ORGANIC LETTERS

2010
Vol. 12, No. 19
4292−4295

Monanchocidin: A New Apoptosis-Inducing Polycyclic Guanidine Alkaloid from the Marine Sponge *Monanchora pulchra*

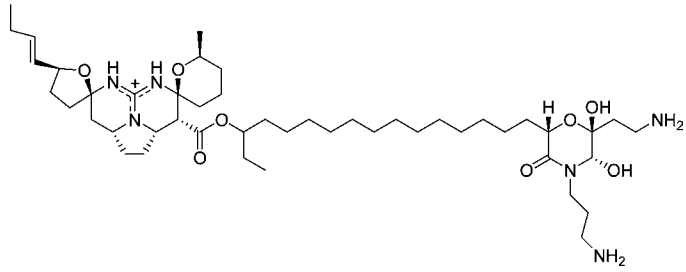
Alla G. Guzii, Tatyana N. Makarieva,* Vladimir A. Denisenko, Pavel S. Dmitrenok, Alexandra S. Kuzmich, Sergey A. Dyshlovoy, Vladimir B. Krasokhin, and Valentin A. Stonik

Pacific Institute of Bioorganic Chemistry, the Far-East Branch of the Russian Academy of Sciences, Vladivostok-22, Prospect 100-let Vladivostoku 159, Russia

makarieva@piboc.dvo.ru

Received July 22, 2010

ABSTRACT



Monanchocidin (1), a guanidine alkaloid with an unprecedented skeleton system derived from a polyketide precursor, (ω-3)-hydroxy fatty acid, and containing a 2-aminoethyl- and 3-aminopropyl-substituted morpholine hemiketal ring, has been isolated from the sponge *Monanhora pulchra*. The structure of 1 was assigned on the basis of detailed analysis of 1D and 2D NMR spectra, mass spectrometry, and results of chemical transformations. Compound 1 shows pro-apoptotic and cytoxic activities.

Polycyclic guanidine alkaloids are a unique class of sponge-derived metabolites exhibiting a broad range of biological activities such as cytotoxic,[1−10] antifungal,[1,11] antiviral,[1,2,6,12−14] antimicrobial,[15] antiprotozoal,[11,15] and antimalarial[7,11] activities. Members of this class include the series of pentacyclic[1−6,10,14,16−18] and tricyclic[7,10,12,13,15,18] guanidine

(1) Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1989**, *111*, 8925–8926.
(2) Jares-Erijman, E. A.; Sakai, R.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 5712–5715.
(3) Rinehart, K. L.; Shi J.-G.; Sun, F. U. S. Patent 6,028,077, 2000.
(4) Jares-Erijman, E. A.; Ingrum, A. L.; Carney, J. R.; Rinehart, K. L.; Sakai, R. *J. Org. Chem.* **1993**, *58*, 4805–4808.
(5) Bensemhoun, J.; Bombarda, I.; Aknin, J.; Vacelet, J.; Gaydou, E. M. *J. Nat. Prod.* **2007**, *70*, 2033–2035.
(6) Palagiano, E.; De Marino, S.; Minale, L.; Riccio, R.; Zollo, F.; Carre, J. B.; Debitus, C.; Lucarain, L.; Provost, J. *Tetrahedron* **1995**, *51*, 3675–3682.
(7) Laville, R.; Thomas, O. P.; Berrue, F.; Marquez, D.; Vacelet, J.; Amade, P. *J. Nat. Prod.* **2009**, *72*, 1589–1594.
(8) Sun, H. H.; Sakemi, S.; Burres, N.; McCarthy, P. *J. Org. Chem.* **1990**, *55*, 4964–4966.
(9) Sorek, H.; Rudi, A.; Gueta, S.; Reyes, F.; Martin, M. J.; Aknin, M.; Gaydou, E.; Vacelet, J.; Kashman, Y. *Tetrahedron* **2006**, *62*, 8838–8843.
(10) Gallimore, W. A.; Kelly, M.; Scheuer, P. J. *J. Nat. Prod.* **2005**, *68*, 1420–1423.
(11) Hua, H.-M.; Peng, J.; Fronczek, F. R.; Kelly, M.; Hamann, M. T. *Bioorg. Med. Chem.* **2004**, *12*, 6461–6464.
(12) Patil, A.; Kumar, D.; Kokke, N. V.; Bean, W. C.; Freyer, M. F.; De Brosse, A. J.; Mai, C.; Truneh, A.; Faulkner, D. J.; Carte, B.; Breen, A. L.; Hertzberg, R. P.; Johnson, R. K.; Westley, J. W.; Potts, B. C. M. *J. Org. Chem.* **1995**, *60*, 1182–1188.
(13) Patil, A. D.; Freyer, A. J.; Taylor, P. B.; Carte, B.; Zuber, G.; Johnson, R. K.; Faulkner, D. J. *J. Org. Chem.* **1997**, *62*, 1814–1819.
(14) Chang, L.; Whittaker, N. F.; Bewley, C. A. *J. Nat. Prod.* **2003**, *66*, 1490–1494.
(15) Hua, H.-M.; Peng, J.; Dunbar, D. C.; Schinazi, R. F.; De Castro Andrews, A. G.; Cuevas, C.; Garcia-Fernandes, L. F.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2007**, *63*, 11179–11188.

10.1021/ol101716x © 2010 American Chemical Society
Published on Web 08/30/2010

alkaloids bearing the (5,6,8b)-triazaperhydroacenaphthalene skeleton.

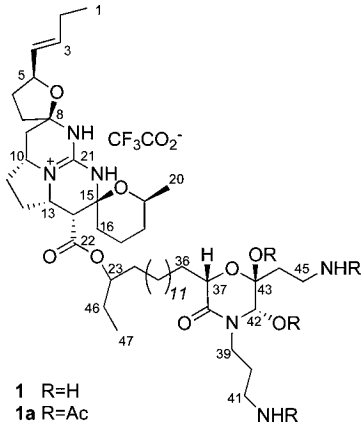
In continuation of our search for new physiologically active marine natural products, we have found that extracts from the Far-Eastern sponge *Monanchora pulchra* (Lambe, 1894)[19] were cytotoxic against a human acute monocytic leukemia cell line (THP-1). Monanchocidin (**1**),[20] a cytotoxic constituent of *M. pulchra*, was isolated from the frozen sponge (0.02% of dry weight) after extraction with EtOH, evaporation, partition between H_2O and *n*-BuOH, partition of the BuOH-soluble materials between aqueous EtOH and hexane, and repeated column chromatography of the ethanol-soluble fraction over Sephadex LH-20 (EtOH) and HPLC (YMC-ODS-A column, 75%EtOH/0.1% aqueous TFA).

Table 1. NMR for Data Monanchocidin (**1**) in DMSO-d_6 and CD_3OD

	1, DMSO-d_6				**1**, CD_3OD	
position	δ_H (mult, *J* in Hz)	δ_C (DEPT)	COSY	HMBC (5 Hz)	δ_H (mult, *J* in Hz)	δ_C
1	0.95 (t, 7.5)	13.2 CH_3	H2	C2, C3	0.99 (t, 7.5)	14.3
2	2.01 (m)	24.5 CH_2	H1, H3	C1, C3, C4	2.05 (m, 2H)	26.8
3	5.72 (dt, 6.4, 15.3)	134.2 CH	H2, H4	C1, C2, C4, C5,	5.77 (dt, 6.4, 15.3)	136.9
4	5.42 (ddt, 1.5, 7.2, 15.3)	129.2 CH	H3, H5	C2, C5	5.45 (ddt, 1.5, 7.2, 15.3)	130.7
5	4.48 (br. q, 7.2)	79.5 CH	H4, H6a, H6b	C3	4.57 (br. q, 7.2)	82.4
6	1.70 (m)	31.2 CH_2	H5, H6b, H7	C4, C5, C7, C8	1.78 (m)	33.5
	2.21 (m)		H5, H6a	C4, C7, C8	2.22 (m)	
7	2.12 (m)	35.8 CH_2	H6a	C5, C6, C8		38.4
8		88.3 C				90.6
9	1.50 (m)	37.6 CH_2	H9b, H10	C8, C10, C11	1.69 (m)	39.9
	2.25 (m)		H9a, H10	C8, C10, C11	2.27 (dd, 4.0, 13.0)	
10	3.91 (m)	53.2 CH	H9a, H9b	C8, C9, C11, C21	4.02 (m)	55.4
11	1.44 (m)	29.8 CH_2	H10, H11b		1.64 (m)	
	2.22 (m)		H10, H11a	C12, C13	2.29 (m)	
12	1.61 (m)	26.2 CH_2	H12b, H13	C14	1.77 (m)	28.2
	2.28 (m)		H12a, H13	C11, C13	2.29 (m)	
13	4.19 (m)	52.5 CH	H14	C12, C14	4.32 (m)	55.3
14	2.99 (d, 5.0)	49.3 CH	H13	C13, C15, C22	3.04 (d, 5.0)	51.5
15		80.7 C				83.2
16	1.64 (m)	31.3			1.69 (m)	33.2
17	1.93 (m)	17.6 CH_2	H16, H18a		1.82 (m)	20.0
18	1.18 (m)	31.5 CH_2			1.27 (m)	33.2
	1.62 (m)		H18a, H19		2.24 (m)	
19	3.74 (m)	66.5 CH	H18, H20	C15, C17, C20	3.86 (m)	69.2
20	1.08 (d, 6.2)	21.6 CH_3	H19	C15, C17, C18, C19	1.13 (d, 6.4)	22.5
21		148.5 C				151.1
21-N<u>H</u>	9.21 (s)			C14, C15, C21		
	9.55 (s)			C8, C9, C21		
22		168.3 C				170.8
23	4.75 (m)	76.5 CH	H46	C22, C24, C46, C47	4.82 (m)	79.2
24	1.48 (m)	32.4	H23		1.58	34.8
25−34	1.20−1.25 (br s)	28.9−29.3				
35	1.22 (m)	28.9 CH_2			1.45 (m)	27.0
36	1.73 (m)	31.9 CH_2	H37	C35, C38	1.79 (m)	34.0
					1.86 (m)	
37	4.08 (dd, 3.6, 7.8)	70.8 CH	H36	C36, C38, C43[a]	4.27 (dd, 3.6, 8.2)	73.5
38		169.2 C				173.6
39	3.25 (m)	41.7 CH_2	H39b, H40	C38, C40, C41, C42	3.46 (dt, 6.0, 14.2)	43.3
	3.45 (m)		H39a, H40	C38, C40, C41, C42	3.66 (m)	
40	1.80 (m)	25.5 CH_2	H39a, H39b, H41	C39, C41	1.98 (m)	27.4
41	2.77 (m)	36.7 CH_2	H40, N<u>H</u>$_2$41		2.95 (m)	38.6
41-NH_2	7.73 (br. s)		H41			
42	4.41 (d, 6.4)	80.9 CH	O<u>H</u>42	C38, C39, C43	4.59 (br. s)	83.3
42-OH	6.52 (d, 6.9)		H42	C42, C43		
43		94.4 C				96.7
43-OH	6.61 (s)			C42, C43		
44	1.94 (m)	34.7 CH_2	H45	C43, C45	2.12 (m)	36.5
	2.04 (m)		H45	C43, C42, C45	2.22 (m)	
45	2.93 (m)	34.3 CH_2	H44a, H44b, N<u>H</u>$_2$45		3.18 (m)	36.7
45-NH_2	7.76 (br. s)			C45		
46	1.52 (m)	26.2 CH_2	H23, H47	C23, C47	1.60 (m)	28.3
47	0.83 (t, 7.4)	9.5 CH_3	H46	C23, C46	0.90 (t, 7.4)	10.6

[a] In CD_3OD. Parameters were optimized for 2 Hz.

The molecular formula of monanchocidin (**1**) was established as $C_{47}H_{83}N_6O_8$ on the basis of HRESIMS data (*m*/*z* 859.6267, M^+, calcd 859.6237, $C_{47}H_{83}N_6O_8$) and ^{13}C NMR data (Table 1). The mass of the fully deuterium-exchanged molecule (*m*/*z* 867) indicated the presence of 8 exchangeable protons. The ^1H and ^{13}C NMR data (DMSO-d_6, Table 1) for **1** revealed the presence of a guanidine group (δ_C 148.5 and δ_H 9.21 and 9.55), three methyl groups (δ_H 0.83, 0.95, 1.08; δ_C 9.5, 13.2, 21.6), one disubstituted double bond (δ_H 5.72, 5.42; δ_C 134.2, 129.2), two *N*-substituted CH carbons (δ_H 3.91, 4.19; δ_C 53.2, 52.5), five oxymethines (δ_H 4.48, 3.74, 4.75, 4.08, 4.41; δ_C 79.5, 66.5, 76.5, 70.8, 80.9), two carbonyl groups (δ_C 168.3 and 169.2), one carbonyl-linked methine (δ_H 2.99; δ_C 49.3), three quaternary carbons (δ_C 88.3, 80.7, and 94.4) and an aliphatic long chain (δ_H 1.20−1.25; δ_C 28.9−29.3).



Substructures **a**−**e** of **1** were established by COSY, HSQC, and HMBC experiments. Fragment **a** has been seen previously in many pentacyclic guanidine alkaloids[1−6,10,14,16−18] isolated from marine sponges and starfish. It was revealed starting from signals of the methyl group in the tetrahydropyran moiety (δ_H 1.13, δ_C 22.5, CH_3-20, CD_3OD) and characteristic signals of the (5,6,8b)-triazaperhydroacenaphthalene core (δ_C 151.1, C-21; δ_H 4.02, δ_C 55.4, CH-10; δ_H 4.32, δ_C 55.3, CH-13, CD_3OD).

Interpretation of the COSY spectrum, in conjunction with the HSQC data, starting from the lower field methyl triplet (δ_H 0.95; CH_3-1) indicated substructure **b**, unusual in guanidine alkaloids (Figure 1), in which the Δ^3-olefin was assigned as *E* on the basis of the coupling constant between H-3 and H-4 (J = 15.3 Hz). The NMR data in DMSO-d_6 showed the absence of OH groups at C-5 and C-8, which

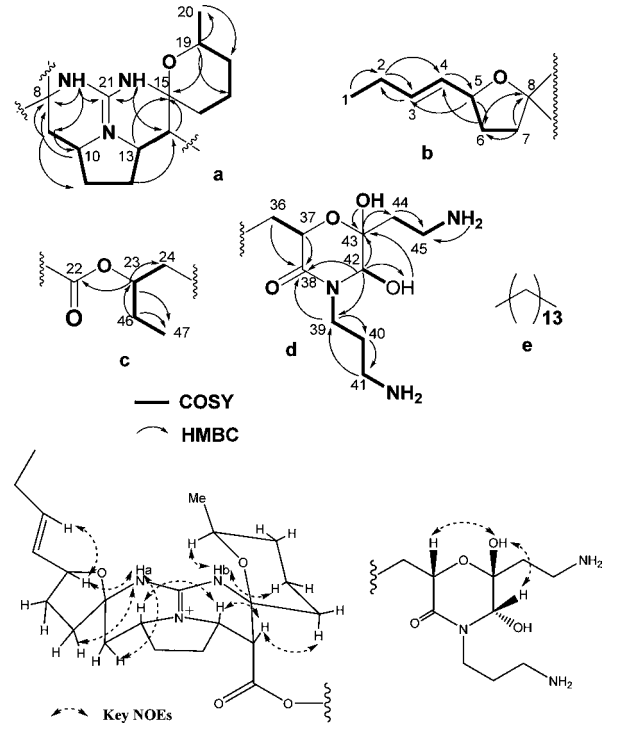


Figure 1. Partial structures of **1** with selected COSY, HMBC, and NOE correlations.

was also confirmed by the peracetylation of **1** (Ac_2O, pyridine). ^1H NMR chemical shifts of the characteristic signals of tetrahydrofuran moiety in **1** and monanchocidin peracetate (**1a**) were the same. Substructures "**c**" and "**d**" were established in the same manner. The position of the ethyl group in the polymethylene chain of **1** was assigned by HMBC experiment, which indicated that the CH_3-47 signal at δ_H 0.83 was correlated to C-46 (26.2) and C-23 (76.5) signals. The H-23 proton at 4.75 was also correlated to C-22 (168.3), C-24 (32.4), C-46 (26.2), and C-47 (9.5). Thus, the ethyl group is located at C-23.

The chemical shift of C-23 suggested an ester linkage at that point. The C-22 ester carbonyl showed correlations with H-14 as well as H-23. Thus, **1** consists of a pentacyclic guanidinium ring system (vessel part) and unusual, containing morpholine ring unit **d** (anchor part) were connected to each other through an ester linkage and a long-chain hydrocarbon moiety. The HRESIMS data of **1** show 13 methylene groups in the connecting chain (substructure **e**). The chemical shifts of the protons and carbons at 39, 40, 41, and 45 positions in the fragment **d** were comparable to those of the spermidine residue of ptilomycalin A.[21] The proton at δ_H 4.08 (H-37) correlated with the carbonyl carbon at δ_C 169.2 (C-38), the carbon signal at δ_C 169.2 correlated with the protons on C-39 and a HMBC correlation between a hydroxyl proton at δ_H 6.61 and quaternary C-43 (94.4) indicated that C-43 is a hemiketal carbon. Analysis of key HMBC correlations then led to the construction of a morpholine hemiketal ring.

(16) Berlinck, R. G. S.; Breakman, J. C.; Daloze, D.; Bruno, I.; Riccio, R.; Ferri, S.; Spampinato, S.; Speroni, E. *J. Nat. Prod.* **1993**, *56*, 1007–1015.

(17) Tavares, R.; Daloze, D.; Breakman, J. C.; Hajdu, E.; Muricy, G.; Van Soest, R. W. M. *Biochem. Syst. Ecol.* **1994**, *22*, 645–646.

(18) Braekman, J. C.; Daloze, D.; Tavares, R.; Hajdu, E.; Van Soest, R. W. M. *J. Nat. Prod.* **2000**, *63*, 193–196.

(19) The sponge was collected by dredging during 36 scientific cruises of R/V "Academic Oparin", August 2008, near Urup Island (45°057,9 N; 150°44,9 E, depth 66 m).

(20) Monanchocidin (**1**): colorless oil; $[\alpha]_D$ −12 (*c* 0.4, EtOH); ^1H, ^{13}C NMR data, Table 1; HRESIMS *m*/*z* 859.6260 $[M]^+$ (calcd for $C_{47}H_{83}N_6O_8$ 859.6267). HRESIMS/MS of the ion $[M]^+$ at *m*/*z* 859.6260: 758.5718 $[M - C_4H_9NO_2 + 2H]^+$, 404.2338 $[M - C_{25}H_{50}N_3O_4 + H]^+$.

(21) Ohtani, I.; Kusumi, T.; Kakisawa, H.; Kashman, Y.; Hirsh, S. *J. Am. Chem. Soc.* **1992**, *114*, 8472–8479.

The relative stereochemistry of **1** was assigned by NOESY and ROESY. Diagnostic NOE correlations between the resonances of NHa 21 (9.55) and H-5, H-7, H-9 and NOEs from H-3 to H-5 were indicative of the relative configurations as 5S* and 8R* in substructure **b**. In addition, 15S* and 19S* relative configurations were suggested by diagnostic NOE correlations between NHb 21 (9.21) and H-19, H-17. An NOE between H-10 and H-13 in the NOESY and ROESY, together with the observed coupling constants between H-13 and H-14 ($J = 5.0$ Hz) located them all on the same side of the molecule. NOEs between OH-43 (6.61) and H-37 and between OH-43 and H-42 were observed, confirming the *trans*-position of hydroxyl groups and *cis*-position of H-37 and OH-43 in the anchor part. Configuration at C-23 was not determined.

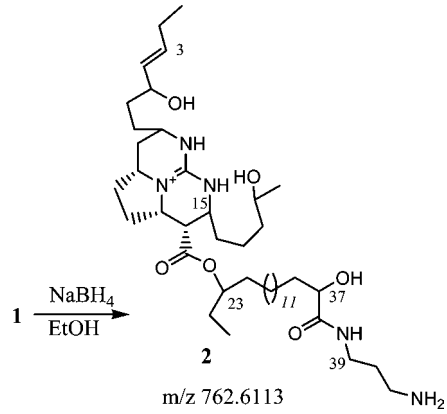


Figure 2. Reductive degradation of **1**.

Treatment of **1** ($NaBH_4$, EtOH, 60 °C, Figure 2) resulted in **2** by reduction of the hemiaminal groups at C-8, C-15 and C-42 with concomitant loss of C-42−C-45. The structure of **2** was established, using NMR, HRESIMS, and HRESIMS/MS data. The ^{1}H NMR spectrum of **2** (DMSO-d_6) indicated the presence of a three new hydroxyl groups at C-5, C-19, and C-37 (δ_H 4.77, 4.30 and 5.48), respectively.

The structure of monanchocidin (**1**) possesses a collection of uncommon features, including a vicinal hemiketal in a substituted 2-morpholinone ring formed by fusion of an α-hydroxy acid and a highly oxidized spermidine unit C-41−C-45. The combination of two contiguous spiro-ring systems, a 1-oxa-6-azaspiro[4.5]decane and 1-oxa-7-azaspiro[5.5]undecane, is unprecedented among guanidine alkaloids. Finally, the long-chain substituted 2-morpholinone unit in **1** is unified with the complex bis-spiro-cyclic moiety through an ester linkage at the ω-3 position of a hydroxy fatty acid residue and not the ω-position, as encountered in related natural products.

Compound **1** demonstrates cytotoxicity against human leukemia THP-1 (IC_{50} 5.1 μM), human cervix epithelioid carcinoma HeLa (IC_{50} 11.8 μM), and mouse epidermal JB6 Cl41 (IC_{50} 12.3 μM) cell lines. It also induces 66% of early apoptosis in THP-1 cells at 3.0 μM concentration.

Acknowledgment. We thank T. Molinski of the University of California, San Diego for reading this manuscript. The research described in this publication was supported by Grant NSS 3531.2010.4 from the President of RF and the Program of Presidium of RAS "Molecular and Cell Biology", Grant 09-04-00015-a from RFBR.

Supporting Information Available: Experimental procedures and full spectroscopic data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL101716X