Notes

Strongylophorine-26, a New Meroditerpenoid Isolated from the Marine Sponge *Petrosia (Strongylophora) corticata* That Exhibits Anti-invasion Activity[⊥]

Kaoru Warabi,[†] Lianne M. McHardy,[‡] Lohi Matainaho,[∥] Rob Van Soest,[#] Calvin D. Roskelley,*[,§] Michel Roberge,*[,‡] and Raymond J. Andersen*[,†]

Departments of Chemistry, Earth and Ocean Sciences, Anatomy and Cell Biology, and Biochemistry and Molecular Biology, University of British Columbia, Vancouver, B.C. V6T 1Z1, Canada, Department of Pharmacy, University of Papua New Guinea, Port Moresby, Papua New Guinea, and Zoologisch Museum, University of Amsterdam, Amsterdam, The Netherlands

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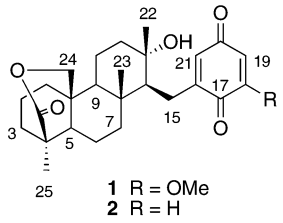
Strongylophorine-26 (**1**), a new meroditerpenoid that shows activity in an anti-invasion assay, has been isolated from the marine sponge *Petrosia* (*Strongylophora*) *corticata* collected in Papua New Guinea. The structure of **1** was elucidated by analysis of spectroscopic data.

Angiogenesis and metastasis are processes involving cell migration that are central to the progression and lethality of solid-tumor cancers.[1] Folkman was the first to propose that antiangiogenic drugs would inhibit the vascularization of solid tumors, thereby starving them of the oxygen and nutrients required for uncontrolled growth and at the same time limiting their access to conduits for metastatic spreading throughout the body.[2] Tissue invasion via cell migration is a shared feature of angiogenesis and metastasis that represents a promising target for the development of new anticancer drugs.[1,3] We have used an anti-invasion assay recently developed in our laboratories to screen extracts of marine invertebrates for their ability to inhibit tissue invasion by human breast cancer MDA-231 cells without being overtly cytotoxic.[3] Extracts of the marine sponge *Petrosia* (*Strongylophora*) *corticata* collected in Papua New Guinea showed strong activity in the assay. Bioassay-guided fractionation of the crude extract identified the new meroterpenoid strongylophorine-26 (**1**)[4] as the active component. Details of the isolation and structure elucidation of strongylophorine-26 (**1**) are presented below.

Specimens of *P. corticata* (450 g) were collected by hand using scuba on shallow reefs (−15 m) near Port Moresby, PNG, frozen on site, and transported to Vancouver over dry ice. Freshly thawed sponge material was extracted successively with MeOH (0.5 L × 3) and CH_2Cl_2/MeOH/H_2O (7:3:0.5; 0.5 L × 3). The extracts were combined, concentrated in vacuo, and partitioned between H_2O (0.3 L) and $CHCl_3$ (0.3 L × 3) to give a bioactive $CHCl_3$-soluble fraction. Further bioassay-guided fractionation of the $CHCl_3$-soluble materials via sequential application of C_{18} reversed-phase flash chromatography (Waters Sep-pak 24 × 59 mm; step gradient elution from MeOH/H_2O (6:4) to CH_2Cl_2/MeOH/H_2O (7:3:0.5)), Sephadex LH-20 chromatography (eluent: MeOH), and reversed-phase HPLC (Whatman Partisil 10 ODS and then Altech Econosil C18) gave pure samples of strongylophorine-8 (**3**) (46 mg) and strongylophorine-26 (**1**) (6 mg). The previously described strongylophorine-8 (**3**) was identified by comparison of its NMR and MS data with the literature values.[5]



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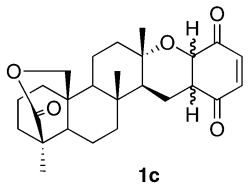
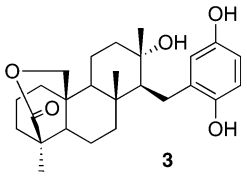
2 R = H



1c



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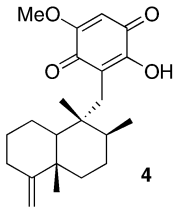

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Strongylophorine-26 (**1**) was isolated as an optically active ($[\alpha]^{28}_D$ +11° (*c* 0.35, CH_3CN)) orange amorphous solid that gave a $[M]^+$ ion at *m/z* 456.2515 in the HREIMS, appropriate for a molecular formula of $C_{27}H_{36}O_6$ (calcd 456.2512), requiring 10 sites of unsaturation. Analysis of the 1D and 2D NMR data obtained for **1** (Table 1) revealed it had a diterpenoid fragment identical to that found in strongylophorine-8 (**3**). The NMR resonances assigned to the nonterpenoid fragment of **1** were complicated by the presence of minor shadow peaks, indicating that strongylophorine-12 exists in three slowly interconverting tautomeric forms (Figure 1). Major-form proton NMR resonances attributed to this fragment included a singlet at δ 3.74 (3H), assigned to a OMe (C-27) residue, and a pair of multiplets at δ 5.86 (d J = 2.3; H-19) and 6.48 (ddd J = 2.3, 1.1, 1.1; H-21), assigned to olefinic protons. Seven carbon resonances were assigned to the nonterpenoid

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* Corresponding authors. R.A.: Tel: 604 822 4511. Fax: 604 822 6091. E-mail: randersn@interchange.ubc.ca.
[†] Chemistry and EOS, UBC.
[‡] Biochemistry and Molecular Biology, UBC.
[§] Anatomy and Cell Biology, UBC.
[∥] University of Papua New Guinea.
[#] University of Amsterdam.

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Table 1. ^{1}H and ^{13}C NMR Data for Strongylophorine-26 (**1**) and the Quinone **2** Recorded in CD_3CN at 500 MHz for ^{1}H and 100 MHz for ^{13}C

	strongylophorine-26 (**1**)			quinone **2**	
C#	δ_H (mult. J^a in Hz)	δ_C	HMBC	δ_H (mult. J^a in Hz)	δ_C
1	2.12 (m)	40.6		1.08 (m)	40.6
	1.08 (m)			2.13 (m)	
2	1.65 (m)	39.8		1.65 (m)	39.8
	1.05 (m)		C4	1.07 (m)	
3	1.69 (m)	41.0		1.70 (m)	41.0
	1.47 (m)		C4, C26	1.50 (m)	
4		43.9			43.9
5	1.27 (m)	50.7	C9, C26	1.30 (m)	50.7
6	1.30 (m)	21.2		1.27 (m)	21.2
	1.22 (m)			1.23 (m)	
7	1.67 (m)	21.8	C6	1.70 (m)	21.7
	1.50 (m)		C14	1.51 (m)	
8		39.5			39.6
9	1.11 (m)	55.9	C8, C10, C11, C23	1.12 (m)	55.9
10		37.6			37.5
11	1.71 (m)	19.9	C9	1.73 (m)	19.8
	1.10 (m)			1.14 (m)	
12	1.77 (m)	45.8	C13, C14	1.78 (m)	45.7
	1.35 (m)			1.35 (m)	
13		73.9			73.8
14	1.60 (m)	62.6	C9, C12, C13, C15, C23	1.61 (m)	62.8
15	2.50 (dd, J = 6.6, 1.1)	24.3	C8, C13, C14, C16, C17, C21	2.50 (m)	24.3
	2.50 (dd, J = 6.6, 1.1)			2.50 (m)	
16		151.5			153.6
17		183.0			188.7
18		160.5		6.72 (d, J = 10.1)	138.0
19	5.86 (d, J = 2.3)	107.6	C17, C18, C20,[b] C21	6.64 (dd, J = 2.5, 10.1)	137.0
20		188.8			189.0
21	6.48 (ddd, J = 2.3, 1.1, 1.1)	133.2	C15, C17, C19	6.59 (ddd, J = 1.3, 1.3, 2.5)	133.0
22	1.10 (s)	24.1	C12, C13, C14	1.12 (s)	24.1
23	0.96 (s)	16.5	C8, C9, C14	0.98 (s)	16.5
24	4.66 (d, J = 12.3)	74.2	C1, C9, C10, C26	4.67 (d, J = 12.3)	74.1
	3.97 (d, J = 12.3)		C1, C5, C10, C26	3.96 (d, J = 12.3)	
25	1.08 (s)	23.6	C3, C4, C5, C26	1.09 (s)	23.5
26		177.0			177.0
27	3.74 (s)	57.1	C18		
13OH	2.35 (s)		C13, C14		2.37(s)

[a] J values were estimated from the ^{1}H NMR spectrum recorded on a Bruker AV400 spectrometer. [b] HMBC correlation was observed with a d6 value of 85 ms.

fragment (Table 1), including the OMe resonance (δ 57.1; C-27), four that could be assigned to olefinic carbons (δ 151.5, C-16; 160.5, C-18; 107.6, C-19; 133.2, C-21), and a pair that had chemical shifts (δ 183.0, C-17; 188.8, C-20) typical of quinone carbonyls. The olefinic proton resonances at δ 5.86 (H-19) and 6.48 (H-21) were strongly correlated in the COSY spectrum, and their mutual scalar coupling of 2.3 Hz required *W* coupling. An additional COSY correlation between the resonance at δ 6.48 (H-21) and the resonance at δ 2.50 (H-15) was assigned to allylic coupling. All the above data were consistent with attachment of a methoxy-*p*-quinone moiety to C-15 of the diterpenoid fragment to give the complete structure of strongylophorine-26 as shown in **1**.

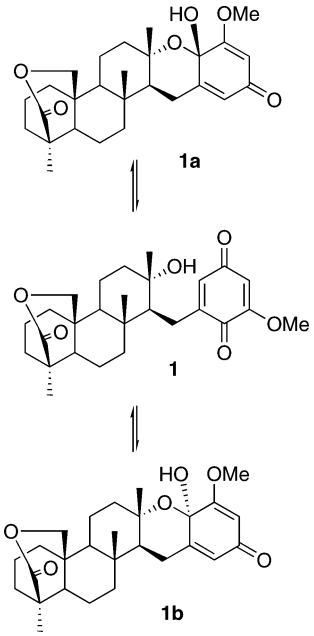

Figure 1. Tautomeric equilibria observed for strongylophorine-26 (**1**).

Analysis of the COSY, HMQC, and HMBC correlations for the minor peaks resulting from the tautomeric equilibrium of **1** demonstrated that the minor isomers were formed by addition of the C-13 hydroxyl to the C-17 carbonyl to give a pair of cyclic diastereomers **1a** and **1b** shown in Figure 1. The open tautomer **1** and the two cycloadducts (**1a/b**) were present in the ratio of 6.4:1.7:1. Partial ^{13}C and ^{1}H chemical shift assignments for the two cyclic forms (**1a/b**) are as follows: C-15 (δ 2.67, m and 2.33, m (major cyclic form); 2.67, m and 2.48, m (minor cyclic form)), C-19 (δ 5.28, d, J = 1.6 Hz; 99.5 (major); 5.26, d, J = 1.6 Hz; 98.5 (minor)), C-21 (δ 5.780, m; 122.2 (major); 5.44, m; 125.4 (minor)), and C-27 (δ 3.71, s (major); 3.72 (minor)). The chemical shifts of the H-15/H-15′ resonances in both minor forms (δ 2.67−2.33) were consistent with the protons being allylic, which ruled out the alternate Michael addition cyclization tautomers **1c**. Similarly, the 1.6 Hz coupling observed between the H-19 and H-21 resonances in both minor forms was consistent with the presence of the same planar *W* coupling geometry found in the open form **1**, once again excluding the stereoisomers of tautomer **1c**.

To provide additional support for the proposed structure of strongylophorine-26 (**1**), a portion of strongylophorine-8 (**3**) was oxidized to the *p*-quinone **2**. Comparison of the NMR data for **2** with that obtained for strongylophorine-12 (**1**) (Table 1) confirmed that the diterpenoid fragments in both molecules were identical. Interestingly, the NMR data obtained for **2** showed no evidence for the presence of cyclic tautomers corresponding to **1a/b**.

Strongylophorine-26 (**1**) had an IC_{50} of ~1 μg/mL in the cell-based anti-invasion assay and showed maximal activity at 2.5 μg/mL (Figure 2). The large decrease in invasion inhibition observed at concentrations of 2.5 μg/mL and above was due to cell death. The hydroquinone strongylophorine-8 (**3**) and the corresponding quinone **2** were both active in the assay, but were significantly less potent than strongylophorine-26 (**1**) (both **2** and **3** had IC_{50}'s $\approx$ 7 μg/mL). The observation of identical IC_{50}'s for **2** and **3** suggests that hydroquinone **3** may be oxidized to quinone **2** during the assay. Ilimaquinone (**4**) was also tested in the anti-invasion assay and found to be completely inactive at concentrations less than 50 μg/mL. Taken together, the

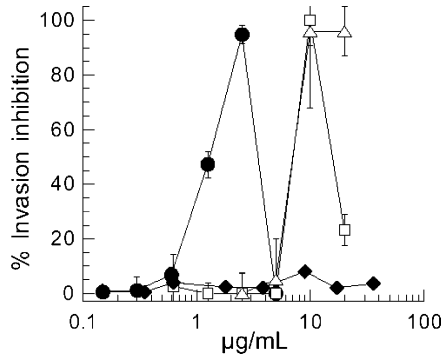

Figure 2. Inhibition of MDA-231 cell invasion by strongylophorine-26 (**1**) (●), **2** (□), **3** (△), and ilimaquinone (◆).

bioassay results for **1**, **2**, and **3** show that the methoxy substituent on the quinone fragment of strongylophorine-26 (**1**) enhances the potency. The observed inactivity of ilimaquinone in the assay implies that the diterpenoid fragment of **1**, **2**, and **3** also plays an important role. Strongylophorine-26 has a potency comparable to that of the recently described invasion inhibitor motuporamine C ($IC_{50} = 0.3\ \mu g/mL$).[3] Further exploration of the structure−activity relationship for the anti-invasion pharmacophore of strongylophorine-26 (**1**) is currently underway in our laboratories.

Experimental Section

General Experimental Procedures. Optical rotation values were measured with a JASCO P-1010 polarimeter. UV spectra were recorded on a Waters 2487 dual λ absorbance detector. ^{1}H spectra were recorded on Bruker AMX500 and AV400 spectrometers, and ^{13}C NMR spectra were recorded on a Bruker AM400 spectrometer. EIMS spectra were recorded on a Kratos MS-50 mass spectrometer.

Animal Material. The sponge was collected by hand using scuba in Papua New Guinea and was kept frozen until extraction. It was identified as *Petrosia* (*Strongylophora*) *corticata*, and a voucher specimen (ZMA POR 17069) has been deposited at the Institute for Systematics and Ecology (Zoological Museum), University of Amsterdam, in The Netherlands.

Isolation of Strongylophorine-26 (1) and Strongylophorine-8 (3). The frozen sponge (453 g) was thawed and successively extracted with MeOH (0.5 L × 3) and CH_2Cl_2/MeOH/H_2O (7:3:0.5, 0.5 L × 3). The extracts were combined, concentrated in vacuo, and partitioned between H_2O (0.3 L) and $CHCl_3$ (0.3 L × 3). The $CHCl_3$ layer was further partitioned between 90% aqueous MeOH (0.5 L) and hexanes (0.2 L × 4). The ratio of MeOH to water was changed from 9:1 to 6:4 by addition of water, and then the 60% aqueous MeOH layer was extracted with $CHCl_3$ (0.2 L × 5). The active $CHCl_3$ extract was fractionated by C_{18} flash chromatography (Waters Sep-pak, 24 × 59 mm), eluting with MeOH/H_2O (4:6), MeOH/H_2O (6:4), MeOH/H_2O (8:2), MeOH, and CH_2Cl_2/MeOH/H_2O (7:3:0.5) in sequence. The active fractions were combined and then chromatographed on LH 20 (22 × 840 mm), eluting with MeOH. Fractionation of the active fractions by reversed-phase HPLC (Whatman Partsil 10 ODS-3, 9.4 × 500 mm) furnished strongylophorine-8 (**3**, 45.8 mg). The final purification of the active fraction by reversed-phase HPLC (Altech Econosil C18 5u, 4.6 × 250 mm) afforded strongylophorine-26 (**1**, 5.5 mg).

Strongylophorine-26 (1): orange amorphous solid: $[\alpha]^{28}_D$ +10.9° (*c* 0.35, CH_3CN); UV (CH_3CN) λ_{max} (ϵ) 202 nm (10000), 241 nm (6200), 276 nm (6900), 347 nm (shoulder); ^{1}H and ^{13}C NMR data, see Table 1; HREIMS *m*/*z* 456.2515, $C_{27}H_{36}O_6$ requires 456.2512.

Oxidation of Strongylophorine-8. A sample of partially purified strongylophorine-8 (**3**) (13 mg) was dissolved in THF (1.0 mL). MnO_2 (59.9 mg) was added to this solution, and the mixture was stirred at room temperature for 55 min before being partitioned between H_2O and Et_2O. The Et_2O layer was dried over Na_2SO_4. Solid Na_2SO_4 was removed with a cotton filter, and the filtrate was concentrated in vacuo. The crude reaction residue was purified by silica gel chromatography (ϕ 12 × 120 mm) eluting in a stepwise gradient using EtOAc/hexane (2:8), EtOAc/hexane (3:7), and EtOAc/hexane (4:6), to afford quinine **2** (4.6 mg) as an orange amorphous solid; ^{1}H NMR (CD_3CN), see Table 1; ^{13}C NMR (CD_3CN), see Table 1; HREIMS *m*/*z* 426.2410, $C_{27}H_{34}O_6$ requires 426.2406.

Invasion Assay. The ability of compounds to inhibit invasion of Matrigel by human breast carcinoma MDA-231 cells was measured exactly as described in ref 3.

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