HETEROCYCLES, Vol. 104, No. 1, 2022, pp. -. © 2022 The Japan Institute of Heterocyclic Chemistry
Received, 4th October, 2021, Accepted, 29th October, 2021, Published online, 4th November, 2021
DOI: 10.3987/COM-21-14565

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

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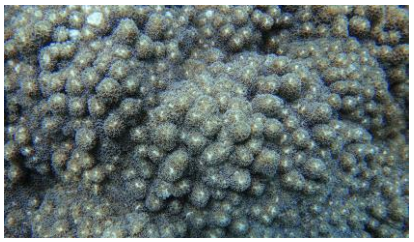
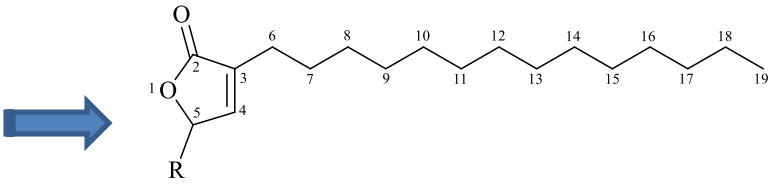
Abstract – A chemical examination of *Cladiella conifera*, octocoral collected in the waters of Taiwan, resulted in isolation of a new natural butenolide, (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**). The structure, including the absolute

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.

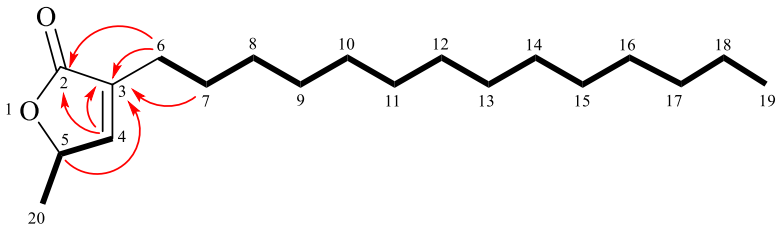
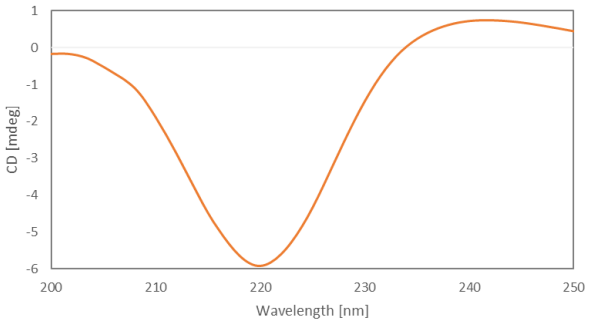


Figure 1. Key COSY (—) and HMBC (↷) correlations of **1**

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** ($[\alpha]_D^{25}$ −23 (*c* 1.33, $CHCl_3$)) with that of (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ($[\alpha]_D^{20}$ −20.1 (*c* 2.06, CH_2Cl_2))[16] and (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ($[\alpha]_D^{20}$ +27.2 (*c* 2.02, CH_2Cl_2);[16] $[\alpha]_D^{23}$ +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**.

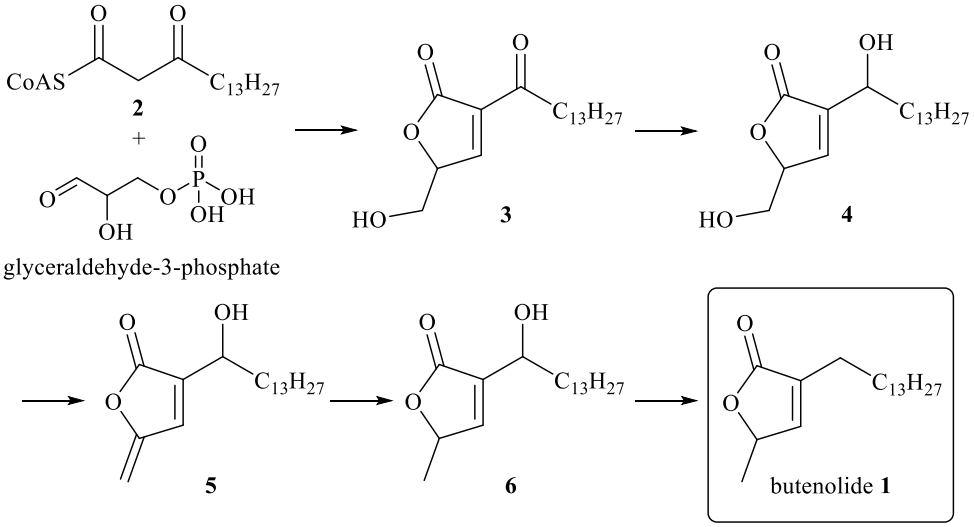


Figure 3. The plausible biosynthetic pathway of **1**

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; $[\alpha]_D^{25}$ –23 (*c* 1.33, $CHCl_3$) (ref.[16] $[\alpha]_D^{20}$ –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]

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Chao-Lien Ho and Hsiao-Ching Yu at the High Valued Instrument Center, National Sun Yat-sen University, for providing the NMR (NMR001100) and mass (MS000600) spectra (MOST 110-2731-M-110-001). This work was mainly funded by grants from the National Museum of Marine Biology and Aquarium, the National Dong Hwa University, the Fooyin University, and the Ministry of Science and Technology (MOST 110-2314-B-242-003, 109-2320-B-291-001-MY3, and 107-2320-B-291-001-MY3), Taiwan, awarded to Shun-Hua Chen and Ping-Jyun Sung. All funding is gratefully acknowledged.

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