–^{1}H COSY spectrum provided two spin systems of protons H-4/H-5/H_3-20 and H_2-6 to H_3-19 (Figure 1). These two fragments were connected by the key HMBC correlations between protons and non-protonated carbons such as H-4/C-2, C-3; H-5/C-3; H_2-6/C-2, C-3; and H_2-7/C-3 (Figure 1). Lastly, consideration of the remaining unsaturation degree determined the α,β-unsaturated γ-lactone bearing a linear chain and a methyl at the α- and γ-positions, respectively.

Table 1. ^{1}H and ^{13}C NMR data for butenolide **1**

Position	δ_H (J in Hz)[a]	δ_C,[b] Mult.[c]
2		173.9, C
3		134.3, C
4	6.98 q (1.2)	148.8, CH
5	4.99 qq (6.8, 1.6)	77.4, CH
6	2.26 tt (7.6, 1.6)	25.2, CH_2
7	1.54 quint (7.6)	27.4, CH_2
8–16	1.25–1.34 m	29.17, CH_2
		29.30, CH_2
		29.34, CH_2
		29.51, CH_2
		29.60, CH_2
		29.63, CH_2
		29.63, CH_2
		29.66, CH_2
		29.67, CH_2
17	1.25 m	31.9, CH_2
18	1.28 m	22.7, CH_2
19	0.88 t (7.2)	14.1, Me
20	1.40 br d (6.8)	19.2, Me

[a] 400 MHz in $CDCl_3$. [b] 100 MHz in $CDCl_3$. [c] Multiplicity deduced by DEPT and HSQC spectra.

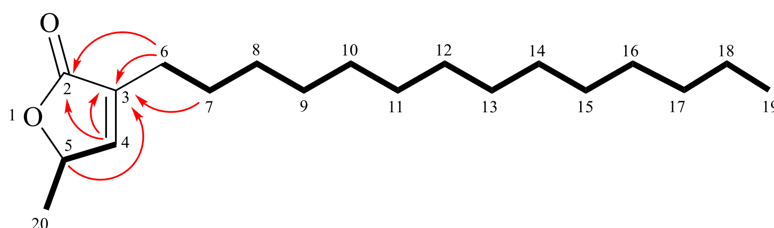
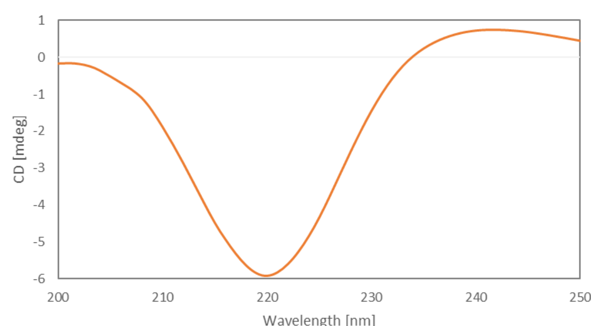


Figure 1. Key COSY (—) and HMBC (⌒) correlations of **1**

Gawronski et al. established a method for determining the absolute configuration of butenolides using CD (circular dichroism) spectra, such is when a butenolide has a negative Cotton effect (π–π*) between 200 and 220 nm, the absolute configuration of C-5 is R.[15] The CD spectrum of butenolide **1** (Figure 2) provided a negative Cotton effect at 220 nm, and the absolute configuration at C-5 of **1** was assigned as R accordingly.

Furthermore, it was found that the planar structure of **1** was identical to that of known synthetic butenolides, (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone and its enantiomer (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**2**) (Chart 1).[16] By comparison of the rotation value of **1** ($[\alpha]^{25}_{D}$ −23 (*c* 1.33, $CHCl_3$)) with that of (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ($[\alpha]^{20}_{D}$ −20.1 (*c* 2.06, CH_2Cl_2))[16] and (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ($[\alpha]^{20}_{D}$ +27.2 (*c* 2.02, CH_2Cl_2);[16] $[\alpha]^{23}_{D}$ +27.7 (*c* 2.3, CH_2Cl_2)[17]), the absolute configuration for C-5 stereogenic center of **1** was further confirmed as *R* form. Therefore, the structure of **1** was determined unequivocally based on the above findings. Butenolide **1** was isolated from a natural source unprecedentedly and so was butenolide analogues obtained from octocorals belonging to genus *Cladiella*.



220 nm (Δε = –5.8, *c* 3.33 ppm, MeOH)

Figure 2. CD spectrum of **1**

According to Klapper et al. study,[18] the generation of butenolide **1** is suggested that starts from a 3-oxo thioester fatty acid derivative **2** with glyceraldehyde-3-phosphate to yield **3** via Knoevenagel condensation. Subsequently, **3** is reduced to alcohol **4** by a short-chain reductase. Further, an oxidoreductase reacts with **4** to produce an exo-methylene moiety to obtain **5**, and a reduction occurs to yield **6**. Finally, the hydroxy group in **6** is removed by deoxidation to generate butenolide **1**.

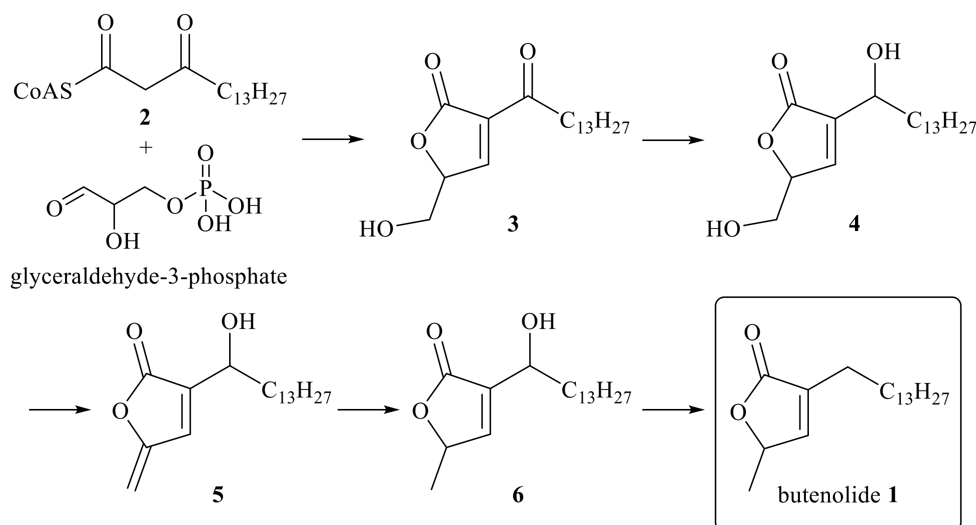


Figure 3. The plausible biosynthetic pathway of **1**

The effect of butenolide **1** on the release of cyclooxygenase-2 (COX-2) from lipopolysaccharide(LPS)-stimulated RAW264.7 macrophage cells was assessed. Butenolide **1** at 10 μM suppressed the release of COX-2 to 84.5 ± 2.8%, as compared to the results of the cells stimulated with LPS only.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured using a JASCO P-1010 digital polarimeter. IR spectra were measured on a Thermo Scientific Nicolet iS5 FT-IR spectrophotometer. CD spectrum was recorded on a JASCO J-815 circular dichroism (CD) spectropolarimeter in MeOH. NMR spectra were taken on a 400 MHz Jeol NMR (model ECZ 400 S) spectrometer operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$ using the residual $CHCl_3$ signal (δ_H 7.26 ppm) and $CDCl_3$ (δ_C 77.1 ppm) as the internal standards for ^{1}H and ^{13}C NMR, respectively; coupling constants (*J*) are given in Hz. ESIMS and HRESIMS were recorded using a Bruker APEX II FTMS system. Column chromatography was carried out with silica gel (230–400 mesh, Merck). TLC was performed on plates precoated with DC-Fertigfolien Alugram® Xtra SIL G/UV_{254} (0.20-mm-thick, Macherey-Nagel) 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. Normal-phase HPLC (NP-HPLC) was performed using a system comprised of a Hitachi L-7100 pump, a Rheodyne 7725i injection port, and a normal-phase column (YMC-Pack SIL, 250 × 20 mm, 5 μm; Sigma-Aldrich). Reverse-phase HPLC (RP-HPLC) was performed using a system comprised of a Hitachi L-2130 pump, a Hitachi L-2455 photodiode array detector, and a Rheodyne 7725i injection port. A reverse-phase column (Luna, 5 μm, C18(2) 100Å, 250 × 21.2 mm) was used for RP-HPLC.

Animal Materials. Specimens of *C. conifera* were collected in May 2017 by hand with SCUBA divers off the coast of Penghu Archipelago, Taiwan (N23.15.203, E119.30.725). A voucher specimen was deposited in the National Museum of Marine Biology & Aquarium, Taiwan (NMMBA-TW-SC-2017-0504).

Extraction and Isolation. *C. conifera* (wet/dry weight = 171/59 g) were sliced and then extracted with a solvent mixture of MeOH and CH_2Cl_2 (1:1). The extract was partitioned between EtOAc and H_2O. The EtOAc layer (3.86 g) was then applied on silica gel column and eluted with gradients of *n*-hexane/EtOAc (from *n*-hexane to 100% EtOAc) to furnish 12 sub-fractions. Among them, fraction 5 was separated by NP-HPLC, using a solvent mixture of *n*-hexane/EtOAc (19:1) to yield 8 sub-fractions 5A–5H. Fraction 5C was further purified by RP-HPLC, using an isocratic solvent system of MeOH/H_2O mixture (87:13; flow rate = 5 mL/min) to afford **1** (5.1 mg).

(5*R*)-3-Tetradecyl-5-methyl-2(5*H*)-furanone (1): amorphous powder; $[\alpha]^{25}_{D}$ –23 (*c* 1.33, $CHCl_3$) (ref.[16] $[\alpha]^{20}_{D}$ –20.1 (*c* 2.06, CH_2Cl_2)); IR ν_{max} 1743 cm^{-1}; ^{1}H ($CDCl_3$, 400 MHz) and ^{13}C ($CDCl_3$, 100 MHz) NMR

data, see Table 1; ESIMS m/z 317 $[M + Na]^+$; HRESIMS m/z 317.24534 (Calcd for $C_{19}H_{34}O_2$ + Na, 317.24510).

***In Vitro* Anti-inflammatory Assay.** The inflammatory assay was employed to evaluate the activity of butenolide **1** related to the release of COX-2 from macrophage cells as the literature reported.[19,20]

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