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Spongiapyridine and Related Spongians Isolated from an Indonesian *Spongia* sp.

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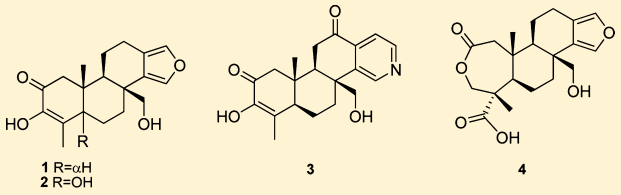
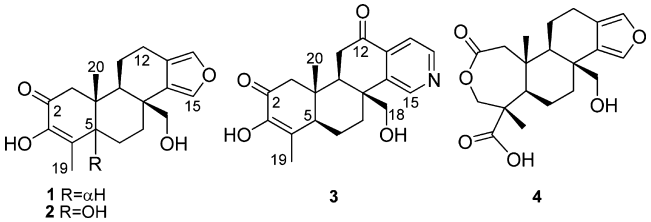
Supporting Information

ABSTRACT: New compounds 18-nor-3,17-dihydroxyspongia-3,13(16),14-trien-2-one (**1**), 18-nor-3,5,17-trihydroxyspongia-3,13(16),14-trien-2-one (**2**), and spongiapyridine (**3**) and the known compound 17-hydroxy-4-*epi*-spongialactone A (**4**) were isolated from an Indonesian sponge of the genus *Spongia*. The structures of **1**−**3** were deduced by analyses of physical and spectroscopic data. Diterpene **3** is unusual, as the D-ring is a pyridyl ring system rather than the standard δ-lactone. The structure elucidation of this compound was complicated by facile exchange of the axial proton at the C-11 methylene with deuterium from methanol-d_4. The isolated compounds were tested for biological activity in a battery of in vitro assays (TNF-α-induced NFκB, LPS-induced iNOS, RXR stimulation, quinone reductase 1 induction, aromatase inhibition, TRPM7 ion channels, and aspartic protease BACE1 inhibition). Norditerpene **2** modestly inhibited aromatase with an IC_{50} of 34 μM and induced quinone reductase 1 activity with a CD (the concentration needed to double the enzymatic response) of 11.2 μM. The remaining isolates were inactive.



Sponges in the genus Spongia are a rich source of structurally interesting terpenoids. Many of these compounds are steroidal-like in structure, but contain a furan ring in place of the typical steroidal cyclopentane ring. These metabolites, termed spongians, are abundant in different species of *Spongia* obtained from Australia, the Caribbean, and the Mediterranean Sea, among other locations.[1,2] Although biological activity has not typically been reported for these spongians, several compounds in this class are cytotoxic against the KB[3] and murine leukemia cell lines.[4]

We report here the chemical investigation of an undescribed species of *Spongia* from Sulawesi, Indonesia, that has led to the isolation of four spongians (**1**−**4**). Spongiapyridine (**3**) contains a unique pyridyl D-ring, while 18-nor-3,5,17-trihydroxyspongia-3,13(16),14-trien-2-one (**2**) displays moderate inhibition of aromatase and induction of quinone reductase 1 (QR1). The structures of these compounds, along with those previously isolated, allow us to propose a plausible biosynthetic pathway for this series of compounds.



RESULTS AND DISCUSSION

Compound **1** was isolated as a white powder after multiple rounds of chromatography. High-resolution mass spectrometry gave a protonated molecule at m/z 317.1747 $[M + H]^+$. This datum is consistent with a molecular formula of $C_{19}H_{24}O_4$ and indicated eight double-bond equivalents. The carbon spectrum showed seven sp^2 carbons, six of which were C=C bonds and one of which was a conjugated carbonyl carbon ($\delta_{C\text{-}2}$ 195.4). Therefore, the compound had four rings to account for the remaining double-bond equivalents.

The planar structure of **1** was assembled by analyses of COSY and HMBC correlations. Beginning with the tertiary methyl group (H_3-20, 0.88 ppm), the observation of HMBC correlations to two methines, a quaternary carbon, and a methylene (C-1, C-5, C-9, C-10) was critical toward the assembly of fragment 1 (Figure 1). This alkyl chain was elongated through COSY correlations between $\delta_{H\text{-}5}$ 2.62 and $\delta_{H\text{-}6a}$ 1.91 and between $\delta_{H\text{-}6a}$ 1.91 and an adjacent methylene ($\delta_{H\text{-}7b}$ 1.41). HMBC correlations from the proton resonance at $\delta_{H\text{-}19}$ 1.88 indicated a chain of two sp^2 carbons connecting to C-5, but were unable to distinguish which of the two sp^2 carbons was attached directly to the methyl group H_3-19. A set of HMBC correlations between the signal at $\delta_{H\text{-}1a}$ 2.73 ppm and signals at $\delta_{C\text{-}2}$ 195.4 ppm and $\delta_{C\text{-}3}$ 145.7 ppm resolved the

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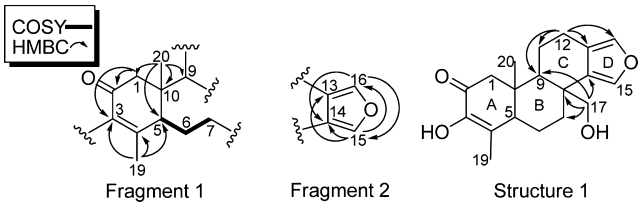

Figure 1. Key HMBC (^{1}H–^{13}C) and COSY correlations for **1**.

ambiguity of the order of sp^2 carbons, creating an α,β-unsaturated ketone moiety within a six-membered ring.

The two downfield protons at $\delta_{H\text{-}16}$ 7.14 and $\delta_{H\text{-}15}$ 7.17, as well as four remaining sp^2 carbons, hinted at the possibility of a heteroaromatic ring. The $^1J_{C-H}$ value for position 16 extracted from the HMBC data suggested the heteroaromatic ring was a furan moiety ($^1J_{C-H}$ = 201 Hz vs a reference value of 202 Hz in furan[5]). With a furan moiety in mind, HMBC correlations from $\delta_{H\text{-}16}$ 7.14 to C-14 and C-15, in addition to correlations from $\delta_{H\text{-}15}$ 7.17 to C-16 and C-13, suggested the furan ring is as shown in Figure 1.

Fragments 1 and 2 were joined based on HMBC correlations observed from the methylene proton resonance of the primary alcohol ($\delta_{H\text{-}17a}$ 3.87) to C-7, C-8, C-9, and C-14. These correlations also indicated the presence of a second six-membered ring. Of the remaining two methylenes, C-12 was placed nearer the sp^2 center (C-13), on the basis of being the more downfield resonance. The more shielded methylene C-11 was then attached to C-12 and C-9 in order to satisfy the last degree of unsaturation. HMBC correlations from the proton resonance at $\delta_{H\text{-}12a}$ 2.80 ppm confirmed the position of C-11 and C-12 in the C-ring. The remaining hydroxy group, required by the molecular formula, was attached to C-3, to provide the structure of **1** depicted, based on the chemical shifts of the carbons in the A-ring.

The relative configuration of **1** was deduced as follows. ROESY correlations between H_3-20 and H_2-17 indicated they were *syn* and axial. The axial orientation of the methine H-5 was evident due to the observation of a 12.9 Hz coupling to H-6. For the fourth stereogenic center, H-9 was assigned as axial due to a ROESY correlation with H-5. It should be noted that the relative configuration deduced for **1** is in accord with the configuration of these centers in all known spongians.

Three other analogues were identified in the extract. Evidence that indicated these compounds belong to the spongian structural class included the resonances for the primary alcohol at C-17, the aromatic proton resonances (H-15, H-16), and the two methyl singlets (H-19, H-20). Carbon and proton resonances for these three analogues are tabulated in Tables 2 and 3, and their structure elucidation is discussed below.

Compound **2** was clearly an analogue of **1**, as it possessed many of the same carbon and proton resonances. A close inspection of the NMR data indicated the quaternary carbon at C-5 was shifted downfield, which suggested an alcohol functional group was present ($\delta_{C\text{-}5}$ 76.8). HMBC correlations from H-19 and H-20 supported C-5 as the oxygenated carbon (Figure 2), while HRMS analyses supported the required molecular formula $C_{19}H_{24}O_5$ through the observed *m*/*z* of 315.1580 $[M + H - H_2O]^+$. Of the four stereocenters of **2**, only three could be determined. Specifically, an NOE correlation between H_3-20 and H_2-17 indicated a *syn* axial relationship, while the 13.2 Hz coupling constant observed at H-9 indicated it was axial as well. The last stereocenter at C-5 could not be assigned due to rapid exchange, on the NMR time scale, of the

Table 1. NMR Spectroscopic Data (500 MHz, CD_3OD) for 1

position[b]	δ_C, type	δ_H (*J* in Hz)	COSY	HMBC (^{1}H to ^{13}C)[a]
1a	53.3, CH_2	2.73, d (16.6)	H-1, H-19	2, 3, 5, 6, 9, 10, 20
1b		2.18, d (16.6)	H-1	2, 5, 9, 10, 20
2	195.4, C			
3	145.7, C			
4	132.9, C			
5	50.1, CH	2.62, dddd (12.9, 3.1, 2.0, 2.0)	H-6b	2, 3, 4, 6, 9, 10, 20
6a	21.7, CH_2	1.91, dddd (13.4, 3.1, 3.1, 3.1)	H-6b	4, 5, 7, 8, 10
6b		1.62, dddd (13.4, 13.4, 13.4, 3.1)	H-6a, H-5	4, 5, 7, 8, 10
7a	34.9, CH_2	2.53, ddd (13.4, 3.1, 3.1)	H-7b	5, 6, 8, 9, 17
7b		1.41, dddd (13.4, 13.4, 3.7, 1.2)	H-7a, H_2-6	5, 6, 8, 14, 17
8	40.8, C			
9	54.4, CH	1.72, m		
10	42.4, C			
11a	18.9, CH_2	1.72, m		
11b		1.72, m		
12a	21.1, CH_2	2.80, dd (16.0, 4.0)	H-12b	9, 11, 13, 14, 16
12b		2.56, m	H-12a	
13	120.8, C			
14	131.3, C			
15	139.2, CH	7.17, d (1.4)		13, 14, 16
16	138.3, CH	7.14, d (1.4)		14, 15
17a	62.8, CH_2	3.87, d (10.9)	H-17b	7, 8, 9, 14
17b		3.48, d (10.9)	H-17a	7, 8, 14
19	13.3, CH_3	1.88, d (2.0)		2, 3, 4, 5
20	15.6, CH_3	0.88, d (0.9)	H-1	1, 5, 9, 10

[a]HMBC correlations optimized for $^nJ_{CH}$ = 7 Hz. [b]Numbering is consistent with previously published compounds of this type.[1]

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Table 2. ^{13}C NMR Spectroscopic Data (125 MHz, CD_3OD) for 2−4

	2	3[a]	4
position	δ_C	δ_C	δ_C
1	50.3	51.9	46.6
2	194.2	194.5	177.0
3	143.0	145.7	74.8
4	132.9	131.9	53.8
5	76.8	49.0[b]	58.0
6	28.7	21.8	22.7
7	36.8	33.8	35.0
8	32.8	42.5	41.7
9	48.6	50.4	55.3
10	44.0	41.8	40.2
11	22.6	35.9	19.9
12	21.4	199.1	21.6
13	122.4	139.5	120.8
14	127.0	145.6[b]	131.6
15	139.9	149.3	139.0
16	138.1	148.3	138.2
17	71.0	120.3	62.8
18		66.2	177.9
19	10.7	13.2	14.9
20	18.7	14.6	17.7

[a]3 was dissolved in CD_3OH. [b]Chemical shifts identified via HMBC correlations.

alcohol proton in aprotic solvents ($CDCl_3$ and CD_3CN) and at lower temperatures (down to −20 °C).

Spongiapyridine (3) had an observed HRESI-TOFMS m/z of 342.1701 $[M + H]^+$, being consistent with a molecular formula of $C_{20}H_{23}NO_4$. Comparison of the NMR data for this compound with those of 1 showed that 3 contained identical

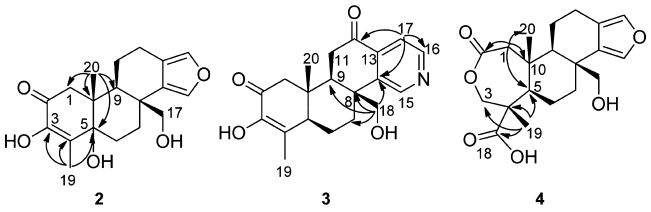

Figure 2. Key HMBC correlations of 2−4.

features in the A- and B-rings and contained a primary alcohol as well. The C- and D-rings, however, included a nitrogen atom, a second carbonyl carbon ($\delta_{C\text{-}12}$ 199.1), a third aromatic proton, and downfield shifts of the aromatic protons ($\delta_{H\text{-}15}$ 8.71, $\delta_{H\text{-}16}$ 8.58, $\delta_{H\text{-}17}$ 7.82). In contrast to the $^1J_{C-H}$ value observed in 1, the resonance at $\delta_{H\text{-}16}$ 8.58 displayed a $^1J_{C-H}$ value of 182 Hz, which was no longer consistent with a furan moiety.[5] Instead, this one-bond coupling constant was consistent with a carbon adjacent to the nitrogen in a pyridine moiety.[5] Consequently, a $^1H-^{15}N$ HMBC experiment was performed to help support this supposition. In this experiment, a correlation was observed from the signal at $\delta_{H\text{-}15}$ 8.58 to a nitrogen resonating at δ_N −68 (referenced to nitromethane), further supporting the inclusion of a pyridine moiety in 3. Additional structural modifications were deduced based on HMBC correlations from $\delta_{H\text{-}18b}$ 3.74 to $\delta_{C\text{-}14}$ 145.6, which connected the pyridine ring to ring B, and between $\delta_{H\text{-}17}$ 7.82 and $\delta_{C\text{-}12}$ 199.1, indicating the carbonyl was at C-12 (Figure 2).

The final structural fragment left unaccounted for in 3, according to a phase-sensitive HSQC experiment, was a putative methine that resonated at $\delta_{H\text{-}11}$ 2.60 and showed a COSY correlation to $\delta_{H\text{-}9}$ 2.36. However, if a methine was present at C-11, then this would result in a molecular formula inconsistent with the observed MW of 341. Upon closer

Table 3. ^{1}H NMR Spectroscopic Data (500 MHz, CD_3OD) for 2−4

	2	3[a]	4
position	δ_H (J in Hz)	δ_H (J in Hz)	δ_H (J in Hz)
1	2.64, d (17.2)	2.62, m	2.86, m
	2.57, d (17.2)	2.20, d (16.4)	2.84, m
3			4.67, brs
			3.95, brs
5		2.66, d (11.4)[b]	2.25, d (11.9)
6	2.19, ddd (13.4, 10.8, 2.2)	2.17, brd (10.2)	1.75, m
	2.00, m	1.74, m	1.29, m
7	2.10, dd (12.4, 6.5)	2.80, brd (9.9)	2.45, brd (13.0)
	1.78, dddd (11.8, 11.8 3.1, 3.1)	1.73, m	1.32, m
9	1.70, brd (13.2)	2.36, dd (14.5, 4.8)	1.51, d (10.7)
11	1.84, m	3.04, dd (18.5, 14.5)	1.83, dd (13.5, 6.9)
	1.62, ddd (12.8, 12.8, 4.2)	2.60, m	1.74, m
12	2.76, ddd (15.2, 3.1, 3.1)		2.82, m
	2.31, dddd (15.2, 12.8, 4.2, 1.9)		2.55, m
15	7.32, d (1.2)	8.71, brs	7.14, s
16	7.15, brs	8.58, d (4.8)	7.14, s
17	4.01, dd (9.0, 3.1)	7.82, d (4.8)	3.88, d (11.0)
	3.70, dd (9.0, 1.6)		3.49, d (11.0)
18a		4.02, d (11.4)	
18b		3.74, d (11.4)	
19	1.83, s	1.90, d (1.9)	1.25, s
20	1.09, s	1.06, brs	1.03, brs

[a]3 was dissolved in CD_3OH. [b]J value extracted from the 1D TOCSY spectrum obtained by irradiating the resonance at 1.75 ppm.

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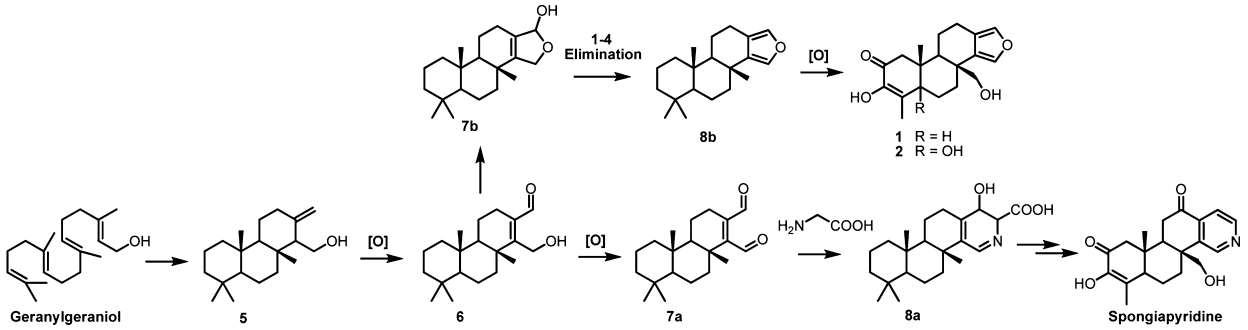

Figure 3. Plausible biosynthetic pathway for spongians originating from geranylgeraniol.

investigation of the ^{13}C spectrum, it became clear that the carbon at $\delta_{C\text{-}11}$ 35.9 was not a singlet, as would be expected in a broad-band-decoupled carbon spectrum, but instead was coupled to another nucleus, displaying a triplet with lines in a ratio of 1:1:1. The multiplicity of this carbon resonance suggested coupling with a nucleus that had a quantum spin number of one. One possible explanation for the observed coupling was that deuterium from the solvent was exchanging with one of the protons alpha to the C-12 ketone. To test this hypothesis of deuterium exchange, **3** was dissolved in CD_3OH, and the NMR spectra were rerecorded. The result was the collapse of the carbon triplet into a singlet and the appearance of an additional proton at $\delta_{H\text{-}11}$ 3.04, showing that C-11 was a methylene. It is interesting to note that only the axial proton adjacent to the ketone, not the equatorial proton, is exchanged, likely due to the well-known stereoelectronic effect, i.e., overlap of the axial C−H bond with the ketone's p*-orbital as documented by Corey and Sneen that favors removal of that proton.[6]

The relative configuration of **3** was determined in a manner similar to that described for **1**. An NOE correlation between H_3-20 and H_2-18 indicated they were *syn* and axial. H-5 and H-9 had *J* values of 11.4 and 14.5 Hz, respectively, suggesting they are also axial, resulting in proposed structure **3**.

HRESI-LCMS of **4** gave a protonated molecule at *m/z* 363.1790 $[M + H]^+$, consistent with the molecular formula $C_{20}H_{26}O_6$. All the major spectral differences were assigned to the A-ring by comparison of the NMR spectra of **1** and **4**. These changes were two ester/carboxylic acid functional groups, an oxygenated methylene, and an sp^3 quaternary carbon. These changes were at the expense of the *α,β*-unsaturated carbonyl and were satisfied via a seven-membered lactone ring. All of the HMBC correlations from $\delta_{H\text{-}19}$ 1.25 and also from $\delta_{H\text{-}1}$ 2.87 supported this structure (Figure 2). An NOE correlation between $\delta_{H\text{-}19}$ 1.25 and $\delta_{H\text{-}20}$ 1.03 confirmed the configuration at C-4, indicating that **4** was the known compound 17-hydroxy-4-*epi*-spongialactone A. This molecule has previously been isolated, but only as the diacetyl methyl ester derivative by Gunasekera and Schmitz from a *Spongia* sp. specimen.[4] For this reason, we included its spectroscopic data in this report.

All of the spongian diterpenes for which the absolute configurations have been determined belong to the same enantiomeric series.[14−17] It is, therefore, believed that compounds **1**−**4** have the absolute configuration 5*R*,8*R*,9*R*,10*R*.

A plausible biosynthetic pathway is proposed in Figure 3. From geranylgeraniol, a polyolefin cyclization cascade could give structure **5**. After oxidation to yield **6**, attack on the carbonyl carbon by the primary alcohol yields **7b**. A 1,4-elimination of H_2O establishes the furan **8b**, which is followed by several oxidations to yield our new compound **1**. Similar known structures could also be accounted for by this biosynthetic pathway. For example, oxidation from **7b** leads to the known compound zimolactone B, and further oxidation of **7a** to a carboxylic acid before cyclization leads to the framework required for zimolactones A and C[7] (not shown). Also, from **8b**, partial oxidation may account for spongiadiol, spongiatriol, and their C-3 epimers.[8]

Compound **7a** may feed into an alternative pathway to produce **3**. Condensation of **7a** with glycine and subsequent cyclization yields **8a**. Decarboxylation, oxidation at C-12, and A-ring oxidation yields spongiapyridine **3**.

There are many strategies for decreasing cancer mortality through chemoprevention,[9] and a variety of assessments were performed with **1**−**4**. Modest inhibition of TNF-*α*-activated NF-*κ*B activity was observed for all compounds with ED_{50} values around 50 *μ*M (data not shown). No significant activity was observed for inhibition of iNOS activity in LPS-induced RAW 264.7 murine macrophage cells, and no significant induction occurred in a retinoic X receptor response element luciferase reporter gene assay.

Another approach is the inhibition or down-regulating aromatase.[10] Aromatase, a key cytochrome P450 enzyme, catalyzes the rate-limiting aromatization step in the conversion of androgens (testosterone and androstenedione) to estrogens (estradiol and estrone). Aromatase inhibitors decrease bioavailable estrogen and have shown considerable activity in the prevention of certain breast cancers. Such compounds ultimately reduce estradiol receptor stimulation and reduce the formation of genotoxic estrogen metabolites. Certain non-steroidal aromatase inhibitors are already in clinical use for the treatment of breast cancer, and several naturally occurring nonsteroidal aromatase inhibitors have shown promising chemopreventive activity.[11] In the current investigation, compound **2** inhibited aromatase in a dose-dependent manner with an IC_{50} value of 34.4 *μ*M. The other compounds did not achieve 50% inhibition at a concentration of 50 *μ*M.

NAD(P)H:quinone reductase 1, a cytoprotective enzyme, can exhibit cancer protective activity by inhibiting the formation of intracellular semiquinone radicals and by generating *α*-tocopherolhydroquinone, which acts as a chemopreventive agent. Cancer chemoprevention can be achieved by activating QR1. One parameter used to compare the QR1 induction potential of different compounds is the CD value, i.e., the concentration of test compound required to double QR1 activity. With cultured Hepa 1c1c7 cells, **2** demonstrated a CD value of 11.2 *μ*M, which is similar to the CD value of resveratrol (21 *μ*M), a weak QR1 inducer.[9]

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Lastly, all of our isolated compounds were screened against BACE 1 as part of our ongoing search for drug leads for Alzheimer's disease (AD). BACE1 is an aspartic protease and one of the major players in the amyloid cascade hypothesis for AD.[12,13] All compounds showed no significant activity toward the aspartic protease BACE1 (<100 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco DIP-370 digital polarimeter at the sodium D-line (589 nm). UV absorbances were measured on a Varian Cary 50 Bio UV−vis spectrophotometer. IR spectroscopy was measured as a thin film on a CaF_2 disk using a Shimadzu IRAffinity-1 FTIR. ^1H, ^{13}C, and 2D NMR experiments were performed on a Varian Unity Inova 500 MHz spectrometer. NMR spectra were referenced to the appropriate residual solvent signal (δ_H 3.30, δ_C 49.1 for MeOH-$d_{3/4}$) with chemical shifts reported in δ units (ppm). The HSQC experiments were optimized for $^1J_{C,H}$ = 140 Hz and HMBC experiments for $^3J_{C,H}$ = 7 Hz. Mixing times for ROESY and NOESY experiments were 500 ms and were generally 80 ms for the 1D TOCSY experiments. High-resolution mass spectrometric data were obtained on an LC-MS-TOF spectrometer using ESI mode.

Collection and Isolation. The sponge was collected from Bunaken Marine Park, Sulawesi, Indonesia, in 1992, and freeze-dried and stored at −20 °C. The freeze-dried voucher appears to have come from a cavernous encrusting sponge with surface conules, the color in life appears to be charcoal gray, and the interior is tan. The texture is soft and compressible, tearing relatively easily. The skeleton is composed of slightly fasciculated primary fibers cored with foreign spicule detritus, and a light dusting of foreign spicules is found in the collagenous ectosome. The secondary spongin network is well developed. The sponge is most closely comparable to species in the genus *Spongia* (order Dictyoceratida, family Spongiidae) with homogeneous spongin fibers, but the primary fibers are slightly fasciculated as in species of the genus *Cacospongia*. A voucher specimen has been deposited at the Natural Museum, London (NHMUK2012.3.27.3).

The freeze-dried sponge (50 g) was extracted overnight three times with a 1:1 mixture of MeOH and CH_2Cl_2. The resulting extract (7.4 g) was subjected to a liquid−liquid partitioning protocol between hexanes, CH_2Cl_2, BuOH, and H_2O. The CH_2Cl_2 partition (2.6 g) was then dry loaded and separated by reversed-phase (C8) chromatography (four steps: 25%, 50%, 75%, 100% MeOH/H_2O) to yield three fractions at each step and 12 fractions in total.

The first 75% MeOH fraction (550 mg) was separated by HPLC (Luna C8; 250 × 10 mm, 5 μ) using a flow rate of 2.75 mL/min and a linear gradient of 30−60% MeOH in H_2O over 30 min, followed by 60−100% over the next 10 min. This afforded pure 18-nor-3,17-dihydroxyspongia-3,13(16),14-trien-2-one (**1**, t_R = 24 min, 12 mg, 0.16% yield) and 17-hydroxy-4-*epi*-spongialactone A (**4**, t_R = 16 min, 8.0 mg, 0.11% yield).

The third 75% MeOH fraction (73 mg) was subjected to reversed-phase HPLC on the aforementioned Luna C-8 semipreparative column using a linear gradient of 30−80% CH_3CN in H_2O over 30 min at a flow rate of 2.75 mL/min. This afforded pure 18-nor-3,5α,17-trihydroxyspongia-3,13(16),14-trien-2-one (**2**, t_R = 26.5 min, 2 mg, 0.03% yield).

The second fraction of the 50% MeOH step (160 mg) underwent reversed-phase HPLC on a Luna C-18 semipreparative column (250 × 10 mm, 5 μm) with the gradient 20−30% CH_3CN in H_2O over 30 min at 2.75 mL/min in order to yield spongiapyridine (**3**, t_R = 24 min, 4.5 mg, 0.061% yield).

*18-Nor-3,17-dihydroxyspongia-3,13(16),14-trien-2-one (**1**):* white, amorphous powder; $[\alpha]^{22}_D$ −7.0 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 281 (3.25), 202 (3.54); IR ν_{max} 3390, 1651 cm^{-1}; see Table 1 for NMR data; HRESI-TOFMS *m*/*z* 317.1747 $[M + H]^+$ (calcd for $C_{19}H_{25}O_4$, 317.1753, 1.9 ppm error).

*18-Nor-3,5,17-trihydroxyspongia-3,13(16),14-trien-2-one (**2**):* white, amorphous powder; $[\alpha]^{22}_D$ −7 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 276 (2.95), 202 (3.65); IR ν_{max} 3394, 1641 cm^{-1}; see Tables 2 and 3 for NMR data; HRESI-TOFMS *m*/*z* 315.1580 $[M + H - H_2O]^+$ (calcd for $C_{19}H_{23}O_4$, 315.1596, 5.1 ppm error).

*Spongiapyridine (**3**):* white, amorphous powder; $[\alpha]^{22}_D$ −0.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 282 (3.39), 203 (3.82); IR ν_{max} 3373, 1693 cm^{-1}; see Tables 2 and 3 for NMR data; HRESI-TOFMS *m*/*z* 342.1701 $[M + H]^+$ (calcd for $C_{20}H_{24}NO_4$, 342.1705, 1.2 ppm error).

*17-Hydroxy-4-epi-spongialactone A (**4**):* white, amorphous powder; $[\alpha]^{22}_D$ −2.0 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 276 (2.99), 202 (3.69); IR ν_{max} 3419, 1696 cm^{-1}; see Tables 2 and 3 for NMR data; HRESI-TOFMS *m*/*z* 363.1790 $[M + H]^+$ (calcd for $C_{20}H_{27}O_6$, 363.1808, 3.4 ppm error).

ASSOCIATED CONTENT

Supporting Information

Copies of the ^1H, ^{13}C, and 2D NMR spectroscopic data for all new compounds associated with this article, bioassay protocols, and a photo of the producing organism are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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