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# Spongiabutenolides A - D: Minor γ-Hydroxybutenolide Diterpenoids from a Philippines *Spongia* sp.

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**Abstract:** Four novel diterpenoids, spongiabutenolides A - D (**2-5**), all of which contain a γ-hydroxybutenolide moiety, have been isolated from a Philippines marine sponge of the genus *Spongia*. The structures of spongiabutenolides A - D (**2-5**) and the methyl esters **6** and **7** were elucidated by interpretation of spectral data. Spongiabuteonolide A (**2**) was synthesized from spongia-13(16),14-diene-19-oic acid (**1**) by singlet oxygen oxidation. © 1999 Elsevier Science Ltd. All rights reserved.

**Keywords:** Sponges, marine metabolites, diterpenoids, NMR.

Dictyoceratid sponges are a rich source of bioactive terpenoid metabolites, including the anti-inflammatory sesterterpene manoalide and the protein phosphatase inhibitor dysidiolide.[1,2] Sesquiterpenes with the pentacyclic scalarin skeleton containing either furan or γ-hydroxybutenolide moieties have previously been described,[3,4] as well as linear and tetracyclic diterpenoid furan derivatives from *Spongia* spp.[5,6] A major class of metabolites isolated primarily from sponges of the genus *Spongia* consists of diterpenoids containing the tetracyclic spongian carbon skeleton, exemplified by (+)-spongia-13(16),14-diene-19-oic acid (**1**).[7] In addition to yielding furan **1** as the major metabolite, a collection of a *Spongia* sp. from the Philippines contained a series of novel γ-hydroxybutenolides based on the spongian skeleton. Spongiabutenolides A - D (**2-5**) consist of two pairs of regioisomeric γ-hydroxybutenolides, which contain either a carboxylic acid or a hydroxymethylene group at C-19. Furthermore, each spongiabutenolide consists of an inseparable mixture of two stereoisomers at the hemiacetal carbon in the γ-hydroxybutenolide ring, which complicated both purification and structural elucidation in this series. In this paper, we report the structural elucidation of the spongiabutenolides A - D (**2-5**) using spectroscopic data that relied heavily on 1D-TOCSY and HMQC experiments to resolve overlapping NMR signals.

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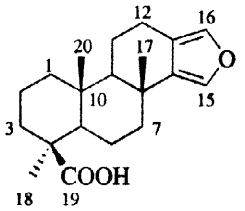
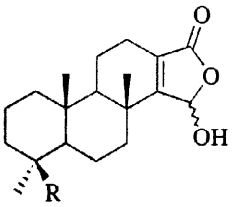
Table. $^{13}C$ and $^{1}H$ NMR data for acids **2** and **3** and methyl esters **6** and **7** in DMSO-$d_6$.

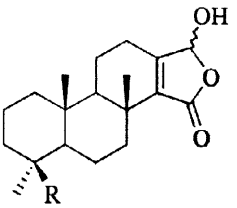
| | **2** | | | | **3** | | | **6** | | **7** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| C# | $\delta_C$ | $\delta_H$ | mult., int. | HMBC | $\delta_C$ | $\delta_H$ | mult., int. | $\delta_C$ | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | 39.5 | 1.79 | m, 1 H | | 39.2 | 1.78 | m, 1 H | 39.5 | 1.76 | 39.7 | 1.78 |
| | | 0.91 | m, 1 H | | | 0.86 | m, 1 H | | 0.90 | | 0.90 |
| 2 | 18.6 | 1.40 | m, 2 H | | 18.6 | 1.40 | m, 2 H | 18.5 | 1.41 | 18.4 | 1.42 |
| 3 | 37.6 | 2.02 | m, 1 H | | 37.4 | 2.01 | m, 1 H | 37.4 | 2.04 | 37.6 | 2.05 |
| | | 0.98 | m, 1 H | | | 0.94 | m, 1 H | | 1.10 | | 1.10 |
| 4 | 42.8 | | | | 42.9 | | | 43.2 | | 43.5 | |
| 5 | 55.9 | 1.10 | m, 1 H | | 56.1 | 1.18 | m, 1 H | 56.0 | 1.14 | 56.1 | 1.15 |
| 6 | 19.0 | 2.00 | m, 1 H | | 19.2 | 1.96 | m, 1 H | 19.0 | 1.90 | 19.2 | 1.90 |
| | | 1.80 | m, 1 H | | | 1.81 | m, 1 H | | 1.84 | | 1.80 |
| 7 | 35.8 | 1.90 | m, 1 H | | 35.5 | 2.48 | m, 1 H | 35.7 | 1.91 | 35.2 | 2.47 |
| | | 1.40 | m, 1 H | | | 1.10 | m, 1 H | | 1.45 | | 1.16 |
| 8 | 36.3 | | | | 34.5 | | | 36.5 | | 35.0 | |
| 9 | 54.5 | 1.03 | d, 14 | | 55.2 | 1.06 | m, 1 H | 54.4 | 1.05 | 55.7 | 1.08 |
| 10 | 38.1 | | | | 37.6 | | | 37.3 | | 37.8 | |
| 11 | 18.6 | 1.84 | m, 1 H | C-13 | 16.6 | 1.84 | m, 1 H | 16.6 | 1.83 | 16.2 | 1.82 |
| | | 1.43 | m, 1 H | | | 1.40 | m, 1 H | | 1.43 | | 1.40 |
| 12 | 20.9 | 2.26 | m, 1 H | C-9, C-13, C-14 | 23.8 | 2.32 | m, 1 H | 20.9 | 2.25 | 24.0 | 2.38 |
| | | 1.11 | m, 1 H | | | 2.20 | m, 1 H | | 1.10 | | ? |
| 13 | 125.8 | | | | 159.6 | | | 125.9 | | 159.0 | |
| 14 | 168.3 | | | | 136.5 | | | 168.0 | | 136.0 | |
| 15 | 97.2 | 6.10 | s, 1 H | | 169.0 | | | 97.5 | 6.10 | 168.0 | |
| 16 | 171.0 | | | C-15 (**3** only) | 96.7 | 5.82 | s, 1 H | 171.0 | | 96.4 | 6.02 |
| 17 | 19.9 | 1.20 | s, 3 H | C-7, C-8, C-9, C-14 | 20.6 | 1.10 | s, 3 H | 20.0 | 1.16 | 20.2 | 1.09 |
| 18 | 28.4 | 1.15 | s, 3 H | C-3, C-4, C-5, C-19 | 28.3 | 1.14 | s, 3 H | 28.1 | 1.14 | 28.0 | 1.13 |
| 19 | 178.3 | | | | 178.4 | | | 176.8 | | 177.0 | |
| 20 | 13.9 | 0.80 | s, 3 H | C-1, C-5, C-6, C-9 | 13.4 | 0.80 | s, 3 H | 13.6 | 0.78 | 13.8 | 0.70 |
| 21 | | | | | | | | 51.1 | 3.60 | 51.4 | 3.58 |




**1**




**2** R =COOH
**4** R =$CH_2OH$
**6** R =COOMe




**3** R =COOH
**5** R =$CH_2OH$
**7** R =COOMe

A sample of *Spongia* sp. (collection # NCI 2416) was collected by hand using SCUBA (-15m) at the southwest point of Haningad Island in the Philippines in May 1997. The $^1$H NMR spectrum of a methanolic extract of the sponge revealed the presence of the furan **1**, but also contained a series of broad singlets between 5 and 7 ppm. Chromatography of the methanolic extract resulted in the isolation of furan **1** ($5 \times 10^{-2}$ % wet wt) as the major secondary metabolite, followed by a 7:4 mixture of the spongiabutenolides A (**2**, $7 \times 10^{-3}$ % wet wt) and B (**3**, $4 \times 10^{-3}$ % wet wt), spongiabutenolide C (**4**, $8 \times 10^{-4}$ % wet wt) and spongiabutenolide D (**5**, $4 \times 10^{-4}$ % wet wt).

The structural elucidation of the mixture of spongiabutenolides A (**2**) and B (**3**) was initially complicated by the absence of a molecular ion in either the EI or FAB mass spectra, and because the hemiacetal hydroxyl signals were too broad to be observed in the $^1$H NMR spectrum in $CDCl_3$. However, the $^1$H NMR spectrum of the mixture of **2** and **3** in DMSO-$d_6$ clearly indicated the presence of four hydroxyl signals, each of which was coupled to a hemiacetal proton signal, and the relative intensities of these peaks suggested that the four pairs of signals could be assigned to two stereoisomeric hemiacetal groups on two regioisomeric γ-hydroxybutenolides. Having recognized the presence of the hemiacetal group, we were able to assign the molecular formula $C_{20}H_{28}O_5$ to both **2** and **3**, based on the mass of the $[M+H]^+$ ion and interpretation of the $^{13}$C NMR spectrum. On treatment with diazomethane, the mixture of acids **2** and **3** was converted into a mixture of the corresponding methyl esters **6** and **7**, which were easily separated by HPLC. After completing the structural assignment of the methyl esters **6** and **7**, we eventually separated the mixture of spongiabutenolides A (**2**) and B (**3**) with difficulty, thus allowing the spectral data of the acids to be acquired.

Both methyl esters **6** and **7** had the same molecular formula, $C_{21}H_{30}O_5$, which gave rise to a pseudo-molecular ion at *m/z* 361 ($[M-OH]^+$) in the mass spectra. Assignment of the NMR spectra of the methyl esters **6** and **7** was complicated both by signal overlap and the multiplicity observed for certain signals due to the presence of two stereoisomers at each hemiacetal center. The use of 1D-TOCSY experiments was critical for assignment of the $^1$H NMR spectra, since every proton spin system could be distinguished by selective irradiation of a well-resolved signal in each system. The $^{13}$C NMR data was then unambiguously assigned using both HMQC and HMBC data. In the HMBC spectrum of methyl ester **6**, there were correlations

between the Me-18 signal at δ 1.14 and both the C-19 ester carbonyl at 176.8 and the C-5 carbon at 56.0, from which a series of correlations, C-5 to Me-20 to C-9 to Me-17, could be traced. The Me-17 signal at δ 1.16 showed a correlation to an olefinic carbon signal at 171.0, which was assigned to the β-carbon of the γ-hydroxybutenolide ring. In the HMBC spectrum of methyl ester **7**, a similar set of correlations established the ABC tricyclic ring system but the Me-17 signal at δ 1.09 showed a correlation to an olefinic carbon signal at 136.0, which was assigned to the α-carbon of the regioisomeric γ-hydroxybutenolide ring.

The relative stereochemistry of the compounds was established by interpretation of the ROESY spectra of butenolides **2** and **6**. In both spectra, ROESY correlations were observed between the Me-20 and Me-17 signals, and in the spectrum of the methyl ester **6** there was a further correlation from the Me-20 signal to the ester signal at δ 3.60, indicating that the two methyl groups and the carboxylic acid or ester were axial with respect to *trans*-fused six-membered rings and on the same face of the molecules. The Me-18 signal showed a ROESY correlation to the H-5 signal, as expected for the stereochemistry illustrated. Having determined the structures of the methyl esters **6** and **7**, were able to assign the NMR data for spongiabutenolides A (**2**) and B (**3**) from the HMQC, HMBC and ROESY spectra (Table 1). The structure of spongiabutenolide A (**2**) was confirmed by singlet oxygen oxidation of spongia-13(16),14-diene-19-oic acid (**1**) using an established procedure.[8] Spongiabutenolide B (**3**) was also observed by $^1$H NMR as a product of this reaction, but no attempt was made to purify it because it was such a minor product (ca. 6%).

Spongiabutenolides C (**4**) and D (**5**) are isomeric and have the molecular formula $C_{20}H_{30}O_4$, which was determined from the low resolution mass spectral data coupled with the $^{13}$C NMR spectrum of alcohol **4**. The $^1$H NMR spectra of the alcohols contained signals at δ 3.70 (d, 1 H, $J$ = 14 Hz) and 3.40 (d, 1 H, $J$ = 14 Hz) for **4** and 3.72 (d, 1 H, $J$ = 16 Hz) and 3.44 (d, 1 H, $J$ = 16 Hz) for **5**, which were assigned to hydroxy methylene groups. The $^{13}$C NMR spectrum of alcohol **4** contained a hydroxymethylene signal at δ 65.1 and four signals at 171.0, 164.0, 127.8 and 98.4 that were assigned to a γ-hydroxybutenolide ring with the same regiospecificity as that of spongiabutenolide A (**2**) but the regiospecificity of the γ-hydroxybutenolide ring in alcohol **5** had to be assumed from the chemical shift of H-16 at δ 5.82 in the $^1$H NMR spectrum. The stereochemistry of **4** was assigned using ROESY data but that of **5** was assumed.

Regioisomeric mixtures of γ-hydroxybutenolides, together with the corresponding furans, have been reported previously.[5] We had earlier demonstrated that regioisomeric mixtures of γ-hydroxybutenolides could be synthesized by singlet oxygen oxidation of 3-substituted and 3,4-disubstituted furans and that regiospecificity could be induced by employing a hindered base to catalyze the rearrangement of the intermediate $^1O_2$ addition product.[8] We used this reaction to synthesize a >10:1 mixture of γ-hydroxybutenolides **2** and **3** from the 3,4-disubstituted furan acid **1**. Schmitz and coworkers recently reported γ-methoxybutenolides corresponding to the γ-hydroxybutenolides **2** and **3**, and speculated about their origin.[9] We suspect that the γ-methoxybutenolides are artifacts resulting from storage in methanol or extraction with

dichloromethane/methanol, either of which may catalyze exchange of the hydroxyl group by a methoxyl group. We believe that the spongiabutenolides A - D (**2-5**) are natural products since they were clearly present in crude methanolic extracts prepared under mild, neutral conditions. All of the compounds above were tested for anti-cancer activity in a 25 cell-line panel but none showed significant cytotoxicity.

## Experimental

*General*: All solvents used in the isolation and purification of the compounds were distilled prior to use. All NMR experiments except $^{13}C$ and DEPT experiments were run using a Varian Inova 300 MHz spectrometer. $^{13}C$ and DEPT spectra were collected on a Varian Gemini 400 MHz spectrometer. HRMS data were obtained from the mass spectrometry facility at the University of Minnesota and LRMS data were obtained using a Hewlett-Packard 5890 GC-mass spectrometer.

*Isolation and Characterization*: The specimen of *Spongia* sp. was collected by hand using SCUBA while diving at depths of 15-20 m at the southwest point of Haningad Island in the Philippines in May 1997 and was kept frozen until extraction with MeOH (4 x 1L). The crude extract was partitioned between equal volumes of EtOAc and $H_2O$ (2 x 200 mL), and the organic extract further partitioned using normal phase silica VLC (vacuum liquid chromatography) separation eluting with a stepwise gradient from hexane to EtOAc. The furan **1** (250 mg) was found as the major metabolite and was eluted from the VLC column using 20% hexane in ethyl acetate. Material eluting using 40% hexane in EtOAc was subjected to normal phase HPLC using 1:1 hexane/EtOAc. The major components of this fraction eluted at 10.6 min, and contained a mixture (53 mg) of spongiabutenolides A (**2**) and B (**3**), while the minor components, spongiabutenolides C (**4**, 2.3 mg) and D (**5**, 1.0 mg), appeared as well resolved peaks at 13 and 15.6 min. Attempts to separate acids **2** and **3** using normal and reversed phase chromatography were initially unsuccessful, but after treatment with diazomethane (MNNG-Diazomethane Kit, Aldrich) the methyl ester derivatives **6** (5.2 mg) and **7** (2.3 mg) were separated on reversed phase HPLC using 30% $H_2O$ in MeOH as eluant. Samples of the purified acids, spongiabutenolide A (**2**) and spongiabutenolide B (**3**), were eventually obtained by reversed phase HPLC using a new column.

*Spongiabutenolide A (2)*: white solid; $[\alpha]_D$ = 28.7 (*c* 7.1, MeOH); UV (MeOH) 215 nm (ε 9330); IR (film) 3260, 2920, 1730, 1705, 1695 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) see Table; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) see Table; CIHRMS *m/z* 349.2016 $[M+H]^+$ (calcd. for $C_{20}H_{29}O_5$ 335.2014).

*Spongiabutenolide B (3)*: white solid; $[\alpha]_D$ = 27.1 (*c* 3.4, MeOH); UV (MeOH) 209 nm (ε 8480); IR (film) 3250, 2945, 1750, 1705, 1020 $cm^{-1}$; $^1H$ NMR (300 MHz, DMSO-$d_6$) see Table; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) see Table; CIHRMS *m/z* 349.2016 $[M+H]^+$ (calcd. for $C_{20}H_{29}O_5$ 335.2014).

*Spongiabutenolide C (4)*: white solid; $^1H$ NMR ($CD_3OD$) δ 6.06 (br s, 1 H, H-15), 3.70 (d, 1 H, *J* = 14 Hz, H-19), 3.40 (d, 1 H, *J* = 14 Hz, H-19'), 2.36 (m, 1 H, H-12), 2.29 (m, 1 H, H-12'), 2.02 (m, 1 H, H-7), 1.87 (m, 1 H, H-11), 1.79 (m, 1 H, H-1), 1.77 (m, 1 H, H-6), 1.77 (m, 1 H, H-3), 1.66 (m, 1 H, H-2), 1.65 (m, 1 H, H-6'),

1.47 (m, 1 H, H-7'), 1.45 (m, 1 H, H-11'), 1.21, (s, 3 H, H-17), 1.12 (m, 1 H, H-9), 1.05 (m, 1 H, H-5), 0.95 (s, 6 H, H-18, H-20), 0.94 (m, 1 H, H-3'), 0.92 (m, 1 H, H-1'); $^{13}$C NMR ($CD_3OD$) δ 171.0 (C-16), 164.0 (C-14), 127.8 (C-13), 98.4 (C-15), 65.1 (C-19), 58.4 (C-5), 57.4 (C-9), 41.0 (C-3), 39.8 (C-4), 38.7 (C-10), 38.3 (C-8), 38.2 (C-7), 36.1 (C-1), 27.6 (C-18), 22.2 (C-12), 20.8 (C-17), 19.3 (C-6), 19.1 (C-2), 18.0 (C-11), 17.4 (C-20). ESIMS *m/z* 333.1 $[M-H]^-$, CIHRMS *m/z* 335.2228 $[M+H]^+$ (calcd. for $C_{20}H_{31}O_4$ 335.2222).

*Spongiabutenolide D (5)*: white solid; $^1$H NMR ($CD_3OD$) selected signals, δ 5.82 (br s, 1 H, H-16), 3.72 (d, 1 H, $J$ = 16 Hz, H-19), 3.44 (d, 1 H, $J$ = 16 Hz, H-19'), 2.60 (m, 1 H, H-7), 2.48 (m, 1 H, H-12), 2.25 (m, 1 H, H-7'), 1.21 (s, 3 H, H-17), 0.97 (s, 3 H, H-18), 0.92 (s, 3 H, H-20); ESIMS *m/z* 333.2.

*Spongiabutenolide A methyl ester (6)*: white solid ; $^1$H NMR (DMSO-$d_6$) see Table; $^{13}$C NMR (DMSO-$d_6$) see Table; ESIMS *m/z* 361.0.

*Spongiabutenolide B methyl ester (7)*: white solid; $^1$H NMR (DMSO-$d_6$) see Table; $^{13}$C NMR (DMSO-$d_6$) see Table; ESIMS *m/z*$^-$ 361.1.

*Singlet oxygen oxidation of spongia-13(16),14-diene-19-oic acid (1)*: A stream of $O_2$ was bubbled through a stirred solution of furan **1** (50 mg), diisopropylethylamine (100 μl), and polymer-bound rose bengal catalyst (1 mg). The solution was cooled to -78 °C and singlet oxygen was generated by shining a 150 watt sodium lamp on the reaction vessel. After 1 h at -78 °C, the solution was allowed to warm and the solvent was evaporated under vacuum, after which the residue was dissolved in EtOAc and passed through a small plug of silica. Final purification of spongiabutenolide A (**2**, 38 mg, 69% yield) was achieved using normal phase HPLC using 55% hexane in THF as eluant.

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