Notes

Antineoplastic Agents. 542. Isolation and Structure of Sesterstatin 6 from the Indian Ocean Sponge *Hyrtios erecta*[1]

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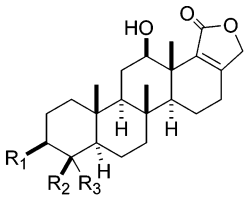
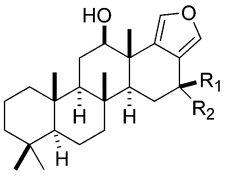
A new scalarane-type pentacyclic sesterterpene, sesterstatin 6 (**6**), was isolated in 8.3×10^{-7}% yield from the Republic of Maldives marine sponge *Hyrtios erecta*. The structure was elucidated by analyses of HRMS and high-field 2-D NMR spectra. Sesterstatin 6 showed significant cancer cell growth inhibition against murine P388 lymphocytic leukemia and a series of human tumor cell lines (ED_{50} 0.17 μg/mL, GI_{50} 1.8−8.9 $\times 10^{-1}$ μg/mL) and proved to be the most inhibitory of the series.

The marine porifera are proving to be an exceptionally productive source of new terpenes exhibiting a great variety of novel structural modifications and biological activities. Illustrative are the recent isolation and structural determination of cyclosmenospongine, a sesquiterpenoid aminoquinone from *Spongia* sp.,[2a] yardenones A and B, cytotoxic triterpenes from an *Axinella* sp.,[2b] cytotoxic furanoterpenoids from *Sarcotragus* sp.,[2c] a sesquiterpenoid from *Axinyssa* sp.,[2d] cancer cell line inhibitory sesterterpenes from *Thorectandra* sp.,[2e] the cancer cell line active theopederins K and L from *Discodermia* sp.,[2f] and especially pertinent here, the cancer cell line active salmahyrtisol A from the Red Sea sponge *Hyrtios erecta*.[2g] We had earlier discovered in a Republic of Maldives collection of *H. erecta* the cancer cell inhibitory pentacyclic sesterterpene sesterstatins 1−5 (**1**−**5**).[3,4] Both 3β-acetyl and 21-acetyl derivatives of compounds **1** and **3**, respectively, were also isolated from a Red Sea collection of the sponge *H. erecta*.[2g] The present study was directed at investigating remaining cancer cell line inhibitory (murine P388 lymphocytic leukemia), albeit very minor, fractions from a scale-up recollection (600 kg) of the Republic of Maldives *H. erecta*. That endeavor resulted in isolation and structural elucidation of a new cancer cell line inhibitory (P388 ED_{50} 0.17 μg/mL) pentacyclic sesterterpene designated sesterstatin 6 (**6**).

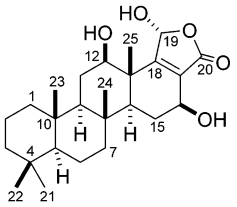
A fraction prepared from the CH_2Cl_2−CH_3OH extract of *H. erecta* (600 kg, wet weight) collected in the Republic of Maldives in 1994 was obtained by bioassay-directed (P388 leukemia cell line) fractionation of the extract by a sequence of solvent partitioning (between 9:1 CH_3OH−H_2O and *n*-hexane, then diluted to 3:2 CH_3OH−H_2O and extracted with CH_2Cl_2) to afford an active (P388 0.31 μg/mL) fraction. An extensive series of column gel-permeation and partition chromatographic separations on Sephadex LH-20 followed by reversed-phase HPLC separations and final purification on a C18 column with acetonitrile−H_2O (1:1) as mobile phase gave sesterstatin 6 (**6**) as colorless fine needles (5.0 mg, 8.3×10^{-7} % yield), mp 253−255 °C.



1, R_1 = OH, R_2 = R_3 = CH_3, Sesterstatin 1
2, R_1 = H, R_2 = CH_2OH, R_3 = CH_3, Sesterstatin 2
3, R_1 = H, R_2 = CH_3, R_3 = CH_2OH, Sesterstatin 3



4, R_1 = H, R_2 = OH, Sesterstatin 4
5, R_1 = OH, R_2 = H, Sesterstatin 5



6, Sesterstatin 6

The HRFABMS of sesterstatin 6 displayed an intense $[M + H]^+$ ion at *m/z* 419.2793 corresponding to the molecular formula $C_{25}H_{38}O_5$, requiring seven sites of unsaturation. A combination of ^{1}H NMR, ^{13}C-APT, and HMQC revealed five singlet methyls, seven methylenes, six methines, four quaternary carbons, two olefinic carbons, and one carbonyl, which counted for two unsaturations. Although interrupted by quaternary carbons and overlapping signals, a sophisticated TOCSY and ^{1}H,^{1}H-COSY analysis led to assignment of the following spin systems: CH_2−CH_2−CH_2 (C1−C3), CH−CH_2−CH_2 (C5−C7), CH_2−CH (O) (C11−C12), and CH−CH_2−CH (O) (C14−C16). On the basis of the HMBC information, the four quaternary carbons (δ_C 33.1 C-4, 37.1 C-8, 37.3 C-10, and 45.2 C-13) were found bonded to these alkyl fragments by a series of cross-peaks between the carbons and adjacent protons (Table 1) to form a substructure comprising three six-membered rings with one hydroxyl each at C-12 and C-16. The fourth ring was deduced by HMBC interpretations of olefinic carbons at C-18 (δ_C 167.4) with H-12 (δ_H 3.68), H-16 (δ_H 4.47), and H-25 (δ_H 1.15) and C-17 (δ_C128.72) with H-16

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Table 1. ^{1}H and ^{13}C NMR Assignments for Sesterstatin 6 (**6**, in $CDCl_3$−DMSO-d_6, J in Hz)[a]

position	δ_C	δ_H	^{1}H,^{1}H-COSY	HMBC
1-CH_2	39.6	1.64 (1H, m)	H-1b	C-3, C-5
		0.74 (1H, m)	H-1a, H-2b	
2-CH_2	18.0	1.54 (1H, m)	H-2b	
		1.32 (1H, m)	H-1b, H-2a	C-1, C-4
3-CH_2	41.9	1.30 (1H, m)	H-2a, H-3b	C-1, C-4, C-5
		1.04 (1H, m)	H-3a	
4-C	33.1			
5-CH	56.5	0.74 (1H, m)	H-6b	
6-CH_2	18.5	1.58 (1H, m)	H-6b, H-7b	C-8
		1.35 (1H, m)	H-5, H-6a	
7-CH_2	41.4	1.77 (1H, m)	H-7b	C-5
		0.84 (1H, m)	H-6a, H-7a	
8-C	37.1			
9-CH	58.4	0.84 (1H, m)	H-11b	C-8, C-14
10-C	37.3			
11-CH_2	25.4	1.73 (1H, m)	H-11b	C-12, C-13, C-8
		1.48 (1H, m)	H-9, H-11a, H-12	C-12
12-CH	73.7	3.68 (1H, dd, 11.0, 4.0)	H-11b	C-18, C-25
13-C	45.2			
14-CH	53.2	1.10 (1H, m)	H-15a	
15-CH_2	26.0	2.11 (1H, dd, 12.5, 6.5)	H-15b, H-16	C-8, C-13, C-14, C-16, C-17
		1.49 (1H, m)	H-14, H-15a, H-16	C-8, C-13, C-14, C-16
16-CH	65.0	4.47 (1H, dd, 18.0, 2.0)	H-15a, H-15b	C-15, C-17, C-18
17-C	128.7			
18-C	167.4			
19-CH	96.3	6.11 (1H, s)		C-17, C-20
19-OH		7.80 (1H, br, s)		
20-CO	170.8			
21-CH_3	33.1	0.78 (3H, s)		C-3, C-4, C-5, C-22
22-CH_3	21.1	0.76 (3H, s)		C-3, C-4, C-5, C-21
23-CH_3	16.0	0.78 (3H, s)		C-1, C-5, C-9
24-CH_3	17.5	0.84 (3H, s)		C-7, C-9
25-CH_3	16.8	1.15 (3H, s)		C-12, C-18

[a] 500 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, 5:1 $CDCl_3$−DMSO-d_6.

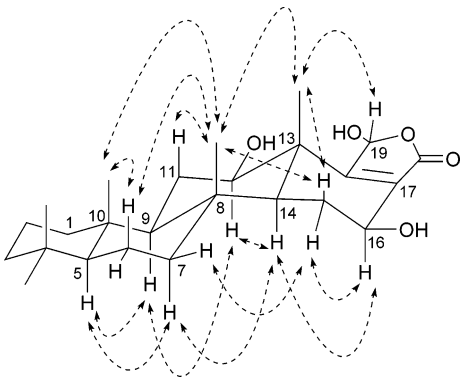

Figure 1. Key ROESY correlations of sesterstatin 6 (**6**).

(δ_H 4.47) and H-15a (δ_H 2.11). The following NMR data analysis revealed a γ-hydroxyl, α,β-unsaturated lactone unit linked to the C-17 and C-18: (1) the ^{1}H,^{1}H-COSY relationship of a hydroxyl (δ_H 7.80) with H-19 (δ_H 6.11); (2) the HMBC coupling between H-19 and a carbonyl (δ_{C-20} 170.8). The ^{13}C shifts (δ_{C-17} 128.7, δ_{C-18} 167.4) showed that the carbonyl was connected to C-17 rather than C-18, which made the lactone ring of sesterstatin 6 (**6**) opposite from that of compounds **1**−**3**.[3a]

The all *trans*-fused A−B−C−D ring system of sesterstatin 6 (**6**) was suggested by the ROESY correlation (Figure 1) including the following key cross signals: H-5 (δ_H 0.74) with H-7b (δ_H 0.84); H-7b with H-14 (δ_H 1.10); H-23 (δ_H 0.78) with H-24 (δ_H 0.84); and H-24 with H-25 (δ_H 1.15). The option of a *cis*-fused A−B ring was excluded by the impossibility of a ROESY correlation between H-5 (or H-1; both at δ_H 0.74) and H-7b (or H-9, both at δ_H 0.84). The stereochemistry of the 12β-OH, 16β-OH, and 19α-OH groups was determined by the ROESY correlations (Figure 1): H-12α (δ_H 3.68) with H-9α (δ_H 0.84) and H-14α (δ_H 1.10); H-16α (δ_H 4.47) with H-14α and H-15α (δ_H 2.11); and H-19β (δ_H 6.11) with H-25 (δ_H 1.15). The α-orientation of the 19-OH was further confirmed via GOESY (gradient Overhauser enhancement spectroscopy) NMR experiments, which caused the signal of 25-H to be increased by irradiation at H-19. Sesterstatin 6 was therefore assigned structure **6**.

After the structure assignment of sesterstatin 6 was completed, its 16α-hydroxy epimer was reported from the Okinawan variety of *H. erectus* and named hyrtiolide by Yamada,[5a] as well as the 12α-acetoxy, 16α-hydroxy epimer by Yan[5b] and co-workers from a South China Sea collection of *H. erecta*. The different axial and equatorial orientations of the 16-H in sesterterstatin 6 and hyrtiolide produced, as expected, different coupling constants: the C-16 β-epimer (**6**), δ_H 4.47 (dd, J = 18, and 2 Hz), and the α-epimer, δ_H 4.42 (d, br, J = 2.9 Hz).

The cancer cell growth inhibitory properties of sesterstatin 6 were evaluated using a minipanel of human cancer cell lines and murine P388 lymphocytic leukemia (Table 2). Sesterstatin 6 was clearly more inhibitory (10×) than sesterstatins 4 (**4**) and 5 (**5**) against the human cancer cell lines. The activity against the P388 lymphocytic leukemia cell line was superior to the sesterterpenes (**2**−**5**) we isolated previously from *H. erecta*, but similar to sesterstatin 1 (**1**, Table 2). The significant cancer cell growth inhibitory properties of sesterstatins 1 (**1**) and 6 (**6**) merit further study; investigations to ascertain molecular targets and also to discover whether associated microorganisms are the source of the sesterstatins should provide useful results.

Table 2. Cancer Cell Growth Inhibitory Activity Comparison of Sesterstatins 1−6 (**1**−**6**) against Murine P388 Lymphocytic Leukemia (ED_{50}) and a Selection of Human Cancer Cell Lines (GI_{50})[a]

cell type	cell line	**1**	**2**	**3**	**4**	**5**	**6**
murine leukemia	P388	0.46	4.2	4.3	4.9	>10	0.17
pancreas-a	BXPC-3				1.6	2.2	0.44
melanoma	RPMI-7951					2.1	
CNS	U251					1.9	
thyroid ca	KAT-4				2.0		0.40
thyroid ca	SW1736				2.1		0.87
lung-NSC	NCI-H460				1.8	2.5	0.18
pharynx-sq	FADU				2.0	1.9	0.89
prostate	DU-145				1.6	1.9	0.37

[a] ED_{50} and GI_{50} in μg/mL.

Experimental Section

General Experimental Procedures. Except as recorded in the following experimental introduction, the general experimental methods have been summarized in ref 3a. Solvents used for chromatographic procedure were redistilled. A specific rotation result was determined using a Perkin-Elmer 241 polorimeter. The ultraviolet spectrum was observed using a Perkin- Elmer Lambda 3β UV/vis spectrophotometer equipped with a Hewlett-Packard Laser Jet 2000 plotter. The IR spectrum was obtained with an AVATAR 360 FT-IR instrument, and the sample was prepared as a $CHCl_3$ film. The high-resolution mass spectrum was obtained using a JEOL LCMate magnetic sector instrument.

***Hyrtios erecta* Collection and Extraction.** The experiments summarized here emanated from the 600 kg (wet weight) of a 1994 collection of *H. erecta* in the Republic of Maldives.[6a] The *H. erecta* was identified by Dr. John N. A. Hooper and corresponds to Queensland Museum voucher number QMG304492. The CH_3OH-H_2O extract was initially separated as summarized in ref 3a and as summarized for our earlier discovery of spongistatins 1−3 in *H. erecta*.[6a]

Isolation of Sesterstatin 6. A CH_2Cl_2 P388 active fraction prepared earlier[3a] from 600 kg (wet weight) of *H. erecta* was used to provide several very minor and partially separated active (P388 leukemia) fractions. The active fraction selected for further investigation was chromatographed on a silica gel column with *n*-hexane−CH_2Cl_2−CH_3OH (6:9:1) as eluent. The third fraction (0.34 g) was further separated employing a preparative reversed-phase HPLC column (Prepex C 8, 10 × 250 mm) with a gradient of CH_3CN-H_2O (48.5% to 52%, 6 mL/min) to give 10 fractions. HPLC purification (LiChrospher 100 RP-18 column, 4.6 × 250 mm, 50% CH_3CN-H_2O, a flow rate of 1 mL/min) of the fourth fraction yielded sesterstatin 6 (**6**, 5.0 mg, 8.3×10^{-7}% yield).

Sesterstatin 6 (6): colorless fine needles, mp 253−255 °C; $[\alpha]^{24}_D$ +11.3° (*c* 0.08, $CHCl_3$); IR (film) ν_{max} 3387, 2931, 1749, 1099, and 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* (%) 418 $[M]^+$ (3), 400 (13), 385 (10), 374 (26), 356 (21), 275 (19), 235 (28), 191 (100), 148 (69); HRFABMS *m/z* 419.2793 $[M + H]^+$ (calcd for $C_{25}H_{39}O_5$ 419.2797).

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References and Notes

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