Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 2127–2129

Carteramine A, an inhibitor of neutrophil chemotaxis, from the marine sponge *Stylissa carteri*

Hirotsugu Kobayashi,[a] Koki Kitamura,[b] Koji Nagai,[c] Yoichi Nakao,[a] Nobuhiro Fusetani,[a] Rob W. M. van Soest[d] and Shigeki Matsunaga[a,*]

[a]*Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan*
[b]*Molecular Medicine Research Labs, Drug Discovery Research, Astellas Pharma Inc. 21, Miyukigaoka, Tsukuba, Ibaraki 305-8585, Japan*
[c]*Fermentation Research Labs, Drug Discovery Research, Astellas Pharma Inc. 5-2-3, Toukoudai, Tsukuba, Ibaraki 305-2698, Japan*
[d]*Institute for Systematics, and Ecology, University of Amsterdam, 1090 GT Amsterdam, The Netherlands*

Received 29 December 2006; revised 18 January 2007; accepted 22 January 2007
Available online 25 January 2007

Abstract—A new dimeric oroidin derivative, carteramine A (**1**), was isolated as a neutrophil chemotaxis inhibitor from the marine sponge *Stylissa carteri*. The structure of **1** was elucidated by the analysis of spectral data.

Neutrophils, the most abundant blood-borne leucocytes, accumulate at the sites of inflammation where they play protective roles and sometimes cause excessive effects leading to tissue injury which leads to a wide range of inflammatory diseases.[1] Therefore, migration of neutrophils provides a promising target for the development of new anti-inflammatory agents.[2–7] In the screening of neutrophil chemotaxis inhibition of the extract of Japanese marine invertebrates, we found that the marine sponge *Stylissa carteri* collected off Kuchinoerabu Island, Kagoshima Prefecture, exhibited potent activity. Bioassay-guided fractionation afforded a new styloguanidine derivative named carteramine A (**1**). This Letter describes the isolation and structural elucidation of this compound.

The combined MeOH and EtOH extracts of the sponge (190 g, wet weight) was partitioned between $CHCl_3$ and H_2O. The aqueous layer was further extracted with *n*-BuOH, while the $CHCl_3$ fraction was partitioned between *n*-hexane and MeOH–H_2O (9:1). The *n*-BuOH and the aqueous MeOH fractions were combined and separated by ODS flash chromatography (MeOH–H_2O). The active fractions (50% and 70% MeOH eluates) were purified by two rounds of ODS HPLC: first with a linear gradient system [H_2O–1-PrOH–TFA (90:10:0.05–40:60:0.05)] and second with an isocratic elution [H_2O–MeOH–TFA (50:50:0.05)]. Carteramine A (**1**, 41 mg) was isolated as the predominant active constituent.[8]

The molecular formula of carteramine A (**1**), $C_{22}H_{21}ClBr_4N_{10}O_3$, suggested that it was a dimeric oroidin derivative.[9] This idea was supported by the ^{13}C NMR spectrum: there were eight sp^2 carbons (δ_C 127.9, 123.0, 122.0, 112.8, 108.7, 104.8, 97.9, and 96.1) and two amide carbonyl carbons (δ_C 159.2 and 154.9), which were ascribed to two units of 4,5-dibromopyrrole-2-carboxylic acid amide, two guanidines [δ_C 157.3 (2C)], and 10 sp^3 carbon signals most of which were substituted by heteroatom(s) (Table 1).

The connectivity of six of eight protonated sp^3 carbons was determined on the basis of the COSY data (Fig. 1a). One of the two remaining methines (C-6) was coupled to an exchangeable proton, whereas the other (C-20) was coupled to two exchangeable protons. The connectivity of the above-mentioned structural units was established by interpretation of the HMBC data (Fig. 1b). We confirmed our assignment by

Keywords: Sponge; Neutrophil; Chemotaxis; Palau'amine; *Stylissa carteri*.
* Corresponding author. Tel.: +81 3 5841 5297; fax: +81 3 5841 8166; e-mail: assmats@mail.ecc.u-tokyo.ac.jp

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.01.113

Table 1. ^{1}H and ^{13}C NMR spectral data in DMSO-d_6 at 600/150 MHz for carteramine A (**1**)

Position	δ_H (mult, J in Hz)	δ_C	HMBC
2		108.7	
3		96.1	
4		123.0	
5		122.0	
6	5.51 (s)	53.4	C2, 3, 4, 5, 8, 10, 11
7	9.01 (s)		C6, 8, 10
8		157.3	
9	9.30 (s)		C6, 8, 10
10		81.5	
11	2.83 (d, 14.4)	55.9	C6, 10, 12, 13, 16, 18, 20
12	2.43 (m)	40.3	C11, 13, 18
13a	2.98 (dd, −9.6, 9.3)	44.8	C12, 18
13b	3.77 (dd, −9.6, 7.6)		C10, 11, 12
15		154.9	
16		70.3	
17	4.29 (d, 9.6)	73.7	C16, 18, 19, 20
18	2.10 (m)	49.2	C12, 17
19a	3.32 (m)	38.8	C12, 17, 18, 6′
19b	3.54 (m)		C12, 17, 18, 6′
20	5.72 (d, 4.9)	82.3	C17, 22
21	9.44 (s)		C16, 20, 22
22		157.3	
23	8.83 (s)		C16, 20, 22
24		104.8	
25		97.9	
26	6.94 (s)	112.8	C2′, 3′, 5′, 6′
27		127.9	
28		159.2	
8-NH	7.69 (br)		
19-NH	8.35 (t, 6.2)		C19, 6′
20-OH	7.66 (d, 4.9)		C16, C20
22-NH	7.69 (br)		

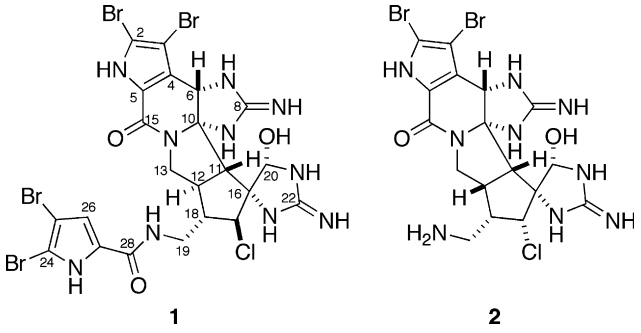

Figure 1. Structures of carteramine A (**1**) and 2,3-dibromostyloguanidine (**2**).

^{1}H–^{15}N HSQC spectrum which displayed that the protons at δ 8.35, 8.83, 9.01, 9.30, and 9.44 were attached to a nitrogen atom. A chlorine atom was placed on C-17 by considering the molecular formula and ^{13}C NMR chemical shift. The resulting planar structure of **1** was a 2,3-dibromostyloguanidine (**2**) derivative extended by 4,5-dibromopyrrole-2-carboxylic acid amide at 19-N (Fig. 2).[10]

The relative stereochemistry of the eight stereogenic centers in **1** was assigned on the basis of the NOESY data (Fig. 3). 9-NH and 23-NH were both correlated to

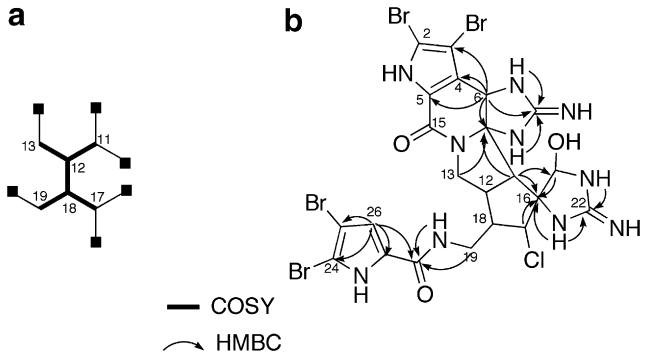

Figure 2. (a) Substructures of **1** and (b) key HMBC correlations of **1**.

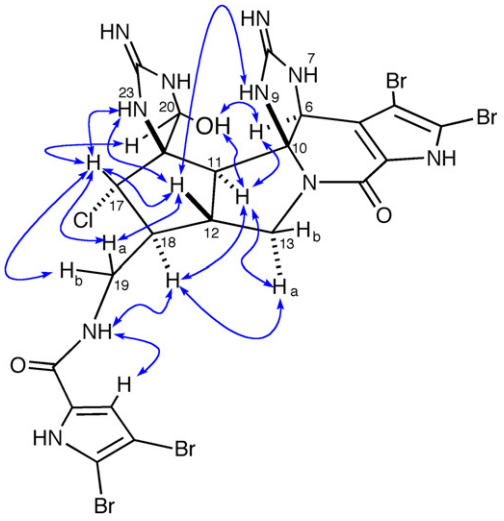

Figure 3. Key NOESY correlations and the relative stereochemistry of **1**.

H-12, whereas 23-NH was correlated to H-17, indicating that these four protons were on the same face of the 7-azabicyclo[3.3.0]octane ring system. On the other hand, H-11, H-18, and H-13a were mutually correlated, suggesting that the three protons were on the opposite face of the 7-azabicyclo[3.3.0]octane ring system. A correlation between H-6 and H-11 showed that the cyclic guanidine was cis-fused at C-6 and C-10. 20-OH was correlated to H-6 and H-11, whereas H-20 was correlated to H-11, H-17, and H-18. In conjunction with the absence of a correlation between H-6 and H-20, we assigned the stereochemistry of C-20 as shown in Figure 3. Therefore, the relative stereochemistry of carteramine A was assigned as 6*S**,10*R**,11*S**,12*S**, 16*R**,17*S**,18*S**,20*S**.

Carteramine A (**1**) belongs to the palau'amine class of metabolites characterized by the presence of a chlorinated 7-azabicyclo[3.3.0]octane ring system.[10–13] Because carteramine A was the homologue of 2,3-dibromostyloguanidine (**2**),[10] we compared the ^{13}C NMR data of the two compounds and found that their spectra were almost superimposable for the common portion except for C-18 and C-19, which were influenced by the substi-

tution. However, there were discrepancies in the assignments of relative stereochemistry between cateramine A and 2,3-dibromostyloguanidine, most notably in the mode of junction in the 7-azabicyclo[3.3.0]octane ring system. Unfortunately the NOESY data of 2,3-dibromostyloguanidine (**2**) was not present in the report and its stereochemistry was apparently assigned by comparing the NMR data with those of palau'amine.[10] Because the NOESY data of carteramine A (**1**) is only consistent with our proposed structure, we suggest that the stereochemistry of 2,3-dibromostyloguanidine (**2**) should be reinvestigated. We also suggest that the stereochemical assignments of palau'amines[11,12] and konbu'acidin[13] require reconsideration.

Carteramine A (**1**) inhibits the chemotaxis of neutrophils ($IC_{50} = 5\ \mu M$). Several synthetic compounds, such as cyclic peptides,[2–5] a phenyl benzylamide derivative,[6] and glutarimide derivatives,[7] have been reported as inhibitors of leucocyte chemotaxis. Because carteramine A (**1**) has structural resemblance to none of these compounds, our results provide a novel platform to develop a new class of anti-inflammatory agents.

Acknowledgments

Thanks are due to the crew of R/V Toyoshio-maru for assistance in collecting the sponge. This work was partially supported by Grant-in-Aid from MEXT (Priority Area 16073207).

References and notes

1. Luster, A. D.; Alon, R.; von Andrian, U. H. *Nat. Immunol.* **2005**, *6*, 1182–1190.
2. Konteatis, Z. D.; Siciliano, S. J.; Van Riper, G.; Molineaux, C. J.; Pandya, S.; Fischer, P.; Rosen, H.; Mumford, R. A.; Springer, M. S. *J. Immunol.* **1994**, *153*, 4200–4205.
3. Wong, A. K.; Finch, A. M.; Pierens, G. K.; Craik, D. J.; Taylor, S. M.; Fairlie, D. P. *J. Med. Chem.* **1998**, *41*, 3417–3425.
4. Paczkowski, N. J.; Finch, A. M.; Whitmore, J. B.; Short, A. J.; Wong, A. K.; Monk, P. N.; Cain, S. A.; Fairlie, D. P.; Taylor, S. M. *Br. J. Pharmacol.* **1999**, *128*, 1461–1466.
5. Finch, A. M.; Wong, A. K.; Paczkowski, N. J.; Wadi, S. K.; Craik, D. J.; Fairlie, D. P.; Taylor, S. M. *J. Med. Chem.* **1999**, *42*, 1965–1974.
6. Sumichika, H.; Sakata, K.; Takeshita, S. S.; Ishibuchi, S.; Nakamura, M.; Kamahori, T.; Ehara, S.; Itoh, K.; Ohtsuka, T.; Ohbora, T.; Mishina, T.; Komatsu, H.; Naka, Y. *J. Biol. Chem.* **2002**, *277*, 49403–49407.
7. Fox, D. J.; Reckless, J.; Warren, S. G.; Grainger, D. J. *J. Med. Chem.* **2002**, *45*, 360–370.
8. Compound **1**: Light yellow powder; $[\alpha]_D^{17}$ −44 (*c* 0.50, MeOH); UV (MeOH) λ_{max} 217.5 nm (ε 11,700), 277.5 (13,700); HRFABMS *m*/*z* 832.8191 $(M+H)^+$ (calcd for $C_{22}H_{22}{}^{37}Cl^{79}Br^{81}Br_3N_{10}O_3$, Δ −1.7 mmu).
9. Mourabit, A. A.; Potier, P. *Eur. J. Org. Chem.* **2001**, 237–243.
10. Kato, T.; Shizuri, Y.; Izumida, H.; Yokoyama, A.; Endo, M. *Tetrahedron Lett.* **1995**, *36*, 2133–2136.
11. Kinnel, R. B.; Gehrken, H.-P.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 3376–3377.
12. Kinnel, R. B.; Gehrken, H.-P.; Swali, R.; Skoropowski, G.; Scheuer, P. J. *J. Org. Chem.* **1998**, *63*, 3281–3286.
13. Kobayashi, J.; Suzuki, M.; Tsuda, M. *Tetrahedron* **1997**, *53*, 15681–15684.