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Bioactive Terpenes from *Spongia officinalis*

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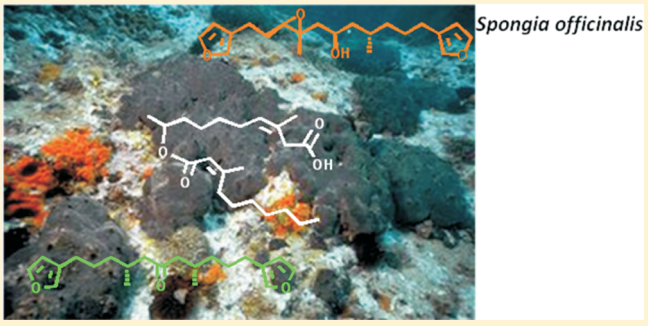
Supporting Information

ABSTRACT: The terpene metabolite pattern of Mediterranean *Spongia officinalis* was chemically investigated. This study resulted in the isolation of a series of sesterterpenes and C_{21} furanoterpenes, according to the literature data on this sponge. Four new oxidized minor metabolites (compounds **1**, **2**, **3**, and **4**) were isolated along with six known compounds of the furospongin series (compounds **5**−**8**, **9**, and **10**) and three scalarane sesterterpenes (compounds **11**−**13**). Interestingly, tetrahydrofurospongin-2 (**6**) and dihydrofurospongin-2 (**7**), which were among the main metabolites, induced biofilm formation by *Escherichia coli*. All compounds isolated were also assayed for antibacterial and antifungal properties.



Marine sponges have been the focus of much recent interest due to two main reasons: (a) they are a rich source of bioactive secondary metabolites[1] and (b) they form close associations with a wide variety of microorganisms.[2] These two facts are strictly related because in several cases the production of the bioactive molecules isolated from the sponges can be ascribed to the associated bacteria.[3] This increasing research interest has greatly improved our knowledge about the communication between sponges and their microbial associates even though many gaps remain in the understanding of such interactions. An interesting aspect of studies in this field is the chemistry of bacterial biofilms and in particular the identification of the molecules that could mediate by either induction or inhibition such sponge−microbe interactions.[4] In this light, in the continuation of our studies on bioactive compounds from marine organisms, we have investigated the chemistry of a specimen of Mediterranean *Spongia officinalis* (Spongiidae), collected off the Sicily coast (Mazara del Vallo). Some selected terpene fractions from the ether extract of the sponge were observed to induce biofilm formation by *Escherichia coli*.

Previous chemical studies on *S. officinalis* from different sites have resulted in the isolation of linear furanosesterterpenes, C_{21} furanoterpenes, and scalarane sesterterpenes.[5] These groups of compounds have also been reported from other genera of Spongiidae families[5a,c,6] and from nudibranch mollusks feeding on them.[7]

The terpene metabolites identified in this study included all three groups of compounds. Four new molecules were chemically characterized including a C_{21} furanoterpene, 7,8-epoxyfurospongin-1 (**1**), two linear carboxylic acids [officinoic acid A (**2**) and officinoic acid B (**3**)], and a linear furanosesterterpene, isofurospongin-4 (**4**). These molecules were isolated together with five known C_{21} furanoterpenes of the furospongin series, compounds **5**−**9**, the linear furanosesterterpene **10**, and three scalarane sesterterpenes, **11**−**13**. Tetrahydrofurospongin-2 (**6**) and dihydrofurospongin-2 (**7**), which were among the main metabolites of the extract, were shown to be responsible for the biofilm induction activity observed in two selected crude terpene fractions of the extract.

RESULTS AND DISCUSSION

The Et_2O-soluble portion from the acetone extract of *S. officinalis* was fractionated by a silica gel column using a light petroleum ether/Et_2O gradient system with increasing polarity as eluent. By this chromatographic step, four fewer polar furospongin metabolites, anhydrofurospongin-1 (**5**),[5c] tetrahydrofurospongin-2 (**6**),[5c] dihydrofurospongin-2 (**7**),[5c] and furospongin-1 (**8**),[5a] were obtained with only minor impurities. Compounds **6** and **7** were further purified by reversed-phase HPLC chromatography. An additional, more polar fraction constituted of a complex mixture of terpenes was also recovered from the silica gel column and subsequently submitted to further purification steps by silica gel and reversed-phase HPLC chromatography to give 7,8-epoxyfurospongin-1 (**1**), furospongolide (**9**),[8] furospongin-4 (**10**),[5d] officinoic acid A (**2**), officinoic acid B (**3**), isofurospongin-4 (**4**), 12α-deoxoscalarin (**11**),[5f] 16-deacetoxy-12-epi-scalarafuranacetate

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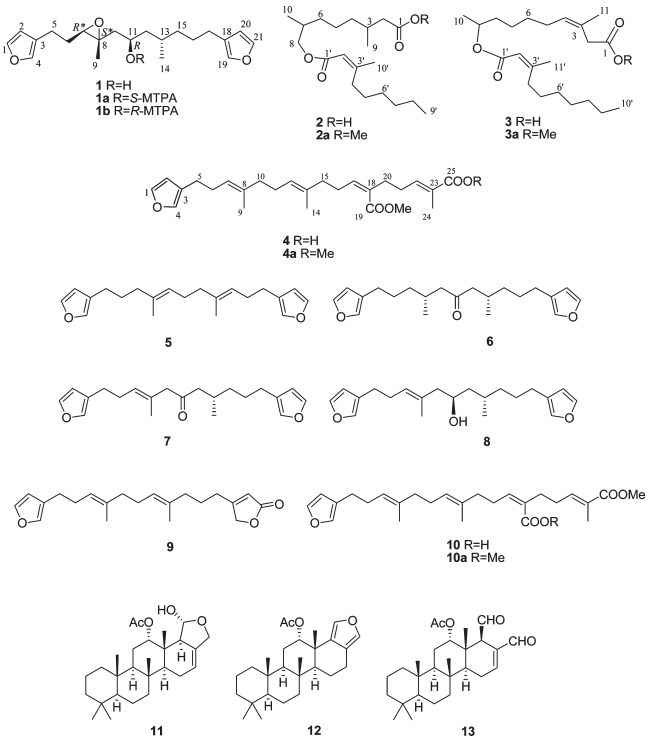
(**12**),[5g] and scalaradial (**13**)[9] (see Experimental Section). Known compounds were identified by comparison of their spectroscopic data with literature values, whereas the structures of unprecedented compounds **1**, **2**, **3**, and **4** were established as follows.



A preliminary analysis of the NMR spectra of new molecules **1**, **2**, **3**, and **4** showed structural relationships of both **1** and **4** with the co-occurring furanoterpenes **5**−**8**, **9**, and **10**, whereas officinoic acid A (**2**) and officinoic acid B (**3**) appeared to be unrelated to either furanoterpenes or scalarane metabolites.

Compound **1** was isolated as an optically active colorless oil and showed a positive Ehrlich reaction, suggesting a furan ring. It had the molecular formula $C_{21}H_{30}O_4$, as suggested by the sodiated molecular peak at m/z 369.2040 in the HRESIMS spectrum. The ^{13}C and ^{1}H NMR spectra of **1** were consistent with a typical structure of a C_{21} difuranoterpene (Table 1) and showed close similarities with those of co-occurring furospongin-1 (**8**),[5a,10] suggesting a related structure. The only difference was the presence of an epoxide ring in **1** replacing the double bond at C-7 in **8**. In fact, the ^{1}H NMR spectrum of **1** contained a methyl singlet at δ 1.30 (H_3-9) and an epoxide proton signal at δ 2.80 (t, J = 6.2 Hz, H-7) rather than the vinyl signals due to the trisubstituted double bond present in **8**. Accordingly, in the ^{13}C NMR spectrum of **1** two oxygenated carbon signals at δ 63.2 (CH, C-7) and 60.9 (C, C-8) (Table 1) were observed in the place of the olefinic carbons in **8**.[10] Thus compound **1** was 7,8-epoxyfurospongin-1. The structure was confirmed by 2D-NMR data. The relative configuration of the epoxide moiety was defined as drawn in formula **1** by NOE difference experiments. Steric effects were observed between the methylene at δ 1.81 (H_2-6) and the singlet methyl at δ 1.30 (H_3-9) as well as between the epoxide proton at δ 2.80 (H-7) and the methylene at δ 1.75 (H-10a) and 1.46 (H-10b). The absolute configuration at C-11 was established by applying the modified Mosher's method. (*S*)- and (*R*)-MTPA esters of **1** were obtained by treating **1** with

Table 1. NMR Spectroscopic Data[a] for 7,8-Epoxyfurospongin-1 (1)

position	δ_C, type	δ_H (J in Hz)	HMBC[b]
1	142.7, CH	7.37, bs	2, 4
2	110.7, CH	6.30, bs	1
3	124.1, C		2, 4
4	138.9, CH	7.25, bs	1
5a	21.5, CH_2	2.64, m	2, 6
5b		2.59, m	
6	28.8, CH_2	1.81, q (6.2)	5, 7
7	63.2, CH	2.80, t (6.2)	9
8	60.9, C		9
9	17.2, CH_3	1.30, s	7, 10
10a	45.2, CH_2	1.75, m	9, 11
10b		1.46, m	
11	66.7, CH	4.06, m	10, 12
12a	45.2, CH_2	1.48, m	11, 14
12b		1.08, m	
13	28.6, CH	1.71, m	14, 15
14	18.9, CH_3	0.93, d (6.6)	13, 15
15a	37.3, CH_2	1.32, m	13, 14
15b		1.26, m	
16a	26.9, CH_2	1.59, m	15, 17
16b		1.51, m	
17a	24.9, CH_2	2.40, t (7.3)	16, 20
18	125.1, C		17, 19, 20
19	138.5, CH	7.21, bs	21
20	110.7, CH	6.28, bs	19, 21
21	142.3, CH	7.34, bs	19, 20

[a] 600 MHz for ^{1}H NMR spectra and 75.47 MHz for ^{13}C NMR spectra ($CDCl_3$); assignments aided by COSY, HSQC, and HMBC. [b] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

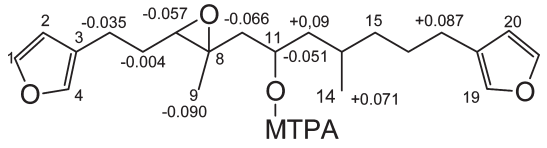


Figure 1. Chemical shift differences ($\Delta\delta = \delta_{S\,\mathrm{ester}} - \delta_{R\,\mathrm{ester}}$) for (*S*)- and (*R*)-MTPA derivatives of compound **1**.

(*R*)- and (*S*)- MTPA chlorides, respectively, and characterized by 2D-NMR experiments [$\Delta\delta$ ($\delta_{S\ \mathrm{ester}} - \delta_{R\ \mathrm{ester}}$) are reported in Figure 1]. The $\Delta\delta$ values observed for the signals of protons close to the hydroxy group at C-11 indicated the *R* configuration, the same as that reported for furospongin-1 (**8**), the absolute configuration of which has been determined by chemical and spectroscopic methods.[5c,6a,11] Accordingly, by biogenetic considerations, the absolute configuration at C-13 in **1** was tentatively assigned to be the same as in **8**.

The molecular formula $C_{20}H_{36}O_4$ of officinoic acid A (**2**) was deduced by the sodiated molecular ion peak at m/z 363.2498 in the HRESIMS spectrum. The IR spectrum showed two intense bands at 1710 and 1651 cm^{-1}, suggesting the presence of two carbonyl groups. This was confirmed by the ^{13}C NMR spectrum, containing signals at δ 174.7 and 167.1 attributable to a

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Table 2. NMR Spectroscopic Data[a] for Officinoic Acids A (2) and B (3)

	2			3		
position	δ_C mult.	δ_H (J in Hz)	HMBC[c]	δ_C mult.	δ_H (J in Hz)	HMBC[c]
1	174.7, C		2	173.8, C		2
2	40.8, CH_2	2.34, dd (15.0, 5.5)	3, 9	36.8, CH_2	3.06, br s	4, 11
		2.16, dd (15.0, 7.6)				
3	30.1, CH	1.95, m	2, 9	127.9, C		2, 4, 11
4	29.6, CH_2	1.30, m	2, 5, 9	129.4, CH	5.36, br t (7.3)	5, 11
5	31.5, CH_2	1.40, m	6	28.2, CH_2	2.01, m	4, 6
6	30.3, CH_2	1.21, m	5, 10	29.3,[b] CH_2	1.32, m	5
7	32.8, CH	1.76, m	6, 10	29.8,[b] CH_2	1.30, m	5
8	68.4, CH_2	3.96, dd (10.6, 6.2)	6, 10	36.0, CH_2	1.60, m	10
		3.87, dd (10.6, 6.6)			1.48, m	
9	19.3, CH_3	0.97, d (6.7)	2, 3	69.9, CH	4.92, dq (6.2,5.5)	8,10
10	17.1, CH_3	0.93, d (6.7)	7	20.1, CH_3	1.21, d (6.2)	8
11				24.0, CH_3	1.80, br s	2,4
1′	167.1, C		10, 2′	165.7, C		9, 2′
2′	115.8, CH	5.65, br s	10′	116.5, CH	5.62, br s	4′, 11′
3′	160.8, C		2′,4′,10′	160.3, C		2′, 4′, 11
4′	33.3, CH_2	2.61, t (7.3)	5′, 10′	33.5, CH_2	2.61, t (7.0)	5′, 11′
5′	28.8, CH_2	1.45, m	4′, 6′	28.0, CH_2	1.48, m	4′
6′	29.6, CH_2	1.32, m	4′	29.2,[b] CH_2	1.30, m	4′
7′	31.9, CH_2	1.31, m	9′	29.3,[b] CH_2	1.30, m	5′
8′	22.4, CH_2	1.28, m	9′	31.8, CH_2	1.30, m	10′
9′	14.2, CH_3	0.88, t (6.2)	8′	22.7, CH_2	1.27, m	10′
10′	25.2, CH_3	1.88, br s	2′, 4′	14.1, CH_3	0.89, t (6.6)	8′, 9′
11′				25.1, CH_3	1.87, s	2′, 4′

[a] 600 MHz for ^{1}H NMR spectra and 75.47 MHz for ^{13}C NMR spectra ($CDCl_3$); assignments aided by COSY, HSQC, and HMBC. [b] Interchangeable values. [c] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

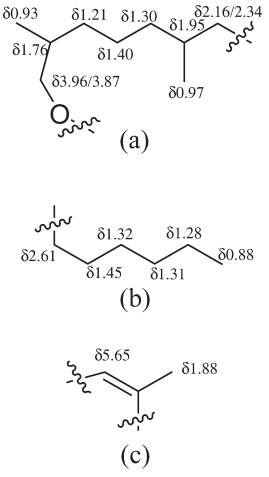

Figure 2. Spin systems for compound **2**.

carboxylic acid and an α,β-unsaturated ester, respectively (Table 2). The carbon spectrum also displayed signals due to a trisubstituted double bond [δ 115.8 (CH, C-2′) and δ 160.8 (C, C-3′)] and 16 sp^3 resonances (4 × CH_3, 10 × CH_2, 2 × CH) including a signal due to an oxygenated methylene [δ 68.4 (CH_2, C-8)], which were consistent with an acyclic carbon skeleton (Table 2). The ^{1}H NMR spectrum showed a vinyl signal at δ 5.65 (1H, br s, H-2′); multiplets at δ 3.96 (1H, dd, J = 10.6 and 6.2 Hz, H_2-8a) and 3.87 (1H, dd, J = 10.6 and 6.6 Hz, H_2-8b), which were assigned to the oxygenated methylene; and four methyl signals at δ 1.88 (br s, H_3-10′), 0.97 (d, J = 6.7 Hz, H_3-9), 0.93 (d, J = 6.7 Hz, H_3-10), and 0.88 (t, J = 6.2 Hz, H_3-9′). The spectrum was completed by aliphatic multiplets at δ 1.45−1.21, 1.76, and 1.95, two doublets of doublets at δ 2.34 (H-2a) and 2.16 (H-2b), and a triplet at δ 2.61 (H_2-4′), all integrating for 20 protons. Analysis of the ^{1}H−^{1}H COSY experiment aided us to easily identify three spin systems, **a**, **b**, and **c** (Figure 2), which were connected by HMBC correlations. In particular, the −CO signal at δ 174.7 (C-1) showed cross-peaks with the methylene protons at δ 2.34/2.16 (H_2-2), thus defining one of the two terminals of the molecule, whereas the second −CO resonating at δ 167.1 (C-1′) was correlated with both the methylene at δ 3.96/3.87 (H_2-8) and the olefinic proton at δ 5.65 (H-2′), implying the connection of the two partial structures **a** and **c**. Diagnostic HMBC correlations were also observed between C-3′ (δ 160.8) and H_3-10′ (δ 1.88), H-2′ (δ 5.65), and H_2-4′ (δ 2.61), leading to structure **2**.

The geometry of the double bond at C-2′ was established as Z by both the carbon value of the vinyl methyl C-10′ (δ 25.2) and a NOE effect between H_3-10′ (δ 1.88) and H-2′ (δ 5.65). The configurations at C-3 and C-7 remain unassigned. Methylation of **2** gave the methyl ester **2a**, which was characterized by 2D-NMR experiments (see Experimental Section). The spectroscopic data were in agreement with the proposed structure. In particular, a long-range correlation between the introduced methoxy group

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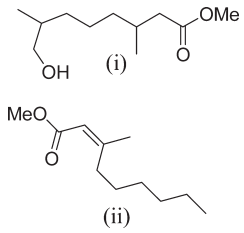

Figure 3. Methyl esters from the alkaline methanolysis of **2a**.

Table 3. NMR Spectroscopic Data[a] for Isofurospongin-4 (4)

position	δ_C mult.		δ_H (J in Hz)	HMBC[b]
1	142.4,	CH	7.33, br s	4
2	110.8,	CH	6.27, br s	1
3	124.9,	C		2, 4
4	138.4,	CH	7.20, br s	2
5	25.2,	CH_2	2.44, t (7.0)	2, 6
6	28.3,	CH_2	2.23, bq (7.0)	5, 7
7	123.5,	CH	5.16, br t (6.5)	6, 9
8	135.7,	C		7, 9, 10
9	15.6,	CH_3	1.58, s	7
10	39.6,	CH_2	1.97, t (7.8)	7, 9
11	26.5,	CH_2	2.05, m	10, 12
12	124.6,	CH	5.10, br t (6.3)	11, 14
13	135.4,	C		12, 14, 15
14	15.8,	CH_3	1.56, s	12, 15
15	39.0,	CH_2	2.02, m	12, 14
16	27.7,	CH_2	2.54, m	15, 17
17	143.1,	CH	5.95, br t (7.2)	16, 20
18	nd,	C		17
19	168.5,	C		17, OCH_3
20	33.5,	CH_2	2.33, m	17, 21
21	28.4,	CH_2	2.29, m	22
22	142.2,	CH	6.74, br t (6.8)	21, 24
23	127.7,	C		22, 24
24	12.0,	CH_3	1.79, s	22
25	169.3,	C		24
OCH_3	51.7,	CH_3	3.70, s	

[a] 600 MHz for ^{1}H NMR spectra and 75.47 MHz for ^{13}C NMR spectra ($CDCl_3$); assignments aided by COSY, HSQC, and HMBC. [b] HMBC correlations, optimized for 10 Hz, are from proton(s) stated to the indicated carbon.

(δ 3.66, OMe) and the terminal ester carbonyl carbon (δ 173.5, C-1) was observed in the HMBC spectrum of **2a**, along with all other expected correlations. To further confirm the proposed structure, compound **2a** was submitted to methanolysis by NaOMe solution to obtain a reaction mixture, which was analyzed by GC-MS. Diagnostic molecular ion peaks at m/z 202 and 184 due to methyl esters (i) and (ii) (Figure 3) produced by methanolysis were observed in the GC-MS spectrum.

Officinoic acid B (**3**) showed spectroscopic similarities with compound **2**. The molecular formula of **3**, $C_{22}H_{38}O_4$ as deduced by HRESIMS, contained an additional C_2H_2 unit with respect to acid **2**. The IR spectrum showed CO stretching bands at 1716 and 1667 cm^{-1} due to the presence of ester and terminal acid functions as in compound **2**. This was also supported by ^{13}C NMR resonances at δ 165.7 (CO, C-1′) and 173.8 (CO, C-1). The ^{1}H and ^{13}C NMR spectra of **3** indicated the presence of two trisubstituted double bonds, one of which is conjugated to an ester carbonyl, two vinyl methyls, two methyls linked to sp^3 carbons, an isolated methylene, and an oxygenated methine (Table 2). These data were consistent with a structure similar to compound **2** exhibiting different alkyl chains. The ^{1}H−^{1}H COSY and HMBC data (significant correlations are reported in Table 2) led us to formulate compound **3** as shown.

The geometries of the double bonds at C-3 and C-2′ were established as *Z*, *Z* by both the carbon values of the vinyl methyls C-11 and C-11′ and diagnostic NOE effects observed between these methyls and the respective olefin protons H-4 and H-2′. The configuration of C-9 remains unassigned. Analogous with **2**, compound **3** was converted into the corresponding methyl ester **3a**, which was characterized by 2D-NMR experiments (see Experimental Section). The subsequent methanolysis of **3a** under the same conditions as reported above for **2a** gave a reaction mixture, which was analyzed by GC-MS. Diagnostic molecular ion peaks at m/z 214 and 198 due to the two methyl esters produced in the reaction were observed in the spectrum, further confirming the proposed structure.

Compound **4** was isolated as a colorless, optically inactive oil and showed a positive Ehrlich reaction. The molecular formula $C_{26}H_{36}O_5$ was indicated by the sodiated molecular ion peak at m/z 451.2478 in the HRESIMS spectrum. The ^{1}H and ^{13}C NMR data of compound **4** closely resembled those of co-occurring furospongin-4 (**10**)[5a,d] (Table 3), which has the same molecular formula, suggesting that **4** is a linear furanosesterterpene with two oxidized methyl groups, analogous with **10**. Analysis of ^{1}H−^{1}H COSY and HSQC experiments confirmed this hypothesis, indicating that **4** differed from **10** only in the esterification site. Accordingly, the proton chemical shift value of H-17 was observed at δ 5.95 in **4** and at δ 6.01 in furospongin-4 (**10**). Diagnostic correlations in the HMBC spectrum of **4** between the carboxy carbon at δ 168.5 (C-19) and both the proton signals at δ 3.70 (3H, s, −OMe) and 5.95 (1H, br t, J = 7.2 Hz, H-17) inferred the esterification at C-19 rather than C-25. Compound **4** was thus named isofurospongin-4. The structure was definitively confirmed by conversion of **4** into the corresponding dimethyl ester **4a**. This compound was identical in all respects with **10a**, the derivative obtained by methylation of **10**. All ^{1}H and ^{13}C NMR resonances of the dimethyl ester **4a** (= **10a**) were assigned as reported in the Experimental Section.

Compounds **4**−**10** were tested for antibacterial and antifungal activities against *E. coli*, *Staphylococcus aureus*, and *Candida albicans*. Only furospongin-4 (**10**) showed weak activity against *S. aureus* at 100 μg/mL.

A very interesting biofilm induction activity was observed for selected terpene fractions containing tetrahydrofurospongin-2 (**6**) and dihydrofurospongin-2 (**7**). Both compounds were purified by HPLC and shown to be responsible for the observed activity (Figure 4). These data suggested that, even at a lower concentration (50 μg/mL), compounds **6** and **7** induced biofilm formation by *E. coli* PHL628 by a factor of 1.57 and 1.93, respectively. However, as the concentration increases, tetrahydrofurospongin-2 (**6**) becomes more efficient in inducing biofilm formation. Conversely, an increase of dihydrofurospongin-2 (**7**) concentration over 50 μg/mL had no additional effect. To our knowledge, this is the first case reported of sponge-produced molecules having bacterial biofilm induction properties. This phenomenon could be related to the symbiosis that marine

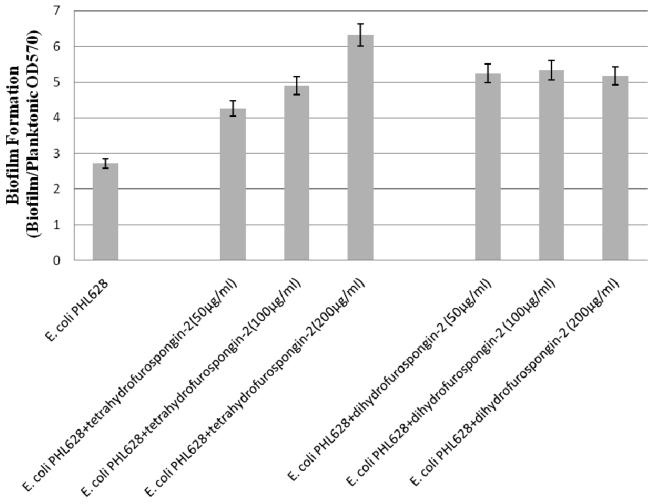

Figure 4. Biofilm formed by *Escherichia coli* PHL628 when incubated in the presence or absence of tetrahydrofurospongin-2 and dihydrofurospongin-2 in microtiter wells. The plate was incubated for 40 h, followed by crystal violet staining and spectrophotometric absorbance measurements (OD_{570}). The ratio of biofilm absorbance/planktonic absorbance was calculated, and this value is presented as the "biofilm formation" on the *y* axis. Bars represent means ± standard errors for six replicates.

organisms (i.e., algae and sponges) are able to form with some strains of bacteria that do not allow biofouling stratification on their surfaces.[12]

Indeed biofouling generally begins with the formation of a biochemical conditioning film onto which bacteria and other microorganisms colonize.[13] Closely following microbial fouling is the colonization by various eukaryotic organisms, including marine invertebrates and algae.[14] If the first bacterial biofilm does not allow the subsequent stratification, the sponge (or alga, or other marine organism) surface will not be contaminated by biofouling. Further studies will be necessary to understand the mechanism of biofilm induction.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco DIP 370 digital polarimeter. IR spectra were measured on a Biorad FTS 155 FTIR spectrophotometer. 1D- and 2D-NMR spectra were recorded on a Bruker Avance-400 (400.13 MHz) and on a Bruker DRX-600 equipped with a TXI CryoProbe in $CDCl_3$ (δ values are referenced to $\underline{C}HCl_3$ at 7.26 ppm), and ^{13}C NMR spectra were recorded on a Bruker DPX-300 (75.47 MHz) (δ values are referenced to $\underline{C}DCl_3$, 77.0 ppm). HRESIMS was conducted on a Micromass Q-TOF micro. GC-MS was carried out on an ion-trap MS instrument in EI mode (70 eV) (Thermo, Polaris Q) connected with a GC system (Thermo, GCQ) by a 5% diphenyl (30 m × 0.25 mm × 0.25 um) column using helium as gas carrier. For HPLC, Waters 501 pumps with a refractometer detector were used with a reversed-phase column (Kromasil C-18, 5 μ, 250 × 4.60 mm, Phenomenex). TLC plates (Kieselgel 60 F_{254}) and silica gel powder (Kieselgel 60, 0.063−0.200 mm) were from Merck.

Biological Material. The sponge *Spongia officinalis* Linnaeus 1759 was collected in May 1999 off Mazara del Vallo along the Sicily coast by one of us (G.V.) at a depth of 80 m, immediately frozen, and transferred to ICB. The sponge was classified by R.v.S. A voucher specimen is stored at the Zoological Museum, University of Amsterdam (numbered as ZMAPOR 21294).

Extraction and Isolation Procedures. The frozen sponge *S. officinalis* (dry weight, 15.2 g) was chopped and then extracted exhaustively with Me_2CO (400 mL × 4) using ultrasound. After filtration and evaporation *in vacuo* of the organic solvent, the residue was subsequently extracted with Et_2O (200 mL × 4) and BuOH (100 mL × 4). The evaporation of the Et_2O and BuOH extracts gave two gummy residues (0.680 and 0.722 g, respectively). The Et_2O extract was analyzed by TLC. Two groups of molecules with different polarity were evidenced by positive reactions with the Ehrlich reagent: a nonpolar fraction at R_f 0.9−0.7 (light petroleum ether/Et_2O, 1:1) and a polar fraction at R_f 0.5−0.4 (light petroleum ether/Et_2O, 1:1). This extract was subjected to silica gel column chromatography using a gradient of light petroleum ether and Et_2O, then $CHCl_3$, and finally MeOH as eluents, to give anhydrofurospongin-1 (**5**) (25 mg), tetrahydrofurospongin-2 (**6**) (65 mg), dihydrofurospongin-2 (**7**) (40 mg), furospongin-1 (**8**) (140 mg), and a more polar fraction (350 mg), which consisted of a complex terpene mixture. This mixture was further fractionated by Si gel column chromatography, using a gradient of light petroleum ether and Et_2O as eluent, obtaining three fractions, A−C. The less polar fraction, A (90 mg), was purified by reversed-phase HPLC (MeOH/H_2O, 9:1) to give 7,8-epoxyfurospongin-1 (**1**) (1.2 mg), furospongolide (**9**) (1.8 mg), furospongin-4 (**10**) (1.6 mg), officinoic acid A (**2**) (0.8 mg), and officinoic acid B (**3**) (0.9 mg). Fraction B (87 mg) was purified by reversed-phase HPLC (MeOH/H_2O gradient), giving isofurospongin-4 (**4**) (2.5 mg). Fraction C (110 mg) was purified by reversed-phase HPLC (MeOH/H_2O, 95:5) to give 12α-deoxoscalarin (**11**) (0.9 mg), 16-deacetoxy-12-epi-scalarafuranacetate (**12**) (1.3 mg), and scalaradial (**13**) (1.0 mg). Tetrahydrofurospongin-2 (**6**) and dihydrofurospongin-2 (**7**) were further purified by reversed-phase HPLC (MeOH/H_2O, 95:5).

Furospongin-1 (***8***)*:* colorless oil; $[\alpha]_D$ +22 (*c* 1.2, $CHCl_3$), $[\alpha]_D$ lit.[5a] +8.8 (*c* 1.0, $CHCl_3$).

7,8-Epoxyfurospongin-1 (***1***)*:* colorless oil; R_f 0.45 (light petroleum ether/Et_2O, 1:1); $[\alpha]_D$ −12 (*c* 0.1, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 220 (2.82); IR (liquid film) ν_{max} 1620, 1575, 1160, 1020 cm^{-1}; ^1H and ^{13}C NMR data in Table 1; HRESIMS *m*/*z* 369.2040 $[M + Na]^+$ (calcd for $C_{21}H_{30}O_4Na$, 369.2042).

Officinoic acid A (***2***)*:* colorless oil; R_f 0.48 (light petroleum ether/Et_2O, 1:1); $[\alpha]_D$ +3.2 (*c* 0.08, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 215 (2.43); IR (liquid film) ν_{max} 1710, 1651 cm^{-1}; ^1H and ^{13}C NMR data in Table 2; HRESIMS *m*/*z* 363.2498 $[M + Na]^+$ (calcd for $C_{20}H_{36}O_4Na$, 363.2511).

Officinoic acid B (***3***)*:* colorless oil; R_f 0.50 (light petroleum ether/Et_2O, 1:1); $[\alpha]_D$ −5.7 (*c* 0.09, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 218 (2.72); IR (liquid film) ν_{max} 1710, 1667 cm^{-1}; ^1H and ^{13}C NMR data in Table 2; HRESIMS *m*/*z* 389.2679 $[M + Na]^+$ (calcd for $C_{22}H_{38}O_4Na$, 389.2668).

Isofurospongin-4 (***4***)*:* colorless oil, R_f 0.42 (light petroleum ether/Et_2O, 1:1); UV ($CHCl_3$) λ_{max} (log ε) 208 (3.12); IR (liquid film) ν_{max} 1705, 1620 cm^{-1}; ^1H and ^{13}C NMR data in Table 3; HRESIMS *m*/*z* 451.2478 $[M + Na]^+$ (calcd for $C_{26}H_{36}O_5Na$, 451.2460).

Preparation of Methylated Compounds 2a, 3a, 4a, and 10a. A 1 mL amount of diazomethane in Et_2O was added to 0.8 mg of compound **10**. After 0.5 h the Et_2O was evaporated *in vacuo*, and compound **10a** was quantitatively obtained. The same procedures were repeated for 0.8 mg of compound **2**, 0.9 mg of compound **3**, and 1 mg of compound **4** to afford **2a**, **3a**, and **4a**, respectively.

Officinoic acid A methyl ester (***2a***)*:* colorless oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.65 (1H, br s, H-2′), 3.85 (1H, dd, *J* = 10.6, 5.9 Hz, H-8a), 3.87 (1H, dd, *J* = 10.6, 6.6 Hz, H-8b), 3.66 (3H, s, OCH_3), 2.61 (2H, t, *J* = 7.3 Hz, H_2-4′), 2.30 (1H, dd, *J* = 14.6, 6.2 Hz, H-2a), 2.13 (1H, dd, *J* = 14.6, 7.6 Hz, H-2b), 1.93 (1H, m, H-3), 1.88 (3H, s, H_3-10′), 1.78 (1H, m, H-7), 1.45 (2H, m, H_2-5′), 1.40 (2H, m, H_2-5), 1.32 (2H, m, H_2-6′), 1.31 (2H, m, H_2-7′), 1.29 (2H, m, H_2-4), 1.29 (2H, m, H_2-8′), 1.19 (2H, m, H_2-6), 0.93 (3H, d, *J* = 6.7 Hz, H_3-9), 0.92 (3H, d, *J* = 6.6 Hz, H_3-10),

0.88 (3H, t, J = 7.0 Hz, H_3-9′). ^{13}C NMR ($CDCl_3$): δ 173.5 (C, C-1), 165.9 (C, C-1′), 160.9 (C, C-3′), 116.0 (CH, C-2′), 68.4 (CH_2, C-8), 51.4 (OCH_3), 41.7 (CH_2,C-2), 33.8 (CH_2, C-4′), 32.7 (CH, C-7), 31.8 (CH_2, C-7′), 30.8 (CH_2, C-6), 30.7 (CH, C-3), 30.6 (CH_2, C-5), 29.7 (CH_2, C-6′), 29.7 (CH_2, C-4), 28.3 (CH_2, C-5′), 25.4 (CH_3, C-10′), 22.6 (CH_2, C-8′), 19.6 (CH_3, C-9), 16.8 (CH_3, C-10), 14.1 (CH_3, C-9′); HRESIMS m/z 377.2671 $[M + Na]^+$ (calcd for $C_{21}H_{38}O_4Na$, 377.2668).

*Officinoic acid B methyl ester (**3a**):* colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 5.62 (1H, br s, H-2′), 5.33 (1H, br t, J = 7.3 Hz, H-4), 4.91 (1H, dq, J = 6.3, 5.5 Hz, H-9), 3.67 (3H, s, OCH_3), 3.04 (2H, br s, H_2-2), 2.61 (2H, t, J = 7.0 Hz, H_2-4′), 2.01 (2H, m, H_2-5), 1.87 (3H, s, H_3-11′), 1.76 (3H, br s, H_3-11), 1.60 (1H, m, H-8a), 1.49 (H, m, H-8b), 1.48 (2H, m, H_2-5′), 1.33 (2H, m, H_2-6), 1.30 (2H, m, H_2-7), 1.30 (2H, m, H_2-7′), 1.30 (2H, m, H_2-8′), 1.29 (2H, m, H_2-6′), 1.28 (2H, m, H_2-9′), 1.21 (3H, d, J = 6.2 Hz, H_3-10), 0.89 (3H, t, J = 6.6 Hz, H_3-10′). ^{13}C NMR ($CDCl_3$): δ 171.8 (C, C-1), 165.7 (C, C-1′), 160.1 (C, C-3′), 128.9 (CH, C-4), 128.5 (C, C-3), 116.3 (CH, C-2′), 69.7 (CH, C-9), 51.6 (OCH_3), 37.1 (CH_2, C-2), 35.9 (CH_2, C-8), 33.3 (CH_2, C-4′), 31.7 (CH, C-8′), 29.8 (CH_2, C-7), 29.3 (CH_2, C-6), 29.3 (CH_2, C-7′), 29.2 (CH_2, C-6′), 28.1 (CH_2, C-5), 27.8 (CH_2, C-5′), 24.9 (CH_3, C-11′), 23.8 (CH_3, C-11), 22.4 (CH_2, C-9′), 20.0 (CH_3, C-10), 14.1 (CH_3, C-10′); HRESIMS m/z 403.2827 $[M + Na]^+$ (calcd for $C_{23}H_{40}O_4Na$, 403.2824).

*Methyl isofurospongin-4 (**4a**):* colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 7.32 (1H, br s, H-1), 7.21 (1H, br s, H-4), 6.73 (1H, br t, J = 7.3 Hz, H-22), 6.28 (1H, br s, H-2), 5.91 (1H, br t, J = 7.3 Hz, H-17), 5.16 (1H, br t, J = 6.6 Hz, H-7), 5.12 (1H, br t, J = 6.3 Hz, H-12), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.54 (2H, q, J = 7.3 Hz, H_2-16), 2.44 (2H, t, J = 7.0 Hz, H_2-5), 2.36 (2H, t, J = 7.3 Hz, H_2-20), 2.30 (2H, t, J = 7.4 Hz, H_2-21), 2.25 (2H, br q, J = 7.0 Hz, H_2-6), 2.07 (2H, m, H_2-15), 2.04 (2H, m, H_2-11), 1.98 (2H, t, H_2-10), 1.81 (3H, s, H_3-24), 1.59 (3H, br s, H_3-9), 1.59 (3H, br s, H_3-14). ^{13}C NMR ($CDCl_3$): δ 168.3 (C, C-19), 168.3 (C, C-25), 143.3 (CH, C-17), 142.3 (CH, C-1), 141.0 (CH, C-22), 138.5 (CH, C-4), 135.6 (C, C-8), 135.4 (C, C-13), 128.1 (C, C-23), 124.9 (CH, C-12), 124.7 (C, C-3), 123.5 (CH, C-7), 111.0 (CH, C-2), 51.5 (OCH_3), 51.5 (OCH_3), 39.6 (CH_2, C-10), 39.0 (CH_2, C-15), 33.4 (CH_2, C-20), 28.5 (CH_2, C-21), 28.4 (CH_2, C-6), 27.8 (CH_2, C-16), 26.6 (CH_2, C-11), 25.2 (CH_2, C-5), 15.9 (CH_3, C-9), 15.9 (CH_3, C-14), 12.3 (CH_3, C-24); HRESIMS m/z 465.2626 $[M + Na]^+$ (calcd for $C_{27}H_{38}O_5Na$, 465.2617).

Preparation of MTPA Esters of 7,8-Epoxyfurospongin-1 (1). *7,8-Epoxyfurospongin-1 (S-MTPA ester) (**1a**).* The *S*-MTPA ester was prepared by treating 0.6 mg of **1** with 0.005 mL of *R*-(−)-MTPA chloride in dry CH_2Cl_2 (0.5 mL) with a catalytic amount of DMAP under stirring for 16 h at room temperature. The ester was purified by chromatography in a Pasteur pipet (SiO_2, $CHCl_3$). Selected ^{1}H NMR values ($CDCl_3$, 600 MHz) δ 5.2090 (H-11), 2.6921 (H-7), 2.5384 (H_2-5), 2.3583 (H_2-17), 2.0721 (H_2-10), 1.7282 (H_2-6), 1.4900 (H_2-12), 1.1914 (H_3-9), 0.8978 (H_3-14); ESIMS m/z 562 $[M + Na]^+$.

*7,8-Epoxyfurospongin-1 (R-MTPA ester) (**1b**).* The *R*-MTPA ester was prepared by treating 0.6 mg of **1** with 0.005 mL of *S*-(+)-MTPA chloride in dry CH_2Cl_2 (0.5 mL) with a catalytic amount of DMAP under stirring for 16 h at room temperature. The ester was purified by chromatography in a Pasteur pipet (SiO_2, $CHCl_3$). Selected ^{1}H NMR values ($CDCl_3$, 600 MHz) δ 5.2600 (H-11), 2.7490 (H-7), 2.5732 (H_2-5), 2.2714 (H_2-17), 2.1380 (H_2-10), 1.7720 (H_2-6), 1.4000 (H_2-12), 1.2810 (H_3-9), 0.8265 (H_3-14); ESIMS m/z 562 $[M + Na]^+$.

Alkaline Methanolysis of Officinoic Acid A and Officinoic Acid B Methyl Esters (2a and 3a). Compounds **2a** and **3a** were treated with 1 mL of a methanolic solution of sodium methoxide (3 M). The reaction mixture was stirred overnight at room temperature. After evaporation, the mixture was filtered over Si gel in a Pasteur pipet, the products were eluted with Et_2O, and the crude filtrate was analyzed by GC-MS.

Biological Assays. The antifungal assay was performed by the broth macrodilution method following the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) document M27-P.[15,16] The antibacterial assay was performed by using the same method as the antifungal test, differing only in the assay medium (Luria−Bertani medium: 10 g/L Bactotryptone, 5 g/L Bactoyeast, and 10 g/L NaCl, pH 7.5) and in the incubation temperature (37 °C for 24 h).

Biofilm Assay. The assay method used was a modified version of that described by Djordjevic et al.[17] *Escherichia coli* PHL628 strain was grown overnight at 37 °C in 5 mL of defined minimal medium, M63, containing kanamycin (50 μg/mL). Overnight cultures were then refreshed again in M63 medium, incubated at 37 °C for 5 to 6 h, and vortexed; then 200 μL of inocula was introduced in the 96-well polystyrene microtiter plate with an initial turbidity at 600 nm of 0.05 in the presence or absence of compound to be tested. The microtiter plate was then left at 30 °C for 40 h under static conditions.

To correlate biofilm formation with planktonic cell growth in each well, the planktonic cell fraction was transferred to a new microtiter plate, and the OD_{570} was measured using a microtiter plate reader (Multiscan Spectrum, Thermo Electron Corporation). To assay biofilm formation, the remaining volume from the incubated microtiter plate was removed and the wells were washed five times with sterile distilled H_2O to remove loosely associated bacteria. Plates were air-dried for 45 min, and each well was stained with 200 μL of 1% crystal violet solution for 45 min. After staining, the plates were washed with sterile distilled H_2O five times. The quantitative analysis of biofilm production was performed by adding 200 μL of EtOH/acetone solution (4:1) to destain the wells. The level (OD) of the crystal violet present in the destaining solution was measured at 570 nm. Biofilm formation was calculated by dividing the total biofilm by the bacterial growth. Six replicate wells were made for each experimental parameter, and each data point was an average from the six replicate wells.

■ ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ DEDICATION

Dedicated to Dr. Guido Cimino on his 70th birthday.

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