Antimycobacterial Scalarane-Based Sesterterpenes from the Red Sea Sponge *Hyrtios erecta*

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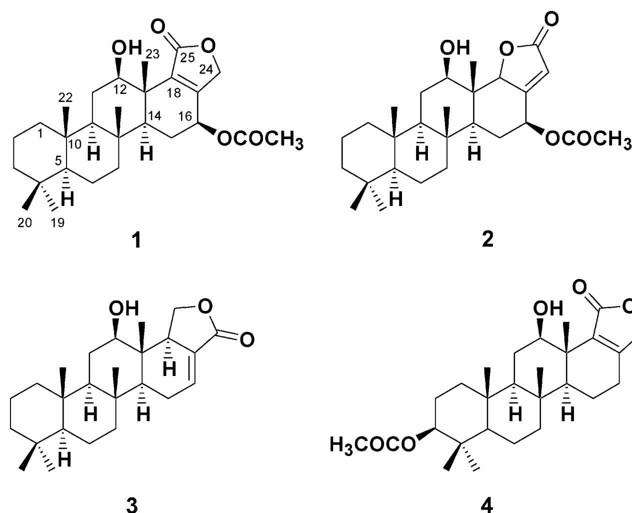
A new scalarane-type pentacyclic sesterterpene, sesterstatin 7 (**1**), was isolated from the Red Sea sponge *Hyrtios erecta*, together with 16-*epi*-scalarolbutenolide (**2**), 25-dehydroxy-12-*epi*-deacetylscalarin (**3**), 3-acetylsesterstatin 1 (**4**), and 21-acetoxydeoxyscalarin. The structure of **1** was elucidated by spectroscopic data interpretation. Sesterstatin 7 (**1**) showed 63% inhibition of *Mycobacterium tuberculosis* ($H_{37}Rv$) at a concentration of 6.25 μg/mL. Compound **2** displayed moderate inhibitory activity, while **3** and **4** were weakly active against the same biological target.

Marine organisms have attracted considerable attention as a source of novel natural products with intriguing structures and useful biological activities.[1] Many scalarane-type sesterterpenoids have been isolated from marine sponges belonging to the order Dictyoceratida.[1] Scalarane-type sesterterpenes display a variety of biological activities such as cytotoxic,[2−4a,b] antimicrobial,[5,6] antifeedant,[7] ichthyotoxic,[8] anti-inflammatory,[9,10] and platelet-aggregation inhibitory effects,[11] as well as nerve growth factor synthesis-stimulating action.[12] The genus *Hyrtios* has proven to be a rich source of structurally diverse substances with potentially useful biological activities including terpenoids,[2−4] macrolides,[3a,13] and tryptamine-derived alkaloids.[14]

In the course of our ongoing efforts to locate drug leads from Red Sea marine invertebrates, we have studied an extract of the Red Sea sponge *Hyrtios erecta*. We report herein the identification and antimycobacterial activity of several scalarane-type sesterterpenes including the new compound sesterstatin 7 (**1**), together with the previously reported compounds, 16-*epi*-scalarolbutenolide (**2**),[15] 25-dehydroxy-12-*epi*-deacetylscalarin (**3**),[16] 3-acetylsesterstatin 1 (**4**),[2] and 21-acetoxydeoxyscalarin.[17] Sesterstatins 1−6 were reported from the sponge *H. erecta* collected in the Republic of Maldives.[3] In addition, 3β-acetylsesterstatin 1 and 21-acetylsesterstatin 3 were isolated from a Red Sea collection of the sponge *H. erecta*.[2] The previously known sesterstatins exhibit cytotoxic activity against several cancer cell lines.[2,3]

Sesterstatin 7 (**1**) was isolated as a white solid with a molecular formula of $C_{27}H_{40}O_5$, as established from its HRFABMS and ^{13}C NMR data. Its ^1H NMR spectrum (Table 1) displayed resonances for 40 protons including six singlets assigned to six methyl groups (δ 0.83, 0.80, 0.89, 0.83, 1.19, and 2.12), eight methylenes, five aliphatic methines, and an exchangeable singlet at δ 5.75 for an OH moiety. The ^{13}C NMR spectrum revealed signals for 27 carbons including six methyls, eight methylenes, five methines, and eight quaternary carbons. Analysis of the ^1H,^1H-COSY and the HMQC NMR experiments led to the assembly of the following structural fragments: C-1 to C-3; C-5 to C-7; C-9 to C-12 with a hydroxyl group at C-12; and C-14 to C-16 with an acetyl moiety at C-16. The proton



signals at δ 4.76 (H-24a) and 4.71 (H-24b) ($J_{24a/b}$ = 18.3 Hz) together with the carbon resonances at δ 70.5 (CH_2, C-24), 158.5 (qC, C-17), 139.2 (qC, C-19), and 174.9 (qC, C-25) were representative of an α,β-unsaturated butenolide moiety in **1**.[2,3a] This was supported by interpretation of the HMBC spectrum (Table 1), which indicated that C-16 and C-24 were connected to the sp^2 carbon C-17, with these situated, along with C-18 and C-25, in the α,β-unsaturated lactone ring.[2,3a] Connectivities of the five ring systems of **1**, as well as the assignments of all quaternary carbons, were supported unequivocally by HMBC data (Table 1). In addition, the placements of the OH and acetyl moieties at C-12 and C-16, respectively, were secured from HMBC correlations of H-11/C-12, H-16/C-17, and H-16/C-18. Moreover, COSY coss-peaks of H-12/H-11a, H-12/H-11b, H-16/H-15a, and H-16/H-15b supported these assignments. The equatorial geometry of OH at C-12 was established from the large coupling constant of H-12 (J = 11.5 Hz).[2,3a] Similarly, the β-configuration of the acetyl moiety at C-16 was established from the coupling constant (J = 10.3 Hz).[15] From the above discussion, compound **1** was assigned as 16β-acetoxyscalarolide.

Compounds **2**−**4** and 21-acetoxydeoxyscalarin were identified by interpretation of their spectroscopic data, including HRMS and high-field NMR, and by comparing these data with literature values.[2,15−17]

Compounds **1**−**4** were evaluated for their in vitro antituberculosis activity at a concentration of 6.25 μg/mL,

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Table 1. ^1H and ^{13}C NMR Data of **1** ($CDCl_3$)

position	δ_C (mult.)	δ_H [mult., J (Hz)]	HMBC[a]
1	39.6, CH_2	1.73, m, 0.79, m	C-10
2	18.5, CH_2	1.56, m, 1.42, m	C-4, C-10
3	42.0, CH_2	1.79, m 1.11, ddd (13.5, 13.5, 4.0)	C-4
4	33.2, qC		
5	56.6, CH	0.79, m	C-4
6	18.1, CH_2	1.57, m, 1.41, m	
7	41.6, CH_2	1.41, m, 0.94, m	C-8
8	37.1, qC		
9	58.0, CH	0.88, m	C-10, C-12
10	37.4, qC		
11	25.7, CH_2	1.88, ddd (11.5, 4.2, 1.8) 1.50, m	C-10, C-12
12	75.2, CH	3.66, dd (11.5, 4.4)	C-13
O*H*		5.75, s	
13	42.8, qC		
14	54.1, CH	1.21, m	C-8, C-13, C-16
15	23.8, CH_2	2.25, dd (11.7, 7.1) 1.74, m	C-16
16	69.0, CH	5.50, brdd (10.3, 7.1)	C-17, C-18
17	158.5, qC		
18	139.2, qC		
19	33.2, CH_3	0.83, s	C-4
20	20.8, CH_3	0.80, s	C-4
21	17.2, CH_3	0.89, s	C-8
22	15.9, CH_3	0.83, s	C-1, C-10
23	16.5, CH_3	1.19, s	C-12, C-13, C-18
24	70.5, CH_2	4.76, dd (18.3, 1.3) 4.71, d (18.3)	C-17, C-18, C-25
25	174.9, qC		
26	170.5, qC		
27	21.3, CH_3	2.12, s	C-26

[a] Protons that correlated with carbons.

against *Mycobacterium tuberculosis* ($H_{37}Rv$) (ATCC 27294), in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[18] Compound **1** displayed 63% inhibition at this concentration. Compounds **2**−**4** showed inhibitory activities of 40%, 16%, and 13%, respectively. A minimum inhibition of 90% against *M. tuberculosis* (H_{37}-Rv) is needed to progress compounds to an in vivo study.[19]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Jasco-DIP-700 digital polarimeter using CH_2Cl_2 at 20 °C at the sodium D line (589 nm). NMR spectra were determined on a Varian Unity 500 instrument (500 MHz for ^1H and 125 MHz for ^{13}C NMR). Homonuclear ^1H connectivities were determined by using the 2D double-quantum filtered COSY technique. One-bond heteronuclear $^1H-^{13}C$ connectivities were determined by a 2D proton-detected HMQC experiment; two- and three-bond $^1H-^{13}C$ connectivities were determined by a 2D proton-detected HMBC experiment. HRFABMS were determined on a Finnigan MAT-312 spectrometer using 3-NBA/NaCl as matrix. Reversed-phase HPLC was conducted on a semipreparative C_{18} Ultracarb column (5 μm ODS 30, 250 × 10 mm, Phenomenex).

Biological Material. The sponge was collected by hand using scuba at a depth of 15 m off Safaga at the Egyptian Red Sea coast in December 2002. The sponge material was frozen immediately and kept frozen at −20 °C until workup. The sponge was identified as *Hyrtios erecta* (Keller) (family Thorectidae, order Dictyoceratida).[2] A voucher specimen has been deposited in the Natural History Museum, London, UK, and in the Red Sea invertebrates collection at the Faculty of Pharmacy, Suez Canal University, under the registration number DY-29.

Extraction and Isolation. Freshly collected specimens (800 g, wet wt) of the sponge were frozen immediately. The sponge was extracted with MeOH (3 × 1.5 L) at room temperature. The combined extracts were concentrated under reduced pressure, dissolved in 500 mL of MeOH/H_2O (9:1), and extracted with hexane (4 × 250 mL) to give 585 mg of hexane residue. The remaining methanolic layer was diluted with H_2O to (3:2) MeOH−H_2O and then extracted with CH_2Cl_2 (4 × 250 mL) to afford 431 mg of a CH_2Cl_2 extract. The hexane residue was subjected to silica flash column chromatography using hexane/CH_2Cl_2/acetone. Fractions of 100 mL were collected and monitored by TLC. Similar fractions were pooled to give 10 further fractions. Of these, fraction 5 (110 mg) was purified on a semipreparative C_{18} reversed-phase HPLC column using 80% MeCN (2.0 mL/min at 220 nm) to give **1** (2.3 mg) and **2** (0.8 mg). In turn, fraction 6 (72 mg) was further purified on a semipreparative C_{18} reversed-phase HPLC column using 90% MeCN (2.0 mL/min at 220 nm) to afford **3** (1.9 mg), **4** (2.1 mg), and 21-acetoxydeoxyscalarin {$[\alpha]_D$ +61.1 (*c* 1.0, CH_2Cl_2); HRFABMS *m/z* 511.5041 (calcd for $C_{29}H_{44}O_6Na$, $[M + Na]^+$, 511.5036)} (1.5 mg).

Sesterstatin 7 (1): amorphous solid; $[\alpha]_D$ −19.5 (*c* 1.15, CH_2Cl_2); NMR data, see Table 1; HRFABMS *m/z* 467.2769 (calcd for $C_{27}H_{40}O_5Na$ $[M + Na]^+$, 467.2773).

16-*epi*-Scalarolbutenolide (2): amorphous solid; $[\alpha]_D$ −7.5 (*c* 1.2, CH_2Cl_2); HRFABMS *m/z* 467.2769 (calcd for $C_{27}H_{40}O_5$-Na $[M + Na]^+$, 467.2773).

25-Dehydroxy-12-*epi*-deacetylscalarin (3): amorphous solid; $[\alpha]_D$ −22.5 (*c* 1.0, CH_2Cl_2); HREIMS *m/z* 409.2711 (calcd for $C_{25}H_{38}O_3Na$, $[M + Na]^+$, 409.2719).

3-Acetylsesterstatin 1 (4): amorphous solid; $[\alpha]_D$ +82.5 (*c* 0.6, CH_2Cl_2); HRFABMS *m/z* 467.2769 (calcd for $C_{27}H_{40}O_5Na$ $[M + Na]^+$, 467.2773).

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