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An Anti-Inflammatory 2,4-Cyclized-3,4-Secospongian Diterpenoid and Furanoterpenene-Related Metabolites of a Marine Sponge Spongia sp. from the Red Sea

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Abstract: Chemical investigation of a Red Sea Spongia sp. led to the isolation of four new compounds, i.e., 17-dehydroxysponalactone (1), a carboxylic acid, spongiafuric acid A (2), one hydroxamic acid, spongiafuranoxyhydroxamic acid A (3), and a furanyl trinorsesterpenoid 16-epi-irciformonin G (4), along with three known metabolites (−)-sponalisolide B (5), 18-nor-3,17-di-hydroxy-spongia-3,13(16),14-trien-2-one (6), and cholesta-7-ene-3β,5α-diol-6-one (7). The biosynthetic pathway for the molecular skeleton of 1 and related compounds was postulated for the first time. Anti-inflammatory activity of these metabolites to inhibit superoxide anion generation and elastase release in N-formylmethionyl-leucyl phenylalanine/cytochalasin B (fMLF/CB)-induced human neutrophil cells and cytotoxicity of these compounds toward three cancer cell lines and one human dermal fibroblast cell line were assayed. Compound 1 was found to significantly reduce the superoxide anion generation and elastase release at a concentration of 10 µM, and compound 5 was also found to display strong inhibitory activity against superoxide anion generation at the same concentration. Due to the noncytotoxic activity and the potent inhibitory effect toward the superoxide anion generation and elastase release, 1 and 5 can be considered to be promising anti-inflammatory agents.

Keywords: Red Sea sponge; Spongia; seco-spongian diterpenoid; isoprenoid-derived amide

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1. Introduction

Marine sponges have been considered to be an important source for the discovery of structurally diverse bioactive secondary metabolites [1]. Many natural products from sponges have been shown to exhibit a variety of biological activities, such as antimicrobial [2–5], antiviral [6–8], antiprotozoal [8–10], cytotoxic [6,11–13], anti-inflammatory [14–16], antioxidant [4,17,18], immunosuppressive [1,19,20], and antifeedant [21–23]. The genus *Spongia* (Spongidae) has been chemically investigated since 1971 [24] and the studies have led to the discovery of a series of furanoterpenes [24–26], spongian diterpenoids [27–32], scalarane sesterterpenoids [33–35], sesquiterpene quinones [36,37], along with other kinds of metabolites, for example, sterols [38–40] and macrolides [41].

We report, herein, the chemical investigation of an unidentified *Spongia* species inhabiting along the eastern coast of the Red Sea. This study afforded four new natural products including a rare A-ring contracted diterpenoid, 17-dehydroxysponalactone (1), a C₁₂ carboxylic acid, spongiafuranic acid A (2); a C₁₂ hydroxamic acid, spongiafuranohydroxamic acid A (3); and a furanyl trinorsesterpenoid, 16-epi-irciformonin G (4); along with three known metabolites, (−)-sponalisolide B (5) [42], 18-nor-3,13-dihydroxyspongia-3,13(16),14-trien-2-one (6) [43], and cholesta-7-ene-3β,5α-diol-6-one (7) [40] (Figure 1 and Supplementary Materials Figures S1–S35 for 1–5). Furthermore, in order to discover bioactive lead compounds, assays for the anti-inflammatory activity of the isolated compounds by inhibition of the superoxide anion generation and elastase release in N-formyl-methionyl-leucyl phenylalanine/cytochalasin B (fMLF/CB)-induced human neutrophils, and the cytotoxicity of these compounds against three tumor cell lines, murine leukemia (P388), and human colon adenocarcinoma (DLD-1), and a human dermal fibroblast (CCD-966SK) cell line were undertaken. Compounds 1 and 5 were shown to exhibit the promising anti-inflammatory activity.

![Structures of compounds 1–7 isolated from a Red Sea *Spongia* sp.](image)

2. Results and Discussion

Compound 1 was obtained as a white powder. Its molecular formula C₂₀H₂₆O₅ was established by the molecular ion peak at m/z 369.1672 [M + Na]+ in the HRESIMS, consistent with eight degrees of unsaturation. The IR spectrum showed absorptions of hydroxyl (3455 and 3401 cm⁻¹) and lactone carbonyl (1752 cm⁻¹) functionalities. The ¹³C NMR spectroscopic data of 1 exhibited 20 carbon signals (Table 1), which were assigned by the assistance of DEPT spectrum showing thirteen carbon signals of a diterpene, including...
three ring-juncture methyls ($\delta_C$ 26.9, 22.6, and 14.0; $\delta_H$ 1.24, 1.14, and 0.84) and a 3,4-disubstituted furan ring ($\delta_C$ 137.1, CH; 134.8, CH; 136.8, C; and 119.6, C and $\delta_H$ 7.06, 1H, br s and 7.09, 1H, br s) [30,31,35,44]. On the basis of the number of unsaturations, 1 was, thus, suggested to be a pentacyclic 3,4-disubstituted furan diterpenoid. The NMR spectroscopic data of 1 and 2D NMR correlations (Figure 2) were similar to those of the previously described sponalactone (8) [30], except that a hydroxymethyl in 8 was replaced by a methyl at C-8 in 1. Compound 1 also possesses the same B, C, and D rings as 9 [32] (Scheme 1).

| Position | $\delta_H$, m (J in Hz) | $\delta_C$, Type |
|----------|------------------------|-----------------|
| 1        | 3.87, 1H, br s         | 81.8, CH        |
| 2        | -                      | 83.3, C         |
| 3        | -                      | 180.6, C        |
| 4        | -                      | 47.6, C         |
| 5        | 1.90, 1H, d (11.5)     | 56.0, CH        |
| 6        | 1.63, 1H, br d (10.5, 10.5) | 18.3, CH$_2$ |
| 7        | 1.66, 1H, m            | 40.0, CH$_2$    |
| 8        | 2.16, 1H, br d (10.5)  | 34.4, C         |
| 9        | 1.96, 1H, d (11.5)     | 47.0, CH        |
| 10       | -                      | 46.4, C         |
| 11       | 1.68, 1H, m            | 20.2, CH$_2$    |
| 12       | 1.78, 1H, dq (12.5, 6.5) | 19.7, CH$_2$   |
| 13       | 2.59, 1H, ddd (16.0, 12.5, 6.5) | 2.76, 1H, dd (16.0, 6.0) |
| 14       | -                      | 34.4, C         |
| 15       | 7.09, 1H, br s         | 134.8, CH       |
| 16       | 7.06, 1H, br s         | 137.1, CH       |
| 17       | 1.24, 3H, s            | 26.9, CH$_3$    |
| 18       | 1.14, 3H, s            | 22.6, CH$_3$    |
| 19       | 3.92, 1H, d (12.0)     | 74.6, CH$_2$    |
| 20       | 4.37, 1H, d (12.0)     | 14.0, CH$_3$    |

The relative and absolute configurations of 1 were established on the basis of nuclear Overhauser effect (NOE) correlation analysis (Figure 3) and by comparison of the observed NOE correlations with those of the related compounds [30,31], the observed pyridine-induced solvent shifts [45], and biogenetic consideration. The NOESY spectrum of 1 showed NOE correlations of H$_3$-17/H$_3$-20 and H-5/H-9, depicting the $5R$,8$R$,9$S$,10$R$-configuration. H-1 displayed NOE interactions with the $\beta$-oriented H$_3$-20 and H-11$\alpha$ ($\delta_H$ 1.68, m), indicating the $\alpha$-orientation of the H-1. Furthermore, the NOE correlations of H-5/H$_3$-18, H$_3$-18/H-19$\alpha$ ($\delta_H$ 3.92) and H-19$\beta$ ($\delta_H$ 4.37)/H$_3$-20 disclosed the $\alpha$- and $\beta$-orientations of H$_3$-18 and the \gamma-lactone ring, respectively, and the $\alpha$-orientation of the hydroxyl at C-2, accordingly. The analysis of the pyridine-induced deshielding effect of the axial hydroxy groups was also employed to support the configuration of 1. Therefore, the significant pyridine-induced downfield shifts ($\Delta\delta$ = $\delta_{\text{CDCl}_3} - \delta_{\text{C}_6\text{D}_5\text{N}}$) exerted on H-5 ($\Delta\delta_H = -0.24$ ppm) could only be approached when 1-OH was axially oriented on the same $\alpha$-face of the molecule. Also, H$_3$-18 exhibited pyridine-induced downfield shift ($\Delta\delta_H = -0.14$ ppm) due to the vicinal effect of 2-OH, which should be syn to H$_3$-18 [45]. On the basis of the above findings, we propose that 1 can be derived from an intermediate spongian 9, which was biosynthesized from the mevalonic acid pathway, after oxygenation of the six-membered ring A and a subsequent ring contraction and formation of a five-membered carbocycle, as illustrated in Scheme 1.
Metabolite 2 was isolated as a colorless oil. Its molecular formula was determined to be C_{12}H_{16}O_{3} from the HREIMS (m/z 231.0992 [M + Na]^+), indicating the four degrees of unsaturation. The IR spectrum displayed the absorptions of carboxylic acid (3105–2857 and 1708 cm^{-1}) and olefin (1654 cm^{-1}). The NMR data (Table 2) showed the presence of a monosubstituted furan ring (δC 142.5, CH; 138.8, CH; 111.0, CH; and 124.7, C; δH 7.34, 7.20, and 6.27, each 1H, s) [24–26,42], a trisubstituted olefin (δC 124.7, CH; δH 5.22, 1H, s), a
methyl (δC 15.9; δH 1.61, 3H, s) and a carbonyl group (δC 180.0, C). Other 1H NMR signals in the shielded region (δH 2.25–2.47, 8H) were attributable to four methylene groups, as depicted from the COSY (Figure 2) correlations. The methylene protons H2-6 (δH 2.25, dt, J = 7.6, 7.2 Hz, 2H) was found to be further correlated with the olefinic proton (δH 5.22, dd, J = 7.2, 7.2 Hz, H-7) in 2. The detailed analysis of HMBC correlations (Figure 2) resolved the carbon positions of the furan ring, olefinic double bond, and the carboxyl group to be at C-1-C-4, C-7/C-8, and C-11, respectively. Furthermore, the methyl group was positioned at C-8. The furanyl H-2 (δH 6.27, s), H-4 (δH 7.20, s), and the olefinic proton H-7 (δH 5.22, dd, J = 7.2, 7.2 Hz, 2H) displayed HMBC correlations with the sp3 carbon C-5 (δC 24.8, CH2), and H3-12 (δH 1.61, s) showed HMBC correlations with C-7 (δC 124.7, CH) and C-9 (δC 34.2, CH2), while the signal of H2-9 (δH 2.32, dd, J = 7.6, 7.6 Hz, 2H) was found to be correlated with the carboxyl carbon (C-11, δC 180.0). Moreover, the NOE correlations observed for H3-12 with H2-6 but not with H-5 and the chemical shift of C-12 (δC < 20 ppm) assigned the E-configuration of the 7,8-double bond [46]. Therefore, 2 was determined to be a furanotrinorsesquiterpenoid carboxylic acid with the structure of (E)-7-(furan-3-yl)-4-methylhept-4-enoic acid. The literature search showed that this compound had been prepared as a synthetic intermediate during the total syntheses of the furanosesquiterpenoids and dendrolasins [42,47], however, its NMR data had not been reported. Therefore, this is the first report of 2 as a natural product, with the NMR data assigned and reported for the first time.

Table 2. 1H and 13C NMR data for compounds 2 and 3.

|   | δH, m (J in Hz) | δC     | δH, m (J in Hz) | δC     |
|---|----------------|--------|----------------|--------|
| 2 | 1 | 7.34, 1H, brs | 142.5, CH | 7.35, 1H, brs | 142.9, CH |
|   | 2 | 6.27, 1H, brs | 111.0, CH | 6.27, 1H, brs | 111.2, CH |
|   | 3 | - | 124.7, C | - | 125.1, C |
|   | 4 | 7.20, 1H, s | 138.8, CH | 7.21, 1H, s | 139.1, CH |
|   | 5 | 2.45, 2H, dt (7.6, 7.6) | 24.8, CH2 | 2.40, 2H, dt (7.6, 7.6) | 24.4, CH2 |
|   | 6 | 2.25, 2H, dt (7.6, 7.2) | 28.3, CH2 | 2.25, 2H, dt (7.6, 7.6) | 28.1, CH2 |
|   | 7 | 5.22, 1H, dd (7.2, 7.2) | 124.7, CH | 2.08, 2H, dd (7.2, 7.6) | 39.1, CH2 |
|   | 8 | - | 133.7, C | - | 139.7, C |
|   | 9 | 2.32, 2H, dd (7.6, 7.6) | 34.2, CH2 | 5.34, 1H, dd (6.0, 6.0) | 115.5, CH |
|   | 10 | 2.47, 2H, m | 32.9, CH2 | 3.10, 2H, d (6.8) | 33.2, CH2 |
|   | 11 | - | 180.0, C | - | 176.1, C |
|   | 12 | 1.61, 3H, s | 15.9, CH3 | 1.65, 3H, s | 16.5, CH3 |

Metabolite 3 exhibited almost the same NMR data as those of 2 (Table 2) from C-1 to C-6, with the carbon chemical shifts of the trisubstituted double bond (δC 139.7, C and 115.5, CH; δH 5.34, dd, J = 6.0, 6.0 Hz, 1H) and the carbonyl group (δC 176.1, C) in 3 showing significant differences of ΔδC −6.0, +9.2, and −3.9 ppm as compared with those of the corresponding carbons in 2, respectively. As illustrated by 1H-1H COSY correlations (Figure 2), the double bond has been isomerized from the C-7/C-8 position in 2 to the C-8/C-9 position in 3. However, the IR spectrum displayed the absorptions of the hydroxyl and NH groups (3407-2858 cm−1), carbonyl group (1705 cm−1), and olefin (1634 cm−1) functionalities. Furthermore, the HREIMS m/z 246.1098 [M + Na]+ established the molecular formula of 3 to be C12H17NO3 and the chemical shift of the carbonyl group (176.1 ppm), showing that a hydroxamic acid moiety [48–51] replaced a carboxylic acid group at C-11 in 3.
Compound 4 was isolated as a colorless oil, $[\alpha]_{D}^{25} +4.4$ (c 0.74, CHCl$_3$). The ESIMS and NMR spectroscopic data (Table 3) established the molecular formula C$_{22}$H$_{32}$O$_4$ for 4. The IR absorptions 3432, 1769, and 1647 cm$^{-1}$ revealed the presence of hydroxyl, carbonyl, and olefin functionalities, respectively. Moreover, it was found that the NMR data of 4 was the same as those of irciformonin G (10) \[52\] in all aspects except for those at positions 17 and 18–20 (Table 4), proposing 4 as an isomer of 10. By using Mosher’s method \[53,54\], the 15R absolute configuration in 4 was established based on the calculated $\Delta \delta_H (\delta_S - \delta_R)$ values of protons neighboring C-15 of (S)- and (R)-$\alpha$-methoxy-$\alpha$-(trifluoromethyl)-phenylacetyl (MTPA) esters 4a and 4b, respectively (Figure 4). After the assignment of the 15R configuration, the 13C NMR data of C-15 to C-20 of 4 were further compared with the corresponding data of irciformonin G (10), (+)-sponalisolide A (11), and 8-epi-(+)-sponalisolide A (12) \[42\] of known absolute configurations (Table 4 and Figure 5). The 15R,16R-configuration of 4 was, thus, confirmed as those of the 7R,8R configured 12, while 10 and 11 possessed the same configurations (R,S) at the corresponding asymmetric carbons. From the above findings, compound 4 was, thus, identified as 16-epi-irciformonin G.

| 4 (1H, m) | 5 (-Sponalisolide B) |
|-----------|----------------------|
| $\delta$H, m (J in Hz) | $\delta$C | $\delta$H, m (J in Hz) | $\delta$C |
| 1 | 7.34, 1H, brs | 142.5, CH | 7.34, 1H, brs | 142.6, CH |
| 2 | 6.28, 1H, brs | 111.1, CH | 6.27, 1H, brs | 111.0, CH |
| 3 | – | 124.9, C | – | 124.7, C |
| 4 | 7.21, 1H, s | 138.8, CH | 7.20, 1H, s | 138.8, CH |
| 5 | 2.45, 2H, t (7.5) | 25.0, CH$_2$ | 2.45, 2H, t (7.5) | 24.8, CH$_2$ |
| 6 | 2.24, 2H, dt (7.5, 7.0) | 28.4, CH$_2$ | 2.25, 2H, dt (7.5, 7.0) | 28.3, CH$_2$ |
| 7 | 5.16, 1H, brs (6.0) | 123.9, CH | 5.23, 1H, t (7.0) | 125.1, CH |
| 8 | – | 135.5, C | – | 134.1, C |
| 9 | 2.00, 2H, dd, (7.5, 7.0) | 39.5, CH$_2$ | 1.61, 3H, s | 16.0, CH$_3$ |
| 10 | 2.08, 2H, m | 26.5, CH$_2$ | 2.35, 2H, m | 34.7, CH$_2$ |
| 11 | 5.17, 1H, t (6.0) | 125.4, CH | 2.34, 2H, m | 34.9, CH$_2$ |
| 12 | – | 134.3, C | – | 173.3, C |
| 13 | 2.24, 1H, m; 2.07, 1H, m | 36.2, CH$_2$ | 1’, – | 175.4, C |
| 14 | 1.50, 1H, m; 1.58, 1H, m | 28.9, CH$_2$ | 4.50, 1H, ddd, (11.5, 8.5, 5.5) | 49.3, CH |
| 15 | – | 3.51, 1H, br d (10.5) | 76.7, CH | 3’ |
| 16 | – | 88.7, C | 4’ |
| 17 | 2.63, 2H, dd (9.0, 7.5); 1.92, 1H, ddd (13.0, 8.0, 8.0); 2.20, 1H, m | 29.2, CH$_2$ | 6.00 brs | – |
| 18 | – | 30.6, CH$_2$ | – | 6.16 brs |
| 19 | – | 176.7, C |
| 20 | 1.37, 3H, s | 21.3, CH$_3$ |
| 21 | 1.61, 3H, s | 16.0, CH$_3$ |
| 22 | 1.61, 3H, s | 13.9, CH$_3$ |

\[\text{Table 3.} \] 1H and 13C NMR data for compounds 4, 5, and (-)-sponalisolide B.

- Spectrum recorded at 500 MHz in CDCl$_3$.
- Spectrum recorded at 125 MHz in CDCl$_3$.
- Spectrum recorded at 400 MHz in CDCl$_3$ \[42\].
- Spectrum recorded at 125 MHz in CDCl$_3$ \[42\].
Table 4. Selected $^{13}$C NMR data at C-15-C-20 of 4 and 10 and the correspondent carbons C-7-C-12 of the related compounds 11 and 12.

|       | 4 $^a$ | 10 (15R,16S) $^b$ | C#       | 11 (7R,8S) $^c$ | 12 (7R,8R) $^c$ |
|-------|--------|-----------------|---------|----------------|-----------------|
| C-15  | 76.7   | 75.5            | C-7     | 75.1           | 76.2            |
| C-16  | 88.7   | 88.9            | C-8     | 88.9           | 88.9            |
| C-17  | 29.2   | 27.8            | C-9     | 27.6           | 29.2            |
| C-18  | 30.6   | 29.5            | C-10    | 29.5           | 30.7            |
| C-19  | 176.7  | 177.3           | C-11    | 177.1          | 176.6           |
| C-20  | 21.3   | 23.0            | C-12    | 23.1           | 21.4            |

$a$ Spectrum recorded at 125 MHz in CDCl$_3$. $^b$ Spectrum recorded at 75 MHz in CDCl$_3$. $^c$ Spectrum recorded at 125 MHz in CDCl$_3$.

Figure 4. $^1$H NMR chemical shift differences $\Delta \delta$ (δS – δR) in ppm for α-methoxy-α-(trifluoromethyl)-phenylacetyl (MTPA) esters of 4.

Figure 5. Structures of known compounds 10–12.

(-)-Sponalisolide B (5) was isolated as a colorless oil, [α]$_D^{25}$ −8.5 (c 0.34, CHCl$_3$). Through detailed analysis of NMR spectroscopic data (Table 3), in particular two-dimensional (2D) NMR correlations, the structure of 5 was established to be identical to that of the known (−)-sponalisolide B [42]. However, the coupling constants and spin-spin splitting patterns of the proton H$_2$-6 (δ$_H$ 2.25, dt, 2H, $J = 7.5, 7.0$ Hz at 500 MHz in CDCl$_3$) were wrongly assigned. We, herein, reanalyzed the spectrum and provided the correct NMR data for 5.

With the aim of discovering bioactive compounds from these isolates, the cytotoxic activities of the isolated compounds 1–7 against the proliferation of three cancer cell lines including murine leukemia (P388), human bile duct carcinoma (HuCCT), and human colon adenocarcinoma (DLD-1), and a human dermal fibroblast cell line (CCD-966SK) were evaluated, using the Alamar Blue assay [55,56]. The results indicated that none of the tested metabolites exhibited cytotoxic activity (IC$_{50} > 20 \mu$g/mL).

The anti-inflammatory activities of compounds 1–7 on inhibition of superoxide anion (O$_2^-$) generation and elastase release in the FMLF/CB-stimulated human neutrophils [57–59] were also evaluated. The results (Table 5) showed that 1 exhibited potent activity to inhibit the superoxide anion generation (91.38 ± 2.91%) and elastase release (90.29 ± 7.71%) at
10 µM, with the IC$_{50}$ values of 3.37 ± 0.21 and 4.07 ± 0.60 µM, respectively. Compound 5 was also found to display significant inhibitory activity against the superoxide anion generation (IC$_{50}$ = 5.31 ± 1.52 µM), and the percentage of inhibition was 67.12 ± 6.00% at 10 µM. Due to the noncytotoxic character and the potent activity toward the superoxide anion generation and elastase release, 1 and 5 can be considered to be the promising anti-inflammatory agents.

Table 5. Effects of compounds 1–7 on superoxide anion generation and elastase release in N-formyl-methionyl-leucyl phenylalanine/cytochalasin B (fMLF/CB)-induced human neutrophils.

| Compound | Superoxide Anion | Elastase |
|----------|------------------|----------|
|          | IC$_{50}$ (µM) a | Inh %    | IC$_{50}$ (µM) a | Inh %    |
| 1        | 3.37 ± 0.21      | 91.38 ± 2.91 *** | 4.07 ± 0.60      | 90.29 ± 7.71 *** |
| 2        | 3.47 ± 0.68 **   | 14.03 ± 3.28 *  |
| 3        | 8.85 ± 3.73      | 18.00 ± 6.08 *  |
| 4        | 2.61 ± 1.26      | -1.07 ± 7.93    |
| 5        | 5.31 ± 1.52      | 35.18 ± 6.00 ** |
| 6        | 9.44 ± 5.04      | 19.24 ± 3.86 ** |
| 7        | 12.79 ± 6.01     | 25.87 ± 4.18 ** |
| LY294002 c | 1.88 ± 0.77     | 90.27 ± 3.87 *** | 2.58 ± 0.67     | 77.59 ± 2.34 *** |

Percentage of inhibition (Inh %) at 10 µM. Results are presented as mean ± SEM (n ≥ 3). * p < 0.05, ** p < 0.01, *** p < 0.001 as compared with the control (DMSO). a Concentration necessary for 50% inhibition (IC$_{50}$). b The compound is not considered to be anti-inflammatory when IC$_{50}$ value is >10 µM. c A phosphatidylinositol-3-kinase inhibitor was used as a positive control.

3. Materials and Methods

3.1. General Procedures

Measurements of optical rotations and IR spectra were carried out on a JASCO P-1020 polarimeter and FT/IR-4100 infrared spectrophotometer (JASCO Corporation, Tokyo, Japan), respectively. ESIMS and HRESIMS were performed on a Bruker APEX II (Bruker, Bremen, Germany) mass spectrometer. The NMR spectra were recorded on a Varian 400MR FT-NMR at 400 and 100 MHz for $^1$H and $^{13}$C, respectively or a Varian Unity INOVA500 FT-NMR at 500 and 125 MHz for $^1$H and $^{13}$C, respectively (Varian Inc., Palo Alto, CA, USA). Silica gel or reversed-phase (RP-18, 230–400 mesh) silica gel was used for column chromatography and analytical thin-layer chromatography (TLC) analysis (Kieselgel 60 F-254, 0.2 mm, Merck, Darmstadt, Germany), respectively. Isolation and purification of compounds by high-performance liquid chromatography (HPLC) were achieved using an Hitachi L-2455 HPLC apparatus (Hitachi, Tokyo, Japan) equipped with a Supelco C18 column (250 × 21.2 mm, 5 µm, Supelco, Bellefonte, PA, USA).

3.2. Animal Material

The sponge Spongia sp. was collected during March 2016, off the Red Sea Coast at Jeddah, Saudi Arabia (21°22′11.08″ N, 39°06′56.62″ E). A voucher sample (RSS-1) has been deposited at the Department of Pharmacognosy, College of Pharmacy, King Saud University, Saudi Arabia.

3.3. Extraction and Separation

The Spongia sp. was collected and freeze-dried. The freeze-dried material (550 g dry wt) was minced and extracted exhaustively with EtOAc/MeOH/CH$_2$Cl$_2$ (1:1:0.5) (3 × 10 L). The solvent-free extract was suspended in water and partitioned with CH$_2$Cl$_2$, EtOAc, and then n-BuOH saturated with water to obtain CH$_2$Cl$_2$ (18.47 g), EtOAc (0.782 g), and n-BuOH (1.0 g) fractions. The CH$_2$Cl$_2$ fraction was chromatographed over silica gel column, using EtOAc in n-hexane (0% to 100%, stepwise), to yield 12 fractions (F1–F12). F6 (1.21 g), eluted with n-hexan/EtOAc (1:1), was re-chromatographed on a RP-18 column using MeOH in H$_2$O (50% to 100%, stepwise) to give 15 subfractions (F6-1 to F6-15). F6-5 (83.0 mg), F6-8 (85.2 mg), F6-11 (21.1 mg), and F6-14 (23.5 mg) were purified on RP-18
HPLC separately, using MeOH/H$_2$O (1:4:1), CH$_3$CN/H$_2$O (1:1.7), MeOH/H$_2$O (1:5.1), and CH$_3$CN/H$_2$O (1:6:1), in order, to afford 2 (55.5 mg) from F6-8, 6 (6.2 mg) from F6-5, 1 (10.2 mg) from F6-11, and 4 (7.4 mg) from F6-14. F7 (1.1 g), eluted with n-hexane/ EtOAc (1:3), was isolated using RP-18 silica gel column chromatography and MeOH in H$_2$O (50% to 100%, stepwise) as a mobile phase to result in 20 subfractions (F7-1 to F7-20). F7-4 (16.1 mg) and F7-6 (25.7 mg) were further separated on RP-18 HPLC, using CH$_3$CN/H$_2$O (1:1.7) and (1:2.5), separately, to afford 3 (4.6 mg) from F7-4, 5 (9.1 mg) and 7 (4.3 mg) from F7-6.

3.3.1. 17-Dehydroxysponalactone (1)

White powder, $[\alpha]_{D}^{25}$ +27.7 (c = 0.71, CHCl$_3$); IR (neat) $\nu_{\text{max}}$ 3455, 3401, 2962, 2927, 2864, 1752, 1663, 1455, 1387, 1186, 1150, 1111, 1060, 1019, 890.0, and 757 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$); and $^{13}$C (125 MHz, CDCl$_3$) data, see Table 1. ESIMS $m/z$ 369 [M + Na]$^+$; $^1$H NMR (C$_5$D$_5$N, 400 MHz) $\delta$H 7.37 (1H, br s, H-16), 7.26 (1H, br s, H-15), 4.44 (1H, d, $J$ = 9.6 Hz, H-19), 4.30 (1H, br s, H-1), 3.94 (1H, d, $J$ = 9.6 Hz, H-19), 2.66 (1H, m, H-12), 2.60 (1H, m, H-12), 2.26 (1H, m, H-9), 2.14 (1H, d, $J$ = 11.5 Hz, H-5), 2.12 (1H, m, H-7), 1.76 (1H, m, H-11), 1.67 (1H, m, H-6), 1.60 (1H, m, H-11), 1.57 (1H, m, H-6), 1.56 (1H, m, H-7), 1.28 (3H, s, H$_3$-18), 1.24 (3H, s, H$_3$-17), 0.94 (3H, s, H$_3$-20); $^{13}$C NMR (C$_5$D$_5$N, 100 MHz) $\delta$C 181.0 (C-3), 138.0 (CH, C-15), 136.0 (C-14), 135.7 (CH, C-16), 120.5 (C, C-13), 84.3 (C, C-2), 82.7 (CH, C-1), 74.4 (CH$_2$, C-19), 57.0 (CH, C-5), 47.8 (CH, C-9), 47.6 (C, C-4), 47.0 (C, C-10), 40.9 (CH$_2$, C-7), 35.1 (C, C-8), 27.4 (CH$_3$, C-17), 23.8 (CH$_3$, C-18), 20.9 (CH$_2$, C-11), 20.4 (CH$_2$, C-12), 18.9 (CH$_2$, C-6), 14.5 (CH$_3$, C-20). HRESIMS $m/z$ 369.1672 [M + Na]$^+$ (calcd for C$_{20}$H$_{30}$O$_2$Na, 369.1673).

3.3.2. Spongiafuranic Acid A (2)

Colorless oil, IR (neat) $\nu_{\text{max}}$ 3105, 2920, 2918, 2875, 1708, 1654, 1500, 1446, 1386, 1298, 1210, 1163, 1024, and 847 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$); and $^{13}$C (100 MHz, CDCl$_3$) data, see Table 2. ESIMS $m/z$ 231 [M + Na]$^+$*. HRESIMS $m/z$ 231.0994 [M + Na]$^+$ (calcd for C$_{12}$H$_{16}$O$_3$Na, 231.0997).

3.3.3. Spongiafuranohydroxamic Acid A (3)

Colorless oil, IR (neat) $\nu_{\text{max}}$ 3407, 3252, 2918, 2875, 1704, 1634, 1442, 1372, 1298, 1205, 1136, 1027, and 963 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$); and $^{13}$C (100 MHz, CDCl$_3$) data, see Table 2. ESIMS $m/z$ 246 [M + Na]$^+$*. HRESIMS $m/z$ 246.1098 [M + Na]$^+$ (calcd for C$_{12}$H$_{17}$NO$_3$Na, 246.1100).

3.3.4. 16-Epi-Ircifommin G (4)

Colorless oil, $[\alpha]_{D}^{25}$ +4.4 (c 0.74, CHCl$_3$); IR (neat) $\nu_{\text{max}}$ 3432, 2920, 2851, 1769, 1647, 1557, 1456, 1384, 1239, 1162, 1089, 944, 874, and 776 cm$^{-1}$; $^1$H NMR (500 MHz, CDCl$_3$); and $^{13}$C (125 MHz, CDCl$_3$) data, see Table 3. ESIMS $m/z$ 383 [M + Na]$^+$*. HRESIMS $m/z$ 383.2195 [M + Na]$^+$ (calcd for C$_{22}$H$_{33}$O$_5$Na, 383.2198).

3.3.5. Preparation of (S)- and (R)-MTPA Esters of 4

To a solution of 4a (1 mg, 2.8 $\mu$M) in pyridine (100 $\mu$L), R-(−)-MTPA-Cl (5 $\mu$L) was added and left to react overnight at RT. The reaction was ended by addition of water (1.0 mL), and the mixture was further processed, as previously described [53,54], to afford (S)-MTPA ester (4a, 1.4 mg, 2.4 $\mu$M). The correspondent (R)-MTPA ester (4b, 0.9 mg, 1.6 $\mu$M) was similarly obtained from the reaction of S-(−)-MTPA-Cl with 4. $^1$H NMR (CDCl$_3$, 400 MHz) of 4a: $\delta$H 7.340 (1H, br dd, $J$ = 1.8, 1.8 Hz, H-1), 7.208 (1H, br s, H-4), 6.275 (1H, br s, H-2), 5.164 (1H, dd, $J$ = 8.0, 8.0 Hz, H-11), 5.113 (1H, m, H-7), 2.489 (1H, m, H-18a), 2.450 (2H, dd, $J$ = 7.6, 7.6 Hz, H-2), 2.426 (1H, m, H-18a), 1.593 (3H, H$_3$-21), 1.559 (3H, H$_3$-22), and 1.355 (3H, H$_3$-20). $^1$H NMR (CDCl$_3$, 400 MHz) of 4b: $\delta$H 7.338 (1H, br s, H-1), 7.206 (1H, br s, H-4), 6.275 (1H, br s, H-2), 5.174 (1H, ddd, $J$ = 9.2, 9.2, 3.2 Hz, H-11),
5.065 (1H, br dd, J = 7.8, 7.8 Hz, H-7), 2.563 (1H, m, H-18a), 2.541 (1H, m, H-18a), 2.447 (2H, dd, J = 8.0, 8.0 Hz, H_2-5), 1.570 (3H, H_3-21), 1.532 (3H, H_3-22), and 1.376 (3H, H_3-20).

3.4. In Vitro Bioassays

3.4.1. Anti-Inflammatory Activity

Human neutrophils were isolated from the blood of healthy adult volunteers and enriched by using dextran sedimentation, Ficoll–Hypaque gradient centrifugation, and hypotonic lysis, as described previously [59]. Then, neutrophils were incubated in Ca^{2+}-free HBSS buffer (pH 7.4, ice-cold).

Superoxide Anion Generation

Neutrophils (6 × 10^5 cells/mL) incubated (with 0.6 mg/mL ferricytochrome c and 1 mM Ca^{2+}) in HBSS at 37 °C were treated with DMSO (as control) or tested compound for 5 min. Neutrophils were primed by 1 µg/mL cytochalasin B (CB) for 3 min before being activated by 100 nM fMLF for 10 min. The change of superoxide anion generation was spectrophotomically measured at 550 nm (U-3010, Hitachi, Tokyo, Japan) [57,58]. LY294002 [2-(4-morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one] was used as a positive control.

Elastase Release

Neutrophils (6 × 10^5 cells/mL) incubated (with 100 µM MeO-Suc-Ala-Ala-Pro-Val-p-nitroanilide and 1 mM Ca^{2+}) in HBSS at 37 °C were treated with DMSO or the tested compound for 5 min. Neutrophils were, then, activated with fMLF (100 nM)/CB (0.5 µg/mL) for 10 min. The change of elastase release was spectrophotomically measured at 405 nm (U-3010, Hitachi, Tokyo, Japan) [58].

3.4.2. Cytotoxic Activity

P388, HuCCT-1, DLD-1, and CCD-966SK cell lines were purchased from the American Type Culture Collection (ATCC). Cytotoxicities of compounds 1–7 were measured using Alamar Blue assay [55,56], with doxorubicin hydrochloride used as a positive control.

3.4.3. Statistical Analysis

Data are displayed as the mean ± SEM and comparisons were performed by one-way ANOVA with Dunnett analysis. All results were obtained from more than 3 biological replicates. A p value of 0.05 or less was considered to be significant. The software Prism (GraphPad Software, San Diego, CA, USA) was used for the statistical analysis.

4. Conclusions

The chemical investigation of dichloromethane-soluble fraction of the organic extract of a Red Sea sponge _Spongia_ sp. resulted in the isolation and identification of a rare A-ring contracted secospongian diterpenoid 17-dehydroxysponalactone (1) and three new furanornerpenoids 2–4. Compound 1 was found to be noncytotoxic but was shown to exhibit potent inhibitory activity against the superoxide anion generation and elastase release in the fMLF/CB-induced neutrophils, and 5 was also found to display strong inhibitory activity against the superoxide anion generation. Therefore, 1 and 5 are the promising candidates for further development of anti-inflammatory agents.

Supplementary Materials: HRESIMS, ^1^H, ^13^C, DEPT, HMQC, COSY, HMBC, and NOESY spectra of new compounds 1–4 are available online at https://www.mdpi.com/1660-3397/19/1/38/s1, Figure S1: HRESIMS spectrum of 1, Figure S2: ^1^H NMR spectrum of 1 in CDCl_3_ at 500 MHz, Figure S3: ^13^C NMR spectrum of 1 in CDCl_3_ at 125 MHz, Figure S4: HSQC spectrum of 1 in CDCl_3, Figure S5: ^1^H--^1^H COSY spectrum of 1 in CDCl_3, Figure S6: HMBC spectrum of 1 in CDCl_3, Figure S7: NOESY spectrum of 1 in CDCl_3, Figure S8: HRESIMS spectrum of 2, Figure S9: ^1^H NMR spectrum of 2 in CDCl_3_ at 400 MHz, Figure S10: ^13^C NMR spectrum of 2 in CDCl_3_ at 100 MHz, Figure S11: HSQC spectrum of 2 in CDCl_3, Figure S12: ^1^H--^1^H COSY spectrum of 2 in CDCl_3, Figure S13: HMBC spectrum of 2 in CDCl_3, Figure S14: NOESY spectrum of 2 in CDCl_3, Figure S15: HRESIMS spectrum
of 3, Figure S16: $^1$H NMR spectrum of 3 in CDCl$_3$ at 400 MHz, Figure S17: $^{13}$C NMR spectrum of 3 in CDCl$_3$ at 100 MHz, Figure S18: HSQC spectrum of 3 in CDCl$_3$, Figure S19: $^1$H $^1$H COSY spectrum of 3 in CDCl$_3$, Figure S20: HMBC spectrum of 3 in CDCl$_3$, Figure S21: NOESY spectrum of 3 in CDCl$_3$, Figure S22: HRESIMS spectrum of 4, Figure S23: $^1$H NMR spectrum of 4 in CDCl$_3$ at 500 MHz, Figure S24: $^{13}$C NMR spectrum of 4 in CDCl$_3$ at 125 MHz, Figure S25: HSQC spectrum of 4 in CDCl$_3$, Figure S26: $^1$H NMR spectrum of 4 in CD$_3$OD at 400 MHz, Figure S27: $^{13}$C NMR spectrum of 4 in CD$_3$OD at 100 MHz, Figure S28: HSQC spectrum of 4 in CD$_3$OD, Figure S29: $^1$H $^1$H COSY spectrum of 4 in CD$_3$OD, Figure S30: HMBC spectrum of 4 in CD$_3$OD, Figure S31: NOESY spectrum of 4 in CD$_3$OD, Figure S32: HRESIMS spectrum of 5, Figure S33: $^1$H NMR spectrum of 5 in CDCl$_3$ at 125 MHz, Figure S34: $^{13}$C NMR spectrum of 5 in CDCl$_3$ at 125 MHz, Figure S35: HSQC spectrum of 5 in CDCl$_3$

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