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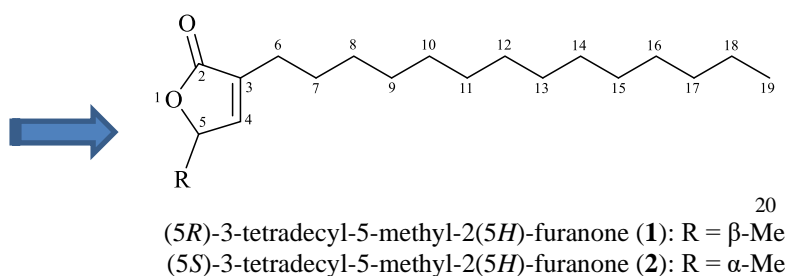
**Abstract** – A chemical examination of *Cladiella conifera*, octocoral collected in the waters of Taiwan, resulted in isolation of a new natural butenolide, (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**1**). The structure, including the absolute

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.<sup>1</sup> 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)<sup>2</sup> 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 (*5R*)-3-tetradecyl-5-methyl-2(*5H*)-furanone (**1**) (Chart 1). Butenolide derivatives are occasionally encountered among various marine invertebrates, such as sponges,<sup>3,4</sup> brittle stars,<sup>5</sup> octocorals,<sup>6–8</sup> and marine microorganisms, including Gram-positive bacterium *Streptomyces* spp.,<sup>9,10</sup> fungus *Aspergillus terreus*,<sup>11,12</sup> *Paecilomyces variotii*,<sup>13</sup> and *Paradendryphiella salina*.<sup>14</sup> These butenolides of marine origin were found to possess potential bioactivities in cytotoxicity,<sup>5</sup> antifouling activity,<sup>8</sup> PPAR $\alpha$  agonistic activity,<sup>9</sup>  $\alpha$ -glucosidase inhibitory activity,<sup>11,12</sup> anti-inflammatory activity,<sup>12</sup> antiradical activity,<sup>12,13</sup> and antibacterial effects.<sup>12,14</sup> As follows is the description of isolation, structural characterization, and bioactivity of butenolide **1**.



*Cladiella conifera*



**Chart 1.** A picture of *Cladiella conifera* and the structures of (*5R*)-3-tetradecyl-5-methyl-2(*5H*)-furanone (**1**) and (*5S*)-3-tetradecyl-5-methyl-2(*5H*)-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  $1743\text{ cm}^{-1}$ , suggesting the presence of an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone carbonyl functional group. The  $^{13}C$  NMR spectrum (Table 1), in combination with DEPT and HSQC spectra, revealed the presence of one  $\gamma$ -lactone moiety ( $\delta_C$  173.9, C-2) and one trisubstituted olefin ( $\delta_C$  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  $^1\text{H}$  NMR and HSQC spectra of **1** showed the presence of one olefinic proton signal at  $\delta_{\text{H}}$  6.98 (1H, q,  $J = 1.2$  Hz), that was attributed to H-4 of a butenolide. Additionally, a signal at  $\delta_{\text{H}}$  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  $\delta_{\text{H}}$  1.40 (3H, br d,  $J = 6.8$  Hz, H<sub>3</sub>-20). The  $^{13}\text{C}$  NMR resonances at  $\delta_{\text{C}}$  173.9 (C-2), 134.3 (C-3), 148.8 (CH-4), 77.4 (CH-5), and 19.2 (CH<sub>3</sub>-20) were characteristic of the  $\alpha,\beta$ -unsaturated  $\gamma$ -methyl- $\gamma$ -lactone of a butenolide. Analysis of  $^1\text{H}$ - $^1\text{H}$  COSY spectrum provided two spin systems of protons H-4/H-5/H<sub>3</sub>-20 and H<sub>2</sub>-6 to H<sub>3</sub>-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<sub>2</sub>-6/C-2, C-3; and H<sub>2</sub>-7/C-3 (Figure 1). Lastly, consideration of the remaining unsaturation degree determined the  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone bearing a linear chain and a methyl at the  $\alpha$ - and  $\gamma$ -positions, respectively.

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

| Position | $\delta_{\text{H}}$ ( $J$ in Hz) <sup>a</sup> | $\delta_{\text{C}}$ , <sup>b</sup> Mult. <sup>c</sup>  |
|----------|---|--|
| 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 <sub>2</sub>  |
| 7        | 1.54 quint (7.6)                              | 27.4, CH <sub>2</sub>  |
| 8–16     | 1.25–1.34 m                                   | 29.17, CH <sub>2</sub><br>29.30, CH <sub>2</sub><br>29.34, CH <sub>2</sub><br>29.51, CH <sub>2</sub><br>29.60, CH <sub>2</sub><br>29.63, CH <sub>2</sub><br>29.63, CH <sub>2</sub><br>29.66, CH <sub>2</sub><br>29.67, CH <sub>2</sub> |
| 17       | 1.25 m  | 31.9, CH <sub>2</sub>  |
| 18       | 1.28 m  | 22.7, CH <sub>2</sub>  |
| 19       | 0.88 t (7.2)                                  | 14.1, Me   |
| 20       | 1.40 br d (6.8)                               | 19.2, Me   |

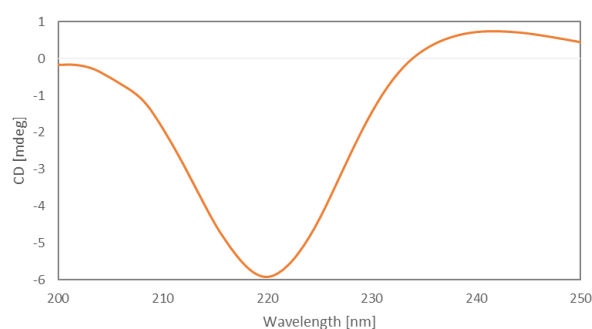
<sup>a</sup> 400 MHz in CDCl<sub>3</sub>, <sup>b</sup> 100 MHz in CDCl<sub>3</sub>, <sup>c</sup> Multiplicity deduced by DEPT and HSQC spectra.



**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 ( $\pi$ - $\pi^*$ ) between 200 and 220 nm, the absolute configuration of C-5 is *R*.<sup>15</sup> The CD spectrum of butenolide **1** (Figure 2) provided

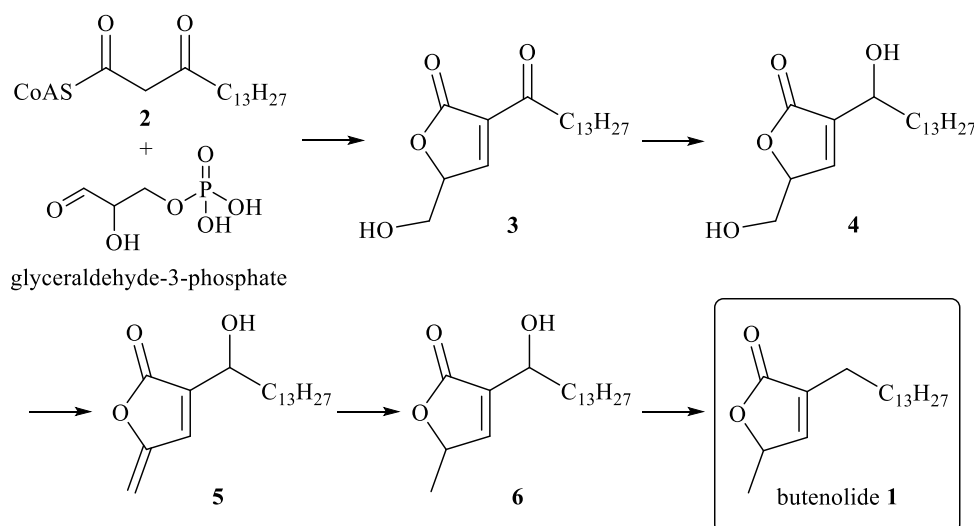
a negative Cotton effect at 220 nm, and the absolute configuration at C-5 of **1** was assigned as *R* accordingly. Furthermore, it was found that the planar structure of **1** was identical to that of known synthetic butenolides, (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone and its enantiomer (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone (**2**) (Chart 1).<sup>16</sup> By comparison of the rotation value of **1** ( $[\alpha]_{\text{D}}^{25} -23$  (*c* 1.33, CHCl<sub>3</sub>)) with that of (5*R*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ( $[\alpha]_{\text{D}}^{20} -20.1$  (*c* 2.06, CH<sub>2</sub>Cl<sub>2</sub>))<sup>16</sup> and (5*S*)-3-tetradecyl-5-methyl-2(5*H*)-furanone ( $[\alpha]_{\text{D}}^{20} +27.2$  (*c* 2.02, CH<sub>2</sub>Cl<sub>2</sub>);<sup>16</sup>  $[\alpha]_{\text{D}}^{23} +27.7$  (*c* 2.3, CH<sub>2</sub>Cl<sub>2</sub>)<sup>17</sup>), 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 ( $\Delta\epsilon = -5.8$ , *c* 3.33 ppm, MeOH)

**Figure 2.** CD spectrum of **1**

According to Klapper et al. study,<sup>18</sup> 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  $\mu\text{M}$  suppressed the release of COX-2 to  $84.5 \pm 2.8\%$ , 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  $^1\text{H}$  and 100 MHz for  $^{13}\text{C}$  in  $\text{CDCl}_3$  using the residual  $\text{CHCl}_3$  signal ( $\delta_{\text{H}}$  7.26 ppm) and  $\text{CDCl}_3$  ( $\delta_{\text{C}}$  77.1 ppm) as the internal standards for  $^1\text{H}$  and  $^{13}\text{C}$  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<sup>®</sup> Xtra SIL G/UV<sub>254</sub> (0.20-mm-thick, Macherey-Nagel) and RP-18W/UV<sub>254</sub> (0.15-mm-thick, Macherey-Nagel), then sprayed with 10%  $\text{H}_2\text{SO}_4$  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,  $250 \times 20$  mm,  $5 \mu\text{m}$ ; 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,  $5 \mu\text{m}$ , C18(2) 100Å,  $250 \times 21.2$  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  $\text{CH}_2\text{Cl}_2$  (1:1). The extract was partitioned between EtOAc and  $\text{H}_2\text{O}$ . 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/ $\text{H}_2\text{O}$  mixture (87:13; flow rate = 5 mL/min) to afford **1** (5.1 mg).

**(5R)-3-Tetradecyl-5-methyl-2(5H)-furanone (1):** amorphous powder;  $[\alpha]_{\text{D}}^{25} -23$  ( $c$  1.33,  $\text{CHCl}_3$ ) (ref.<sup>16</sup>  $[\alpha]_{\text{D}}^{20} -20.1$  ( $c$  2.06,  $\text{CH}_2\text{Cl}_2$ )); IR  $\nu_{\text{max}}$  1743  $\text{cm}^{-1}$ ;  $^1\text{H}$  ( $\text{CDCl}_3$ , 400 MHz) and  $^{13}\text{C}$  ( $\text{CDCl}_3$ , 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.<sup>19,20</sup>

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