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ANTINEOPLASTIC AGENTS 397: ISOLATION AND STRUCTURE OF SESTERSTATINS 4 AND 5 FROM *HYRTIOS ERECTA* (THE REPUBLIC OF MALDIVES)[1a]

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Abstract. The wide ranging marine sponge *Hyrtios erecta* is the source of the spongistatins, a new class of macrocyclic lactone antineoplastic agents. Continuation of a detailed investigation of cancer cell growth inhibitory (P388 lymphocytic leukemia) fractions (trace) from *H. erecta* has revealed the presence (10^{-5} to 10^{-7}% yield) of cytotoxic pentacyclic sesterterpenes. Employing P388 leukemia and human tumor cell line-guided bioassay techniques, two new moderate inhibitors of cancer cells were isolated and named sesterstatins 4 (**1a**, P388 ED_{50} 4.9 μg/mL) and 5 (**1b**, DU-145 prostate GI_{50} 1.9 μg/mL). Similar to other sesterterpenes, sesterstatin 5 inhibited growth of a Gram-positive bacterium. High field (500 MHz) 2-D NMR techniques were primarily employed for initial structural assignments, and structural assignments were confirmed by X-ray crystal structure determination of sesterstatin 4 (**1a**) and 5 (**1b**).

Marine porifera have proven to be very productive sources of various mono- to diterpenes,[2] and the number of such sester- to triterpene constituents is rapidly increasing. Recent examples in the latter two groups include antimicrobial monocyclic furanosesterterpenes,[3a] antifeedant tetracyclic sesterterpenes,[3b] pentacyclic sesterterpenes,[1b,3c,3d] (including antimicrobials and cancer cell growth inhibitors[1b]) and tricyclic triterpenes (stellettins)[3e] that inhibit the growth of human cancer cells. A continuing examination of cancer cell growth inhibitory fractions from a 600 kg (wet wt.) recollection (Republic of Maldives, 1994) of the black marine sponge, *Hyrtios erecta*[1b,3c,4,5] has led to the isolation and structural elucidation of two new pentacyclic furanosesterterpenes, designated sesterstatins 4 (**1a**) and 5 (**1b**). **1a** and **1b** inhibited growth of a number of human cancer cell lines (Table 1), and **1b** inhibited growth of the Gram-positive bacterium *Micrococcus luteus*.

Table 1. Cancer Cell Growth Inhibition (GI_{50}, μg/mL) Results

Compound	Murine P388 Leukemia	BXPC-3 Pancreas	RPM1-7951 Melanoma	U251 CNS	KAT-4 Thyroid	SW1736 Thyroid	NCI-H460 Lung NSC	FADU Pharynx	DU-145 Prostate
1a	4.9	1.6	–	–	2.0	2.1	1.8	2.0	1.6
1b	>10	2.2	2.1	1.9	–	–	2.5	1.9	1.9

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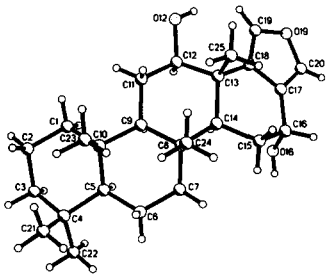


Figure 1. Computer-generated X-ray crystal structure of sesterstatin 4 (**1a**).

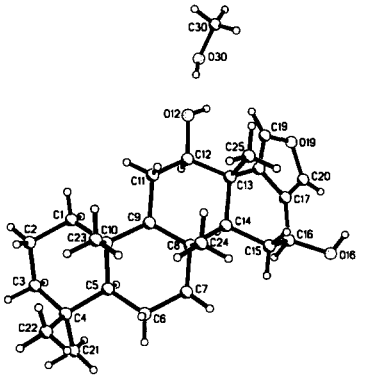
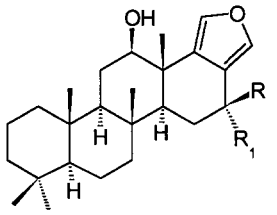


Figure 2. Computer-generated X-ray crystal structure of sesterstatin 5 (**1b**) and methanol solvate



1a, Sesterstatin 4, R = H, R_1 = OH
1b, Sesterstatin 5, R = OH, R_1 = H

The methanol extract from a portion of *H. erecta* from the 1994 recollection (600 kg, wet wt) provided a P388 leukemia cell line active dichloromethane fraction (203 g, ED_{50} 0.31 μg/mL) following solvent partitioning.[1b] Successive chromatography of this fraction on Sephadex LH-20 (methanol → 3:1:1 hexane toluene methanol) followed by chromatography on silica gel (6:9:1, hexane dichloromethane methanol) and reversed-phase HPLC (acetonitrile-water gradients) allowed isolation of sesterstatin 4 (**1a**) as colorless crystals from CH_3CN-H_2O (6.1 mg, 1.0 x 10^{-6} % yield): mp 252 - 254°C, $[\alpha]_D^{25}$ -10 (*c* 0.09, $CHCl_3$). Interpretation of the ^1H, APT, and HMQC NMR (500 MHz) spectra (Table 2) established the presence of five methyl, seven methylene, and five methine groups including two downfield signals (δ 79.95/3.63; 61.56/4.91). Four quaternary carbons and four olefinic carbons (δ139.37/7.52 , 137.85/7.36, 133.97, and 124.53) were also located in the ^{13}C NMR spectrum. Correlation of this information with the low resolution mass spectral molecular ion at *m/z* 386 suggested molecular formula $C_{25}H_{38}O_3$. Analysis (Table 2) of the 2-D NMR spectra (COSY, TOCSY, and HMBC) resulted in the recognition of a pentacyclic sesterterpene framework with two hydroxyl groups (at C-12 and C-16) and a terminal furan ring. In the ROESY spectrum of **1a**, the nOe effects observed between the olefinic protons and the nearby groups (H-19 at δ 7.52 with H-25 at δ 1.12, and H-20 at δ 7.36 with H-16 at δ 4.91 and OH-16 at δ 5.32) indicated that the β- (δ 124.53) and β′ - (δ 133.97) carbons of the furan ring should correspond to C-17 and C-18, respectively. The ROESY spectrum also suggested that the OH-12 was equatorial and the OH-16 axial (from the nOe correlation of H-12 at δ 3.63 and the OH-16 at δ5.32). These interpretations suggested **1a** was a 16-epi-deacetyl-scalarafuran,[6] the structure of which was confirmed by a single crystal X-ray determination (see below).

Another P388 leukemia cell line active dichloromethane fraction (149.09 g, ED_{50} 0.17 μg/mL) was prepared as noted above and separated using a series of Sephadex LH-20 gel permeation and partition chromatographic procedures (methanol → 3:1:1 hexane toluene methanol) followed by column chromatography on Silica gel (6:9:0.5 hexane-dichloromethane-methanol) to yield sesterstatin 5 (**1b**) as colorless needles from dichloromethane methanol (1:1): 242 mg (4 x 10^{-5} % yield); mp -245°C; $[\alpha]_D^{25}$ + 27.0 (*c* 0.288, $CHCl_3$); UV (MeOH) λ_{max} 219 nm, ϵ 4148°; IR (film) 3445, 3358, 2918, 2849, 1456, 1383, 1039 cm^{-1}; EIMS *m/z* 386 corresponding to $C_{25}H_{38}O_3$.

The ^{13}C NMR (Table 2) of **1b** showed the presence of five signals corresponding to quaternary methyl groups; seven methylene groups; five methine groups; and four quaternary carbons. The ^{13}C NMR spectrum indicated the presence of four olefinic carbons with the two fully substituted sp^2 carbons at δ134.6 and 126.31, as well as trisubstituted sp^2 carbon atoms at δ136.40/7.42 (1H) and 137.50/7.32 (H). The ^1H NMR spectrum showed two hydroxyl signals at δ5.09 and 4.79, and this accounted for a total of 38 hydrogen atoms including 36 nonexchangeable atoms.

Interpretation of the COSY and TOCSY spectra revealed four partial structural units, C-1 to C-3; C-5 to C-7; C-9, C-11 to C-12 and C-14 to C-16 with hydroxyl groups bonded to C-12 and C-16. The COSY spectrum showed weak coupling between the olefinic protons, at δ7.42 and 7.32, suggesting they were part of a terminal furan ring.

Table 2. High-field (500 MHz), ^1H and ^{13}C NMR Data Corresponding to Sesterstatin 4 (**1a**) (in CD_2Cl_2) and Sesterstatin 5 (**1b**) (in DMSO-d_6).

	Sesterstatin 4 (1a)			Sesterstatin 5 (1b)			
No.	^{13}C	^1H	NMBC (H to C)	No.	^{13}C	^1H	NMBC (H to C)
1	40.22(t)	1.69 (1H,m) 0.83 (1H,m)	C-3	1	39.30(t)	1.60 (1H,m) 0.75 (1H,m)	C-2 C-3,C-5
2	19.02(t)	1.62 (1H,m) 1.42 (1H,m)		2	17.70(t)	1.55 (1H,m) 1.36 (1H,m)	C-4
3	42.42(t)	1.14 (1H,m) 1.37 (1H,m)	C-2, C-4	3	41.59(t)	1.31 (1H,m) 1.08 (1H,m)	C-5 C-4
4	33.56(s)			4	32.90(s)		
5	56.92(d)	0.84 (1H,m)	C-21	5	55.96(d)	0.76 (1H,m)	C-4
6	18.53(t)	1.58 (1H,m) 1.42 (1H,m)		6	18.10(t)	1.48 (1H,m) 1.36 (1H,m)	C-8
7	41.87(t)	1.83 (1H,m) 0.95 (1H,m)	C-5	7	41.04(t)	1.76 (1H,m) 0.85 (1H,m)	C-5, C-9 C-5, C-8
8	37.25(s)			7	36.88(s)		
9	59.18(d)	1.01 (1H,m)	C-8, C-10,C-11,C-23,C-24	9	57.90(d)	0.83 (1H,m)	C-7,C-11,C-12,C-14
10	37.81(s)			10	36.77 (s)		
11	28.32(t)	1.77 (1H,m) 1.53 (1H,m)		11	27.17 (t)	1.60 (1H,m) 1.41 (1H,m)	C-8,C-9,C-12,C-13,C-9 C-12,C-13
12	79.95(d)	3.63 (1H,dd,11.5/4 Hz)	C-18,C-25	12	77.94 (t)	3.37 (1H,dd,	
12-OH		3.63 (1H)		12-0H		4.79 (1H,d)	
13	40.74(s)			13	39.90 (s)		
14	49.58(d)	1.49 (1H,m)	C-15,C-16,C-25	14	53.86 (d)	0.96 (1H,dd)	C-7,C-9,C-12,C-13,C-15,C 16,C-18
15	28.09(t)	1.88 (1H,m) 1.83 (1H,m)	C-14 C-13,C-14,C-16,C-17	15	28.63 (t)	1.83 (1H,m) 1.47 (1H,m)	C-8,C-13,C-14,C-16,C-17 C-13,C-14,C-16,C-17
16	61.56(d)	4.91 (1H,br,d,*J*=1 Hz)	C-14	16	64.93 (d)	4.45 (1H,m)	C-17
16-0H		5.32 (1H,s)		16-OH		5.04 (1H,dt)	
17	124.53(s)			17	126.31 (s)		
18	133.97(s)			18	134.60 (s)		
19	139.97(d)	7.52 (1H,d,*J*=1.5 Hz)	C-17,C-18,C-20	19	136.40 (d)	7.42 (1H,d,*J*=1.5 Hz)	C-17,C-18,C-20
20	137.85(d)	7.36 (1H,d,*J*=1.5 Hz)	C-17,C-18,C-19	20	137.50 (d)	7.32 (1H,d,*J*=1.5 Hz)	C-17,C-18,C-19,C-15
21	33.37(q)	0.85 (3H,s)	C-5	21	33.04 (q)	0.81 (3H,s)	C-4,C-5
22	21.44(q)	0.83 (3H,s)	C-4,C-5,C-21	22	21.13 (q)	0.78 (3H,s)	C-4,C-5
23	16.41(q)	0.87 (3H,s)	C-1,C-9,C-10	23	15.96 (q)	0.79 (3H,s)	C-5,C-9
24	17.90(q)	0.91 (3H,s)	C-7,C-8,C-9,C-14	24	17.49 (q)	0.82 (3H,s)	C-7,C-9,C-14
25	18.63(q)	1.12 (3H,s)	C-13,C-14	25	19.86 (q)	1.10 (3H,s)	C-12,C-13,C-14,C-18

A ROESY experiment confirmed this showing crosspeaks between the proton at δ7.42 and H-12 (δ3.3), OH-12 (δ4.79) and the methyl group at C-25 (δ1.1). These crosspeaks were not seen for the proton at δ7.32. Therefore these protons were placed on either side of the oxygen atom. HMBC data revealed crosspeaks between a fully substituted sp2 carbon at δ126.31 and the protons H-16 (δ4.45) and H-15 (δ1.47 and 1.83). Crosspeaks between this carbon and the olefinic protons were also observed. Also, the crosspeak between the proton at δ7.32 and the carbon at δ126.31 was larger in intensity than the crosspeak between this carbon and the proton at δ7.42, and visa versa with the carbon at δ134.6. The HMBC results also indicated that a crosspeak correlation existed between the carbon at δ134.6 and the C-24 methyl protons C-24 (δ1.1) and H-14 (δ0.96). Thus, the resonance at δ126.31 was assigned to C-17 and the resonance at δ134.6 was assigned to C-18. The remaining three segments were connected by the quaternary carbons which showed distinctive crosspeaks with the methyl groups in HMBC spectrum. For example, the protons on the methyl groups at C-21 had crosspeaks with the quaternary carbon at C-4, as well as the methine carbon C-5, the methyl group at C-22, and the methylene carbons C-1 and C-3.

The stereochemistry of sesterstatin 5 (**1b**) was established by a ROESY experiment. The hydroxyl group at C-16 was assigned as equatorial. This was based on crosspeak correlation between the protons at H-16 (δ4.45) and H-20 (δ7.32), and intense crosspeaks involving H-16 and H-14 (δ0.96) protons. The H-12 proton at δ3.33 was assigned as axial owing to the intense crosspeaks with H-14 (δ0.96), H-11 (axial, δ1.60), and H-9 (δ0.83). That led to equatorial assignment of the 12-OH group. These interpretations indicated **1b** was a 16-deacetyl-scalarafuran,[6] differing from sesterstatin 4 (**1a**) only in the configuration at C-16. Again, this was confirmed by a single crystal X-ray determination.

The proposed structures of both sesterstatins 4 and 5 were confirmed by independent single crystal X-ray crystallographic analyses. In the case of sesterstatin 4 (**1a**), data were collected at 26 ± 1 °C on a crystal (0.24 x 0.16 x 0.06 mm) grown by evaporation of a solution of the compound in CH_3CN-H_2O. Linear and anisotropic decay corrections were applied to the intensity data as well as an empirical absorption correction (based on a series of psi-scans).[7] Structure determination was readily accomplished with the direct-methods program SIR92[8] and anisotropic refinement was carried out by full-matrix least-squares methods using SHELXL-93,[9] yielding a conventional R_1 [2σ(I) cutoff] of 0.0779. The absolute stereochemical structure determined for sesterstatin 4 (**1a**) is shown in Figure 1.[10]

In a similar manner, data were collected at 25 ± 1° on a crystal (0.58 x 0.16 x 0.16 mm) of **1b**, grown via slow evaporation of a methylene chloride-methanol solution of the compound. As in the case of **1a**, linear and anisotropic decay corrections were again applied to the intensity data of **1b**, as well as an empirical absorption correction (psi-scan). Structure determination with SIR92 and anisotropic refinement with SHELXL-93 resulted in the structure for **1b**, as shown in Figure 2. A final R_1 [2σ(I) cutoff] of 0.0685 was obtained. In addition to the sesterstatin 5 (**1b**) molecule, each asymmetric unit was also found to contain a molecule of methanol.

Under the same HPLC conditions (C18 column, developed in 70% acetonitrile-water) sesterstatins 4 (**1a**) and

5 (**1b**) displayed different retention times, Rt 13.75 min for **1a** and 14.35 min for **1b**. In addition, their solubility behavior and response to the P388 leukemia cell line also differed (ED_{50} 4.87 *vs.* ED_{50} >10 μg/mL, respectively). Evaluation against a minipanel of human cancer cell lines also showed differences (Table 1). Disk diffusion assays[11] were used to evaluate potential antimicrobial activity of the two new pentacyclic furanosesterterpenes. Sesterstatin 5 (**1b**) inhibited growth of the opportunistic Gram-positive bacterium *Micrococcus luteus* (minimum inhibitory concentration 25-50 μg/disk). A number of related sesterterpenes, including the recently described sesterstatin 2, have similar narrow spectrum anti-Gram-positive action.[1b,3a,12,13]

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References and Notes.

1. (a) For contribution 396 in the series, see Mohammad, R. M.; Varterasian, M. L.; Almatchy, V. P.; Pettit, G. R.; Al-Katib. A. *Clinical Cancer Res.* **1998**, *4*, 1337. (b) Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Hoard, M. S.; Melody, N.; Pettit, R. K. *J. Nat. Prod.*, in press.
2. Pettit, G. R.; Hogan-Pierson, F.; Herald, C. L. *Anticancer Drugs from Animals, Plants, and Microorganisms*; John Wiley-Interscience: New York, 1993, p. 49.
3. (a) Martinez, A.; Duque, C.; Sato, N.; Fujimoto, Y. *Chem. Pharm. Bull.* **1997**, *45*, 181. (b) Carotenuto, A.; Fattorusso, E.; Lanzotti, V.; Magno, S.; Mayol, L. *Liebigs Ann.* **1996**, 77. (c) Pettit, G. R.; Cichacz, Z. A.; Tan, R.; Herald, D. L.; Melody, N.; Hoard, M. S.; Doubek, D. L., Hooper, J. N. A. *J. Chem Soc., Perkin Trans 1*, submitted. (d) Lu, Q.; Faulkner, J. *J. Nat. Prod.* **1997**, *60*, 195. (e) McCormick, J. L.; McKee, T. C.; Cardellina, J. H. II; Leid, M.; Boyd, M. R. *J. Nat. Prod.* **1996**, *59*, 1047.
4. Pettit, G. R.; Cichacz, Z. A.; Herald, C. L.; Gao, F.; Boyd, M. R.; Schmidt, J. M.; Hamel, E.; Bai, R. *J. Chem. Soc., Chem. Commun.* **1994**, 1605.
5. Pettit, G. R.; Cichacz, Z. A.; Gao, F.; Herald, C. L.; Boyd, M. R.; Schmidt, J. M.; Hooper, J. N. A. *J. Org. Chem.* **1993**, *58*, 1302.
6. Walker, R. P.; Thompson, J. E.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 4976.
7. North, A. C.; Phillips, C. C.; Matthews, F. S. *Acta Cryst.* **1968**, *A24*, 351.
8. Altomare, A.; Camalli, M. *SIR92 - A Program for Automatic Solution of Crystal Structures by Direct Methods*, Dipartimento Geomineralogico, University of Bari, Italy.
9. Sheldrick, G. M. *SHELXL93. Program for the Refinement of Crystals Structures*, University of Göttigen, Germany (1993).
10 Preparation of Figure 1 was done with "*SHELXTL-PC Version* 5.03, (1994)" an integrated software system for the determination of crystal structures from diffraction data. Siemens Industrial Automation, Inc., Analytical Instrumentation, Madison, WI 53719.
11. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Disk Susceptibility Tests - Sixth Edition: Approved Standard M2-A6.* NCCLS: Villanova, PA, 1997.
12. Ravi, B. N.; Perzanowski, H. P.; Ross, R. A.; Erdman, T. R.; Scheuer, P. J. *Pure Appl. Chem.* **1979**, *51*, 1893.
13. Schmidt, E. W.; Faulkner, D. J. *Tetrahedron Lett.* **1996**, *37*, 3951.

Supplementary Material Available: X-ray crystallographic tables of atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **1a** and **1b** are available from the authors. These tables have also been deposited with the Cambridge Crystallographic Data Centre and are available, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, U.K.