CYTOTOXIC ISOQUINOLINE QUINONES FROM SPONGES OF THE GENUS *PETROSIA*

IMELDA T. SANDOVAL,[a,b] ROHAN A. DAVIS,[a] TIM S. BUGNI,[a] GISELA P. CONCEPCION,[c] MARY KAY HARPER,[a] and CHRIS M. IRELAND [a,*]

[a]*Department of Medicinal Chemistry, University of Utah, Salt Lake City, Utah 84112, USA,* [b]*Huntsman Cancer Institute, University of Utah, Salt Lake City, Utah 84112, USA,* [c]*Marine Science Institute, University of the Philippines, Diliman, Quezon City 1101, Philippines*

Bioassay-guided fractionation of two Philippine sponges of the genus *Petrosia* has resulted in the isolation of the novel natural product cribrostatin 7 (**1**) and the known compounds renierone (**2**) and *O*-demethylrenierone (**3**). The structures of these isoquinoline quinones were determined by interpretation of spectroscopic data. Compounds **1**, **2** and **3** were cytotoxic against the HCT 116 human colon carcinoma cell line with IC_{50} values of 45, 24 and 34 μg/ml, respectively.

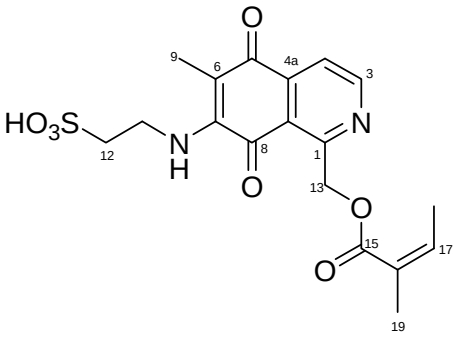
Keywords: Marine sponge; *Petrosia* sp.; Isoquinoline quinone; Cribrostatin 7; Cytotoxic activity

INTRODUCTION

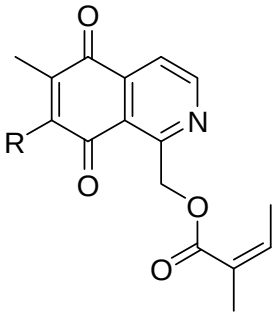
Over the past three decades, marine sponges have proven to be prolific sources of structurally novel bioactive metabolites. Examples include oceanapiside, an antifungal α,ω-bis-aminohydroxylipid glycoside; peloruside A, a cytotoxic polyoxygenated macrolide; and the anti-inflammatory pyridinium alkaloids, spongidines A-D [1-3]. Isoquinoline quinones are another class of sponge-derived compounds that have been shown to possess a wide array of biological activity including antimicrobial, insecticidal, antifungal, and antineoplastic properties [4-

* Corresponding author. Tel.: 1 801 581 8305. Fax: 1 801 585 6208. E-mail: cireland@pharm.utah.edu.

6]. This paper describes the bioassay-guided fractionation of extracts from two Philippine *Petrosia* spp. (Order Haplosclerida, Family Petrosiidae) that displayed cytotoxicity against the HCT 116 human colon carcinoma cell line. Extensive chromatography yielded a new isoquinoline derivative, which we named cribrostatin 7 (**1**) and two known compounds, renierone (**2**) and *O*-demethylrenierone (**3**).



Cribrostatin 7 (**1**)



R = OMe (**2**)
R = OH (**3**)
R = NH_2 (**4**)
R = NHMe (**5**)

RESULTS AND DISCUSSION

The crude MeOH extract from *Petrosia* sp. (PC00-11-149) was subjected to a modified Kupchan partition scheme that resulted in hexanes, $CHCl_3$ and 30% aq MeOH-soluble fractions. The bioactive hexane-soluble material was purified by C_{18} HPLC using a MeOH/H_2O gradient to yield the previously reported metabolites renierone (**2**, 1.1 mg) and *O*-demethylrenierone (**3**, 0.5 mg). Compounds **2** and **3** were readily identified from their spectroscopic data and by comparison with published data [4, 7].

In an attempt to isolate more of compounds **2** and **3** for cytotoxicity screening, another Philippine sponge of the genus *Petrosia* ($PDZ_1$98-4-74) was investigated. The crude MeOH extract was subjected to an identical solvent-partitioning scheme as described previously. The hexane-soluble material was purified by C_{18} HPLC using an CH_3CN/H_2O gradient to yield more renierone (**2**,

0.8 mg) and *O*-demethylrenierone (**3**, 2.3 mg). Analysis of the ^{1}H-NMR spectra of the 30% aq MeOH-soluble fraction indicated the presence of another, potentially novel isoquinoline quinone. This fraction was initially chromatographed on a C_{18} bonded silica flash column using a H_2O/MeOH gradient. Further purification on Sephadex LH-20 (100% MeOH) yielded the novel metabolite cribrostatin 7 (**1**, 7.2 mg).

Cribrostatin 7 (**1**) was obtained as a stable, dark red film. An $[M-H]^-$ ion in the (-)-HRFABMS at *m/z* 407.08969 allowed assignment of the molecular formula $C_{18}H_{20}N_2O_7S$ to **1**. The IR spectrum showed strong absorptions at 1667 cm^{-1} and 1708 cm^{-1}, suggesting compound **1** contained a quinone substructure as well as an unsaturated ester moiety.

Analysis of the NMR spectra of cribrostatin 7 (**1**) revealed similar signals to *O*-demethylrenierone (**3**) allowing a *cis*-angelate ester moiety and an isoquinoline quinone skeleton to be readily assigned to **1** [4]. Additional proton signals were observed at δ 3.11 (2H, t, J = 6.4 Hz) and δ 4.08 (2H, t, J = 6.4 Hz) with corresponding ^{13}C signals at 52.0 ppm and 42.6 ppm, respectively. This 2-carbon unit and the MS data indicated the presence of a taurine moiety, which has previously been observed in the sponge metabolites dysidine, melemeleones A and B, and secoadociaquinones A and B [8-10]. The attachment of the amine end of taurine to the quinone ring was supported by an HMBC correlation observed between H-11 (δ 4.08) and C-7 (149.6 ppm). This amine substitution of the quinone ring was further supported by comparison of the ^{13}C chemical shift of C-7 in **1** to the previously isolated *N*-substituted quinones cribrostatins 3 (**4**) and 5 (**5**) [6]. A ROESY correlation between H-11 and the methyl singlet at δ 2.17 (H-9) further confirmed this assignment. Hence structure **1** was assigned to cribrostatin 7.

All three compounds were tested for cytotoxicity against the HCT 116 human colon carcinoma cell line. Compound **1** has an IC_{50} value of 45 μg/mL, while **2** and **3** have an IC_{50} of 24 and 34 μg/mL, respectively.

EXPERIMENTAL

General

NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer (^{1}H: 399.880 MHz; ^{13}C: 100.559 MHz) at 25 °C. The ^{1}H and ^{13}C chemical shifts are reported in parts per million relative to CD_3OD (δ 3.30, 49.00 ppm). FTIR and UV spectra were recorded on a Jasco FT/IR-420 spectrophotometer and a Hewlett Packard 8452A diode array spectrophotometer, respectively. High- and low-resolution FAB mass spectral measurements were made on a Finnegan MAT 95 high-resolution spectrometer. HPLC was conducted using a Beckman 126 solvent module, a Beckman 168 Diode array detector, and a Rainin Dynamax C_{18} column (10 × 250 mm, 8 μm). Size-exclusion chromatography was performed using a glass column packed with Sigma Lipophilic Sephadex LH-20 (25-200 μm bead size) that was connected to a Spectra/Chrom CF-1 fraction collector. All solvents used for UV and MS were Fisher HPLC grade and the H_2O used was Barnstead E-pure 0.2 μm filtered.

Animal Material

Sponge PC00-11-149 was collected by scuba (-2 m) from Coron (Kalampisauan Island), Philippines. It was dark purple in life and is beige in alcohol. The growth form is rope-like with a firm, slightly compressible texture. Sponge $PDZ_1$98-4-74 was collected by scuba (-6 m) from Zamboanga (Murcielagos Island), Philippines. The sponge was bright blue in life, orange in alcohol. The growth form is upright

clusters of fused bulbs. The texture is firm, barely compressible. These sponges are borderline between the haplosclerid genera *Petrosia* and *Xestospongia*, but fit better into *Petrosia*. The sponges are probably two undescribed species differing in growth form, skeletal density and spicule size. Voucher specimens for PC00-11-149 and PDZ$_1$98-4-74 are retained with the corresponding author.

Extraction and Isolation

Sponge PC00-11-149

The MeOH extract from this *Petrosia* sp. was evaporated to dryness under reduced pressure to yield a dark red-brown gum (2.18 g). This material was dissolved in 10% H_2O/90% MeOH (50 mL) and extracted with hexanes (3 × 50 mL). Subsequently, H_2O (21 mL) was added to the aq MeOH layer followed by extraction with $CHCl_3$ (3 × 50 mL). The hexanes and $CHCl_3$-soluble fractions were evaporated under reduced pressure and yielded 60.5 mg and 111.5 mg of material, respectively.

The hexane-soluble material was chromatographed on Sephadex LH-20 (20 × 370 mm; 1:1 $CHCl_3$/MeOH). A fraction eluting after 50 mL was further purified using C_{18} HPLC (flowrate 2.5 mL/min) employing a gradient from 60% MeOH/40% H_2O to 100% MeOH over 40 min to yield renierone (**2**, 1.1 mg) and *O*-demethylrenierone (**3**, 0.5 mg).

Sponge PDZ$_1$98-4-74

The MeOH extract of this sponge was subjected to an identical solvent partitioning scheme as described above. This resulted in hexanes (188 mg), $CHCl_3$ (387 mg) and aq MeOH (1250 mg) fractions. The hexane-soluble material was chromatographed on Sephadex LH-20 (20 × 370 mm; 1:1 $CHCl_3$/MeOH). A fraction eluting after 50 mL was further purified using C_{18} HPLC (flowrate 2.5 mL/min) employing a gradient from 50% MeOH/50% H_2O to 100% MeOH over

25 minutes to yield a mixture of compounds **2** and **3**. The mixture was separated using C_{18} HPLC (flowrate 3.0 mL/min) employing a gradient from 35% CH_3CN/65% H_2O to 100% CH_3CN over 40 minutes to yield pure renierone (**2**, 0.8 mg) and *O*-demethylrenierone (**3**, 2.3 mg).

The 30% aq MeOH material was chromatographed on a C_{18} bonded silica (40 μM) flash column using 10% stepwise elutions from 20% MeOH/80% H_2O to 100% MeOH to afford 9 fractions. Fraction 3 (24 mg) was further purified on a Sephadex LH-20 column (20 × 370 mm; 100% MeOH) and yielded pure cribrostatin 7 (**1**, 7.2 mg).

Cribrostatin 7 (**1**) Dark red film; UV (MeOH) λ_{max} (ε) 210 (14500), 282 (6500), 338 (2000), 474 nm (1700); IR (NaCl) ν_{max} 3310 (broad), 1962, 1708, 1667, 1256, 1041, 799 cm^{-1}; (-)-LRFABMS *m/z* (rel. ab.) 407 (100); (-)-HRFABMS *m/z* 407.08969 ($C_{18}H_{19}N_2O_7S$ $[M-H]^-$ requires 407.09130); ^{1}H-NMR (400 MHz, CD_3OD): δ 1.93 (3H, dq, *J* = 1.6, 1.6 Hz, H-19), 1.96 (3H, dq, *J* = 7.6, 1.6 Hz, H-18), 2.17 (3H, s, H-9), 3.11 (2H, t, *J* = 6.4 Hz, H-12), 4.08 (2H, t, *J* = 6.4 Hz, H-11), 5.69 (2H, s, H-13), 6.14 (1H, qq, *J* = 7.6, 1.6 Hz, H-17), 7.86 (1H, d, *J* = 5.2 Hz, H-4), 8.81 (1H, d, *J* = 5.2 Hz, H-3); ^{13}C-NMR (100 MHz, CD_3OD): δ 10.7 (C-9), 16.0 (C-18), 20.8 (C-19), 42.6 (C-11), 52.0 (C-12), 66.5 (C-13), 112.6 (C-6), 119.7 (C-4), 124.1 (C-8a), 129.2 (C-16), 139.1 (C-17), 142.3 (C-4a), 149.6 (C-7), 155.0 (C-3), 157.2 (C-1), 169.4 (C-15), 182.1 (C-5), 183.9 (C-8).

Cells and Culture Conditions

The HCT 116 human colon carcinoma cell line was obtained from the American Type Culture Collection. Cells were maintained in McCoy's 5A media supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1 mM MEM sodium pyruvate, 50 units/mL penicillin and 50 μg/mL streptomycin.

Cultures were incubated at 37 °C in a humidified 5% CO_2 atmosphere in T-75 cm^2 tissue culture flasks**.**

Cell Proliferation Assay

HCT 116 cells were seeded in 96-well culture plates at 6000 cells/well. After 24 h, the compounds were added to the cells. After 48 h of drug treatment, cell viability was determined by measuring the metabolic conversion of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-2*H*-tetrazolium bromide) into purple formazan crystals by active cells [11]. MTT assay results were read using a Labsystem multiscan plate reader at 570 nm. All compounds were tested in quadruplicate and were solubilized in 100% DMSO with a final DMSO concentration of 1% in each well.

ACKNOWLEDGMENT

The authors gratefully acknowledge support provided by NIH grant CA36622 (C.M.I.). Funding for the Varian Mercury 400 MHz NMR spectrometer was provided through NIH Grant RR14768. FAB mass spectrometry was performed by Dr. Elliot M. Rachlin on a Finnegan Mat 95 funded by NSF grant CHE-9002690 and the University of Utah Institutional Funds Committee. The authors also thank Dr. J.N.A. Hooper (Queensland Museum) for assistance with sponge taxonomy and Dr. D.A. Jones (Huntsman Cancer Institute) for the use of cell lab facilities.

REFERENCES AND NOTES

[1] G.M. Nicholas, T.W. Hong, T.F. Molinski, M.L. Lerch, M.T. Cancilla, C.B. Lebrilla (1999) *J. Nat. Prod.* **62,** 1678-1681.
[2] L.M. West, P.T. Northcote, C.N. Battershill (2000) *J. Org. Chem*. **65,** 445-449.
[3] S. De Marino, M. Iorizzi, F. Zollo, C. Debitus, J.L. Menoli, L.F. Ospina, M.J. Alcaraz, M. Paya (2000) *J. Nat. Prod*. **63,** 322-326.
[4] J.M. Frincke, D.J. Faulkner (1982) *J. Am. Chem. Soc*.**104,** 265-269.

[5] R.A. Edrada, P. Proksch, V. Wray, R. Christ, L. Witte, R.W.M. Van Soest (1996) *J. Nat. Prod.* **59,** 973-976.
[6] G.R. Pettit, J.C. Knight, J.C. Collins, D.L. Herald, R.K. Pettit, M.R. Boyd, V.G. Young (2000) *J. Nat. Prod*. **63,** 793-798.
[7] D.E. McIntyre, D.J. Faulkner, D. Van Engen, J. Clardy (1979) *Tetrahedron Lett*. **20,** 4163-4166.
[8] C. Giannini, C. Debitus, R. Lucas, A. Ubeda, M. Paya, J.N.A. Hooper, M.V. D' Auria (2001) *J. Nat. Prod*. **64,** 612-615.
[9] K.A. Alvi, M.C. Diaz, P. Crews (1992) *J. Org. Chem*. **57,** 6604-6607.
[10] G.P. Concepcion, T.A. Foderaro, G.S. Eldredge, E. Lobkovsky, J. Clardy, L.R. Barrows, C.M. Ireland (1995) *J. Med. Chem*. **38,** 4503-4507.
[11] J. Carmichael, W.G. De Graff, A.F. Gazdar, J.D. Minna, J.B. Mitchell (1987) *Cancer Res.* **47,** 936-942.