Spongian Diterpenoids from the Sponge *Spongia* (*Heterofibria*) sp.

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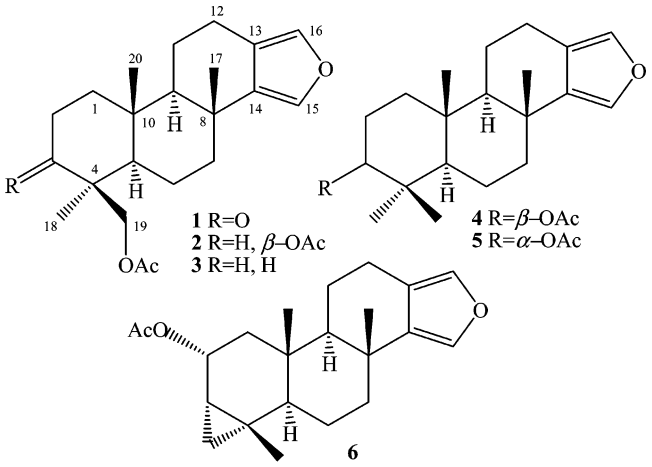
Five new (**1**, **2**, **4**−**6**) and one known (**3**) diterpenoid were isolated from the keratose sponge *Spongia (Heterofibria*) sp. Structures of these compounds and their absolute configurations were proposed on the basis of X-ray analysis of **1**, its CD spectrum, and NMR and MS spectroscopic studies of **1**−**6**. One of the new diterpenoids was shown to be 2(*R*),3(*S*),4(*S*)-3,18-methylene-2-acetoxyspongia-13(16),14-diene (**6**), possessing a novel carbon skeleton system.

Marine sponges of the genus *Spongia* (the family Spongiidae, the order Dictyoceratida) are a source of various terpenoid compounds belonging to the spongian and rearranged spongian series.[1] The first spongian diterpenoid, isogatholactone, was described by Minale and collaborators from the Mediterranean sponge *Spongia officinalis* in 1974.[2] Later a number of other related diterpenoids were isolated from *S. officinalis*,[3] *S. zimocca*,[4] *S. matamata*,[5] *S. arabica*,[6] and *Spongia* sp.[7] Spongian and related diterpenoids isolated from *Spongia* spp. have been reported to exhibit a wide spectrum of biological activites including cytotoxicity,[3d,4,7b,c] antibacterial properties,[3c] and toxicity against some marine macroorganisms.[5a,b]

A keratose sponge identified as *Spongia (Heterofibria*) sp.[8] was collected near Suwarrow atoll (Northern Cook Islands) from a depth of 5 m during an expedition onboard the R/V "Akademic Oparin". In this paper we report the isolation of five new (**1**, **2**, **4**−**6**) and one known spongian diterpenoid (**3**), previously found from an unidentified sponge and shown to be an inhibitor of the lyase activity of DNA polymerase β.[9]



Results and Discussion

The ethanol−chloroform extract of the sponge was fractionated by silica gel flash column chromatography followed by preparative HPLC using an Ultrasphere-Si column to obtain **1**, **2**, and a fraction containing monoacetates **3**−**6**. This fraction was further separated by reversed-phase HPLC using an Ultrasphere ODS column to yield individual compounds **3**−**6**.

Compound **1** was obtained as colorless crystals from an *n*-hexane−ethylacetate mixture. The structure and absolute stereochemistry of **1** were established by spectroscopic analysis and a single-crystal X-ray diffraction study followed by CD spectroscopy. NMR spectra of **1** (Table 1) exhibited signals typical of a β,β'-disubstituted furan ring in the furan-containing spongian diterpenoids isolated from sponges as well as from some nudibranchs[10,11] (δ_C 136.9, 136.6, 135.1, and 119.4; δ_H 7.06 q, J = 1.5 Hz and 7.09 d, J = 1.6 Hz) and signals of three angular methyl groups (δ_H 1.15 s, 1.17 s, 1.25 s), one acetate group (δ_H 2.02, δ_C 170.9), one oxygen-bearing carbon (δ_C 65.9 CH_2), and a ketone group (δ_C 213.1). A quartet signal at δ_H 7.06 was assigned to CH-16 due to coupling with a proton at C-12 in the COSY spectrum. Inspection of the 2D NMR data ($^1H-^1H$ COSY, HSQC, HMBC) and X-ray analysis (for details see Supporting Information) allowed us to assemble the structure as 19-acetoxyspongia-13(16),14-dien-3-one (**1**); the conformations of rings A and B are chairs, while that of ring C is a half-chair.

To distinguish between the two alternative enantiomeric structures, the CD spectrum of **1** was recorded. It showed positive Cotton effects with $[\theta]_{287} = 0.15 \times 10^4$ and $[\theta]_{222} = 2.23 \times 10^4$. Application of the octant rule indicated that **1** has the absolute configuration shown in Figure 1. This 4*S*,5*R*,8*R*,9*R*,10*R*-configuration for **1** is consistent with that for other spongian diterpenoids with established absolute stereochemistry.[1,7a,11]

Compound **1** is an acetylated derivative of the previously known 19-hydroxyspongia-13(16),14-dien-3-one from the sponge *Hyatella intestinalis*.[12] Indeed, the comparison of ^1H and ^{13}C NMR spectra of the product obtained as a result of alkaline hydrolysis of **1** and those of 19-hydroxyspongia-13(16),14-dien-3-one indicated their identity, although their optical rotations were somewhat different ($[\alpha]^{25}_D$ +32.7 (*c* 0.22, $CHCl_3$) for the product obtained by us and $[\alpha]^{21}_D$ +18.8 (*c* 0.6, $CHCl_3$) in the literature[12]).

Comparison of the ^1H and ^{13}C NMR data of **2**−**6** with those of **1** (Table 1) revealed these compounds as belonging to the same class of spongian-based furanoditerpenes. It was suggested that all these metabolites shared with **1** the same absolute configurations in their polycyclic systems.

The HREIMS of **2** displayed a molecular ion at *m*/*z* 402.2414; that along with the NMR data (Table 1) suggested the molecular formula $C_{24}H_{34}O_5$ (calculated *m*/*z* 402.2406). NMR spectra of **2** exhibited signals of the same furan moiety (Table 1): three methyl groups (δ_H 0.95 s, 1.02 s, 1.21 s), two acetoxy groups (δ_H 2.04, δ_C 170.5, 21.2 and δ_H 2.08, δ_C 171.0, 21.1), and two oxygen-bearing

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Table 1. NMR Data of Compounds **1**, **2** (300 MHz), and **4**−**6** (500 MHz) in $CDCl_3$

	1		**2**		**4**		**5**		**6**	
	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	39.8 CH_2	1.43 dt (13.3; 5.8)	38.3 CH_2	1.12 m	38.0 CH_2	1.09 td (4.7; 12.8)	33.7 CH_2	1.24 m	40.3 CH_2	0.52 dd (12.2; 11.3)
		2.16 m		1.88 dt (13.5; 3.9)		1.82 dt (3.7; 13.2)		1.58 m		2.14 dd (12.7; 7.8)
2	34.6 CH_2	2.39 ddd (15.5; 5.6; 3.0)	23.5 CH_2	1.74 m	23.5 CH_2	1.70 m	22.7CH_2	1.94 m	70.0 CH	5.45 dt (10.8; 7.4)
		2.79 m						1.64 m		
3	213.1 C		80.1 CH	4.56 dd (8.7; 7.0)	80.8 CH	4.48 dd (5.3; 11.2)	78.3 CH	4.65 t 2.9	22.8 CH	1.15 m
4	52.0 C		41.2 C		37.9 C		36.9 C		21.4 C	
5	57.4 CH	1.50 m	56.2 CH	1.18 m	55.6 CH	0.98 dd (11.5; 2.4)	50.4 CH	1.43 dd (2.8; 11.4)	52.5 CH	1.09 m
6	19.8 CH_2	1.68 m	19.6 CH_2	1.66 m	18.4 CH_2	1.66 m	18.3 CH_2	1.50 m	22.1 CH_2	1.64 m
				1.76 m		1.57 m		1.57 m		1.79 m
7	40.8 CH_2	1.58 m	41.4 CH_2	1.52 m	41.0 CH_2	1.60 m	41.1 CH_2	1.64 m	39.7 CH_2	1.55 m
		2.17 m		2.12 dt (12.6; 3.0)		2.12 m		2.12 dt (12.7; 3.1)		2.10 dt (12.6; 3.0)
8	34.1 C		34.2 C		34.2 C		34.3 C		33.9 C	
9	55.5 CH	1.25 dd (11.4; 2.3)	56.2 CH	1.11 dd (11.7; 3.0)	56.0 CH	1.18 dd (1.8; 11.8)	55.9 CH	1.30 dd (1.5; 11.8)	51.9 CH	1.09 m
10	37.1 C		37.1 C		37.2 C		37.3 C		39.1 C	
11	18.7 CH_2	1.77 m	18.5 CH_2	1.76 m	18.3 CH_2	1.66 m	18.0 CH_2	1.78 brdd (7.4; 13.6)	18.8 CH_2	1.79 m
		1.67 m		1.61 m		1.76 m		1.62 m		1.62 m
12	20.6 CH_2	2.47 m	20.7 CH_2	2.44 m	20.6 CH_2	2.45 m	20.6 CH_2	2.48 m	20.4 CH_2	2.44 m
		2.80 m		2.77 brdd (16.5; 6.3)		2.76 brdd (16.3; 6.4)		2.78 brdd (6.6; 16.0)		2.76 brdd (16.3; 6.5)
13	119.4 C		119.6 C		119.7 C		119.7 C		119.7 C	
14	136.6 C		137.0 C		137.3 C		137.6 C		137.4 C	
15	135.1 CH	7.09 d (1.6)	135.1 CH	7.08 d (1.6)	135.1 CH	7.08 d (1.5)	135.0 CH	7.09 d (1.1)	134.7 CH	7.08 d (1.5)
16	136.9 CH	7.06 q (1.5)	136.9 CH	7.05 q (1.5)	136.8 CH	7.05 q (1.4)	136.8 CH	7.05 brd (1.4)	137.0 CH	7.04 q (1.8)
17	26.0 CH_3	1.25 s	26.0 CH_3	1.21 s	26.2 CH_3	1.22 s	26.2 CH_3	1.23 s	26.0 CH_3	1.25 s
18	20.7 CH_3	1.17 s	22.5 CH_3	1.02 s	28.0 CH_3	0.87 s	27.9 CH_3	0.86 s	21.6 CH_2	0.38 t (5.0)
										0.62 dd (4.5; 9.3)
19	65.9 CH_2	4.02 d (11.3)	65.4 CH_2	4.17 d (11.3)	16.4 CH_3	0.89 s	21.6 CH_3	0.92 s	22.7 CH_3	1.03 s
		4.62 d (11.3)		4.36 d (11.3)						
20	16.3 CH_3	1.15 s	16.1 CH_3	0.95 s	16.4 CH_3	0.93 s	16.1 CH_3	0.93 s	13.4 CH_3	0.92 s
19-Ac	170.9 C		171.0 C		170.1 C		170.1 C			
19-Ac	20.8 CH_3	2.02 s	21.1 CH_3	2.08 s	21.3 CH_3	2.05 s	21.3 CH_3	2.04 s		
3-Ac			170.5 C							
3-Ac			21.2 CH_3	2.04 s						
2-Ac									171.2 C	
2-Ac									21.5 CH_3	2.04 s

carbons (δ_C 80.1, CH and 65.4 CH_2). The HMBC correlations between the signals at δ_H 4.17 (d, $J = 11.3$ Hz) and 4.36 (d, $J = 11.3$ Hz), which were assigned by HSQC to CH_2−O (δ_C 65.4), and carbon signals at δ_C 22.5 (CH_3-18), 41.2 (C-4), 56.2 (CH-5), and 80.1 (CH-3) and between signals at δ_H 4.56 (CH-3) and δ_C 22.5 proved the acetoxy groups to be attached to C-19 and C-3. In the NOESY experiments, the signals of H_2-19 showed a cross-peak with the signal of CH_3-20 (δ_H 0.95 s). Consequently, CH_2-19 is β-oriented. The orientation of the C-3 acetoxy group was concluded to be β from the splitting pattern of a signal at δ 4.56 (H-3, dd, $J = 8.7$, 7.0 Hz) and from its NOESY correlations with H-5 (1.11 m) and CH_3-18 (1.02 s). Thus, the structure of **2** was established as 3β,19-diacetoxyspongia-13(16),14-diene.

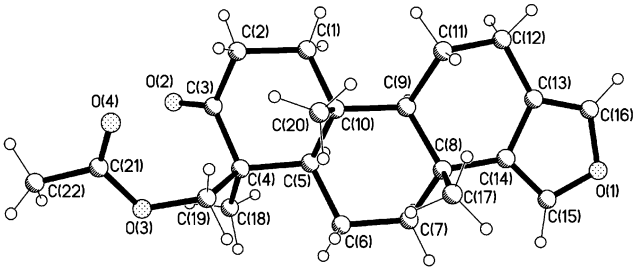

Figure 1. X-ray molecular drawing of compound **1**.

The HREIMS of **3** displayed a molecular ion at *m*/*z* 344.2372, suggesting a molecular formula of $C_{22}H_{32}O_3$ (calculated *m*/*z* 344.2351). Its NMR spectra (Supporting Information, Table S1) exhibited signals of the furan moiety, three methyl groups (δ_H 0.92 s, 0.96 s, 1.20 s), and an acetoxy group (δ_H 2.06, δ_C 171.4) attached to a methylene carbon (δ_C 67.0 CH_2). The HMBC cross-peaks between δ_H 3.94 and 4.23 (which were assigned to protons of CH_2 indicating δ_C 67.0 at HSQC) and δ_C 27.4 CH_3, 36.4 CH_2, 37.1 C, and 57.3 CH proved the acetoxy group to be attached to C-19. The orientation of the CH_2OAc fragment as β was predicted on the same arguments as outlined above for **2**. The assignments of all signals in the NMR spectra were carried out by $^1H-^1H$ COSY, HSQC, and HMBC experiments (Supporting Information, Table S1). On the basis of all the obtained data, the structure of **3** was established as 19-acetoxyspongia-13(16),14-diene. The same structure was proposed for a diterpenoid previously isolated from an unidentified sponge species of the order Dictyoceratida.[9] However, there was some difference in the EIMS and NMR data of **3** in comparison with those reported by Chaturvedula et al.[9] (see Supporting Information).

On the basis of HREIMS and NMR data, the same molecular formula of $C_{22}H_{32}O_3$ was established for compounds **4** and **5**. The HREIMS of these compounds displayed molecular ion peaks at *m*/*z* 344.2364 and 344.2335, respectively (calculated *m*/*z* 344.2351).

Both compounds showed NMR resonances of a furan moiety, an acetoxy group (δ_C 171.4 and δ_H 2.05 for **4**; δ_C 170.1 and δ_H 2.04 for **5**) attached to a methine carbon (δ_C 80.8 CH, δ_H 4.48 for **4**; δ_C 78.3 CH, δ_H 4.65 for **5**), and four methyl groups (δ_H 0.87 s, 0.89 s, 0.93 s, 1.22 s for **4**; δ_H 0.92 s, 0.86 s, 0.93 s, 1.23 s for **5**) (see Table 1). Protons of two geminal methyl groups (δ_H 0.87 and 0.89 s for **4**; δ_H 0.92 s, 0.86 s for **5**) showed the HMBC cross-peaks with a quaternary carbon (C-4: δ_C 37.9 for **4**; 36.9 for **5**), a tertiary carbon (C-5: δ_C 55.6 for **4**; 50.4 for **5**), and an oxygen-bearing carbon (δ_C 80.8 CH for **4**; 78.3 CH for **5**). Therefore, the acetoxy groups in **4** and **5** are attached to C-3. The orientation of this group in **4** was concluded to be β from the coupling constants of the signal at δ 4.48 (H-3, dd, $J = 11.2, 5.3$ Hz) and its NOESY correlations with H-5 (0.98 dd $J = 11.5, 2.4$ Hz) and CH_3-18 (0.87 s). The orientation of the C-3 acetoxy group in **5** was concluded to be α from the coupling constants of a signal at δ 4.65 (H-3, t, $J =$ 2.9 Hz). Thus, the structures of **4** and **5** were established as 3β-acetoxyspongia-13(16),14-diene and 3α-acetoxyspongia-13(16),-14-diene, respectively. Both compounds are new natural products.

The HREIMS of **6** displayed a molecular ion at *m*/*z* 342.2208, suggesting a molecular formula of $C_{22}H_{30}O_3$ (calculated 342.2194). Its NMR spectra indicated, besides the resonances characteristic of the same B, C, and D rings (Table 1), the signals of three methyl groups (δ_H 0.92 s, 1.03 s, 1.25 s), an acetoxy group (δ_C 171.2, δ_H 2.04 s) attached to a methine carbon (δ_C 70.0 CH), and two proton signals in a higher field part of the ^{1}H NMR spectrum (δ_H 0.62 dd, $J = 4.5, 9.3$ Hz and 0.38 t, $J = 5.0$ Hz), which were unusual for spectra of spongiane diterpenoids. This peculiarity suggested the presence of a cyclopropane fragment in **6**. Correlations observed for **6** indicated the presence of the sequence CH_2-1 (δ_C 40.3, δ_H 0.52, dd, $J = 12.2, 11.3$ Hz; 2.14, dd, $J = 12.7, 7.8$ Hz), CH(O)-2 (δ_C 70.0, δ_H 5.45, dt, $J = 10.8, 7.4$ Hz), CH-3 (δ_C 22.8, δ_H 1.15, m), CH_2-18 (δ_C 21.6, δ_H 0.62 dd, $J = 4.5, 9.3$ Hz; 0.38 t, $J = 5.0$ Hz). In the ^{1}H−^{1}H COSY spectrum, there was a cross-peak, CH_3-20/H-1 (δ_H 0.52), due to the *W*-interaction of the corresponding protons. It showed an α-orientation of H-1 with a signal at 0.52 ppm. A methyl group signal at δ_H 1.03 s (CH_3-19) showed HMBC cross-peaks with signals at δ_C 21.4 (C-4), 52.5 (C-5), 22.8 (C-3), and 21.6 (C-18). These results indicated that an acetoxy group is attached to C-2 and a cyclopropane fragment is formed by C-3, C-4, and C-18. Supporting correlations were observed for cyclopropane methylene protons. The orientation of the C-2 acetoxy group was concluded to be α from the coupling constants of a signal at δ_H 5.45 (H-2, dt, $J = 10.8, 7.4$ Hz) and due to an NOE enhancement for CH_3-20 (0.92 s) when the H-2 signal was irradiated. Irradiation of the CH_3-19 signal (1.03 s) caused an NOE enhancement of the CH_3-20 signal (0.92 s), and the irradiation of an H-18 signal (0.38 t) caused an NOE enhancement of the H-5 (1.09 m) and H-1 (δ_H 0.52) signals. Thus, the cyclopropane fragment is α-oriented. In addition, the irradiation of CH_3-20 caused an NOE enhancement of the CH_3-17 signal (δ_H 1.25 s). On the basis of all the above-mentioned data, the structure of **6** was established as 2(*R*),3(*S*),4(*S*)-3,18-methylene-2-acetoxyspongia-13-(16),14-diene. The compound **6** is an unprecedented cyclopropane-containing spongian diterpenoid having a novel carbon skeleton system.

Several rearranged spongian diterpenoids containing an unusual cyclopropyl ring fused to ring B were earlier known, including those from the Palaun sponge *Dendrilla* sp.[13] and the New Zealand sponge *Chelonapsilla* violacea.[14] However, to the best of our knowledge compound **6** is the first spongiane diterpenoid containing a cyclopropyl ring fused to ring A. From a biogenetic point of view, this skeleton system is "unusual", but its formation can be explained by the hypothetic scheme of biogenesis similar to that proposed by Rochfort and Capon for parguerane diterpenoids[15] (see Supporting Informathion, Figure S1).

Compounds **2** and **3** were tested for immunomodulatory properties by the methods reported in literature[16] and demonstrated a slight lysosomal activation (about 130% of control) of mice spleenocytes at concentrations of 100 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined on a Leica Galen III apparatus and were not corrected. Optical rotation was measured on a Perkin-Elmer Model 343 polarimeter in $CHCl_3$. The CD spectrum was recorded on a Jasco J-500A spectropolarimeter. NMR spectra were recorded in $CDCl_3$ on Bruker DPX-300 and Bruker DRX-500 spectrometers using TMS as an internal standard. GLC-MS analyses were performed on a Hewlett-Packard HP6890 GS system, using a HP-5MS capillary column. HREIMS were obtained on an AMD-604S high-resolution mass spectrometer (Germany). X-ray crystallographic data for compound **1** were collected on a Bruker SMART-1000 CCD diffractometer. Silica gel (50−160 μm) for column chromatography was obtained from Sorbpolymer, Russia. TLC was performed on Sorbfil plates (Russia) in *n*-hexane−ethyl acetate, and spots were detected by spraying with sulfuric acid (100 °C, 5 min).

Animal Material. The sponge was collected by scuba near Suwarrow atoll (Northern Cook Islands) at a depth of 5 m in February 1998 and identified as *Spongia (Heterofibria*) sp. (the family Spongiidae, the order Dictyoceratida) by Dr. V. B. Krasokhin.[8] The sponge has a solid base from which arise upright distinct conical fistules (up to 60 mm high) of varying development. The lower part of some fistules is bulbous and tapering to a 2 or 3 terminal oscules (6 mm in diameter); however, some fistules have a cylindrical form with a single osculum. The sponge is moderately firm and compressible. The surface is unarmored and finely conulose. The color is pale inside and dull brown on the upper surface. The specimens are 150 mm long × 100 mm high and wide. This sponge has a reticulate skeleton of sparse, cored primary fibers (100 μm in diameter) and homogeneous secondary (20 μm) and pseudotertiary (8 μm) fibers, which occur among meshes of secondary fiber reticulum. The polygonal fiber network is moderately regular. A voucher specimen (PIBOC O6-102) is on deposit in the collection of the Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia. The sponge was lyophilized immediately after collection and kept at −18 °C.

Extraction and Isolation. The lyophilized specimens (94 g) were extracted with EtOH (3 × 500 mL) and a mixture of EtOH−$CHCl_3$ (2:1, v/v) (3 × 500 mL) by refluxing successively. The combined extracts were concentrated *in vacuo*, dissolved in a minimum volume of aqueous EtOH (90%), and then partitioned with *n*-hexane (3 × 150 mL) and $CHCl_3$ (3 × 150 mL) in series. The *n*-hexane and $CHCl_3$ layers were concentrated *in vacuo* to obtain a yellow, amorphous residue, which was separated by low-pressure column chromatography on Si gel using *n*-hexane with increasing amounts of EtOAc. Fractions eluted with *n*-hexane−EtOAC (90:1) were combined, concentrated *in vacuo*, and subjected to semipreparative HPLC (Shimadzu 10Avp, Ultrasphere-Si column, RID-10A detector, 2.6 mL/min, *n*-hexane−EtOAc, 12:1) followed by RP HPLC (Shimadzu 10Avp, UV detector at 230 nm, Ultrasphere ODS column, MeOH−H_2O gradient from 95% up to 100% in 20 min, flow 0.7 mL/min) to give individual monoacetates **3**−**6**. Fractions, eluted with *n*-hexane−EtOAC (17:1), were purified by HPLC (Shimadzu 10Avp, Ultrasphere-Si column, RID-10A detector, 2.6 mL/min, *n*-hexane−EtOAc, 5:1) to give **1** and **2**.

19-Acetoxyspongia-13(16),14-dien-3-one (1): colorless needles (62.0 mg), mp 132.0−133.7 °C; $[\alpha]^{27}_D$ −57.7 (*c* 0.09, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (%) 358 $[M]^+$ (100), 343 $[M - CH_3]^+$ (15), 298 $[M - AcOH]^+$ (6), 283 $[M - AcOH - CH_3]^+$ (70), 265 (20); HREIMS *m*/*z* 358.2146 (calcd for $C_{22}H_{30}O_4$, 358.2144).

3β,19-Diacetoxyspongia-13(16),14-diene (2): colorless solid (4.8 mg), mp 84−89 °C; $[\alpha]^{27}_D$ −7.3 (*c* 0.055, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (%) 402 $[M]^+$ (62), 387 $[M - CH_3]^+$ (3), 342 $[M - AcOH]^+$ (1), 327 $[M - AcOH - CH_3]^+$ (10), 285 (4), 267 $[M - 2AcOH - CH_3]^+$ (100); HREIMS *m*/*z* 402.2414 (calcd for $C_{24}H_{34}O_5$, 402.2406).

3β-Acetoxyspongia-13(16),14-diene (4): colorless solid (1.9 mg), mp 96−100 °C; $[\alpha]^{27}_D$ −16.9 (*c* 0.065, $CHCl_3$); ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* (%) 344 $[M]^+$ (70), 329 $[M - CH_3]^+$ (18), 287 (5), 269 $[M - AcOH - CH_3]^+$ (100); HREIMS *m*/*z* 344.2364 (calcd for $C_{22}H_{32}O_3$, 344.2351).

3α-Acetoxyspongia-13(16),14-diene (5): colorless solid (0.7 mg), mp 86−90 °C; $[\alpha]^{27}_D$ −8.2 (*c* 0.085, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 344 $[M]^+$ (60), 329 $[M - CH_3]^+$ (6), 284 $[M - AcOH]^+$ (1), 269 $[M - AcOH - CH_3]^+$ (100); HREIMS *m*/*z* 344.2335 (calcd for $C_{22}H_{32}O_3$, 344.2351).

3,18-Methylene-2α-acetoxyspongia-13(16),14-diene (6): colorless powder (0.2 mg), $[\alpha]^{27}_D$ +133.3 (*c* 0.015, $CHCl_3$); ^1H and ^{13}C NMR data, see Table 1; EIMS *m*/*z* 342 $[M]^+$ (3), 327 $[M - CH_3]^+$ (5), 282 $[M - AcOH]^+$ (70), 267 $[M - AcOH - CH_3]^+$ (100); HREIMS *m*/*z* 342.2208 (calcd for $C_{22}H_{30}O_3$, 342.2195).

Alkaline Hydrolysis of 19-Acetoxyspongia-13(16),14-dien-3-one (1). Treatment of **1** with 0.005 M MeONa in dry MeOH at 20 °C for 10 h gave 19-hydroxyspongia-13(16),14-dien-3-one, which was purified by low-pressure column chromatography on Si gel using *n*-hexane with increasing amounts of EtOAc. ^1H and ^{13}C NMR spectra of the resulting 19-hydroxyspongia-13(16),14-dien-3-one were identical to those reported in the literature;[12] $[\alpha]^{25}_D$ +32.7 (*c* 0.22, $CHCl_3$).

X-ray Crystal Data for 1. The colorless acicular crystal was grown from *n*-hexane−EtOAc. Crystal data: $C_{22}H_{30}O_4$; M_r 358.46; T = 173-(1) K; wavelength 0.71073 Å; crystal system monoclinic; space group $P2_1$; unit cell dimensions a = 9.6564(7) Å, b = 7.2884(6) Å, c = 13.6294(11) Å, β = 102.409(2)°; volume 936.82(13) Å^3; Z = 2; D_{calc} = 1.271 mg/m^3; absorption coefficient 0.086 mm^{-1}; F(000) = 388; crystal size 0.30 × 0.20 × 0.10 mm; reflections collected 6307; independent reflections 2422 [R(int) = 0.0366]. Complete crystallographic data of **1** including atomic coordinates, bond lengths and angles, thermal parameters, and additional experimental details may be found in the Supporting Information. This material has also been deposited with the Cambridge Crystallographic Data Center (CCDC 644954). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk)

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Supporting Information Available: Data of 19-acetoxyspongia-13(16),14-diene (**3**) isolated from *Spongia* (*Heterofibria*) sp. by us in comparison with those for the product isolated from unidentified sponge;[9] hypothetic scheme of biogenesis of compound **6**; crystal and X-ray experimental data for 19-acetoxyspongia-13(16),14-dien-3-one (**1**), and a photograph of *Spongia (Heterofibria*) sp. are provided free of charge via the Internet at http://pubs.acs.org.

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(8) This species is clearly a member of the widely distributed genus *Spongia*, in that it possesses a network of primary fibers without concentric laminations, cored with foreign inclusions, and uncored with secondary fibers. Based on the emended diagnosis for *Spongia* [Cook, S. de C.; Bergquist, P. R. *N. Z. J. Mar. Freshwater Res.* **2001**, *35* (1), 33−58] the sponge belongs to the subgenus *Heterofibria*, which is distinguished by a size dichotomy between elements of the fiber skeleton from other subgenera. The regular polygonal mesh skeleton of *Spongia* (*Spongia*) consists of uniform diameter fibers. The irregular skeleton of uniform in diameter secondary fibers is an inherent feature of another subgenus, *Australospongia*. More than 50 valid species were described in the very extensive genus *Spongia* to date, but many species are still undescribed and others were described in other genera in the past. Until reexamination of type samples described more than a century ago, it is not possible to establish an exact species name of our sponge, but our sample has some resemblance to *Hippospongia canaliculata* [Lendenfeld R. Von. *A Monograph of the Homy Sponges*; Trubner and Co.: London, 1889; pp 1−936].
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