ChemInform Abstract: Merobatzelladines A (I) and B (II), Antiinfective Tricyclic Guanidines from a Marine Sponge Monanchora sp

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Merobatzelladines A and B, Anti-Infective Tricyclic Guanidines from a Marine Sponge *Monanchora* sp.

Shunsuke Takishima,[†] Aki Ishiyama,[‡] Masato Iwatsuki,[‡] Kazuhiko Otoguro,[‡] Haruki Yamada,[‡] Satoshi Ōmura,[‡] Hirotsugu Kobayashi,[†] Rob W. M. van Soest,[§] and Shigeki Matsunaga*[,†]

Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, Kitasato University, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan, and Zoological Museum, University of Amsterdam, 1090 GT Amsterdam, The Netherlands

assmats@mail.ecc.u-tokyo.ac.jp

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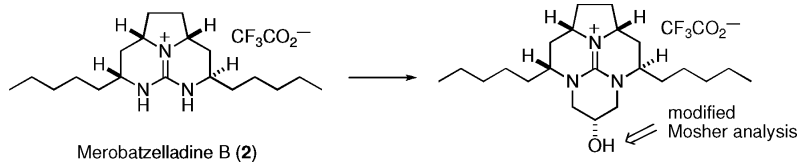
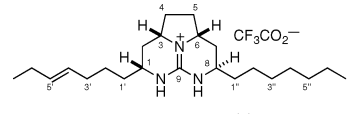
ABSTRACT



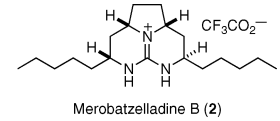
Merobatzelladines A (1) and B (2) have been isolated from a marine sponge *Monanchora* sp. as antibacterial constituents. Their structures including relative stereochemistry were determined by interpretation of spectral data. The absolute stereochemistry of merobatzelladine B (2) was elucidated after introduction of the fourth ring system preinstalled with a secondary hydroxyl group to which the modified Mosher method was applied. Merobatzelladines exhibit moderate anti-infective activity against a bacterium and protozoa.

Even though a variety of secondary metabolites with cytotoxic activity have been discovered from marine invertebrates, those with antibacterial activity are less pronounced.[1] In the course of our search for antibacterial agents against fish pathogenic bacteria,[2] the extract of a marine sponge *Monanchora sp.* (collected off Amami-Oshima, ZMAPOR 19862) exhibited potent activity against *Vibrio anguillarum*. Bioassay-guided fractionation afforded two tricyclic guanidine derivatives merobatzelladines A (**1**) and B (**2**).[3,4]

The MeOH and $CHCl_3$/MeOH (1:1) extract of the sponge *Monanchora* sp. was partitioned between water and $CHCl_3$, and the organic layer was subjected to a modification of the Kupchan's solvent partitioning scheme[5] to give the antibacterial $CHCl_3$ fraction. This material was subjected to ODS flash chromatography followed by reversed-phase HPLC permitting us to isolate merobatzelladines A (**1**) and B (**2**) from a complex mixture of closely related compounds.



Merobatzelladine A (**1**)



Merobatzelladine B (**2**)

Merobatzelladine A (**1**) was isolated as the TFA salt and the molecular formula of $C_{23}H_{42}N_3$ was assigned for the free base by HRESIMS. Interpretation of the ^{1}H NMR, ^{13}C NMR,

[†] The University of Tokyo.
[‡] Kitasato University.
[§] University of Amsterdam.

(1) Donia, M.; Hamann, M. T. *Lancet Infect. Dis.* **2003**, *3*, 338–348.
(2) Kobayashi, H.; Miyata, Y.; Okada, K.; Fujita, T.; Iwashita, T.; Nakao, Y.; Fusetani, N.; Matsunaga, S. *Tetrahedron* **2007**, *63*, 6748–6754.
(3) The major cytotoxic constituent of this sponge was identified as azaspiracid-2.[4] A reexamination of the sponge in ref 4 indicated that the taxonomy of the sponge should be corrected from *Echinoclathria* sp. to *Monanchora* sp .

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and HSQC data (Table 1) showed the presence of 2 terminal methyls, 14 methylenes, 4 methines, 1 disubstituted olefin, and 1 nonprotonated sp^2 carbon.

Table 1. ^1H and ^{13}C NMR Data of Merobatzelladines A (**1**) and B (**2**) in CD_3OD[a]

	1			2	
no.	δ_C (type[b])	δ_H	HMBC #H	δ_C (type)	δ_H
		J (Hz)			
1	51.5, CH	3.43, m	2a,2′	51.5, CH	3.43, m
2a	34.8, CH_2	1.25, m	9,7b	34.8, CH_2	1.26, m
2b		2.25, m			2.27, m
3	57.5, CH	3.76, m	2a,4a,4b	57.5, CH	3.76, m
4a	30.8, CH_2	1.70, m		30.8, CH_2	1.68, m
4b		2.23, m			2.23, m
5a	31.3, CH_2	1.64, m		31.2, CH_2	1.66, m
5b		2.24, m			2.24, m
6	53.5, CH	3.73, m	4a	53.5, CH	3.74, m
7a	31.9, CH_2	1.57, m		31.9, CH_2	1.58, m
7b		2.16, m			2.17, m
8	50.1, CH	3.49, m		50.1, CH	3.49, m
9	150.6, C		8	150.6, C	
1′a	35.5, CH_2	1.54, m	3′	35.9, CH_2	1.54, m
1′b		1.59, m			1.59, m
2′	26.2, CH_2	1.45, m	3′	25.8, CH_2	1.40, m
3′	27.7, CH_2	2.09, m	4′,5′	32.9, CH_2	1.35, m
4′	129.3, CH	5.32, m	2′,3′,6′	23.6, CH_2	1.35, m
5′	133.3, CH	5.38, m	3′,6′,7′	14.3, CH_3	0.92, m
6′	21.5, CH_2	2.04, quint (7)	4′,5′,7′		
7′	14.7, CH_3	0.95, t (7)	5′,6′		
1″a	36.2, CH_2	1.48, m		36.2, CH_2	1.49, m
1″b		1.56, m			1.57, m
2″	27.1, CH_2	1.36, m		26.8, CH_2	1.40, m
3″	30.3, CH_2	1.30, m		32.9, CH_2	1.35, m
4″	30.4, CH_2	1.30, m		23.6, CH_2	1.35, m
5″	33.0, CH_2	1.29, m	7″	14.3, CH_3	0.92, m
6″	23.7, CH_2	1.30, m	5″,7″		
7″	14.4, CH_3	0.89, t (7)	6″		

[a] 600 MHz for ^1H and 150 MHz for ^{13}C. [b] Multiplicity assigned by the HSQC data.

Interpretation of the COSY spectrum, in conjunction with the HSQC data, starting from the lower field methyl triplet (δ 0.95; H_3-7′) indicated unit "a" (Figure 1) in which $\Delta^{4'}$-olefin was assigned as *E* on the basis of the carbon chemical shifts of an allylic carbon (δ 27.7; C-3′).[6] Analysis of the COSY data also suggested units b and c. Although the linkage between units a and b was not assigned by the COSY data due to degenerate ^1H signals for H_2-1′ and H_2-2′, a TOCSY cross peak between H_2-3′ and H-1 demonstrated the connection between C-1′ and C-2′. In the same way, the linkage between units b and c was displayed on the basis of a TOCSY cross peak between H-3 and H-5a. The ^1H and ^{13}C shifts for the methine carbons (C-1, C-3, C-6, and C-8) suggested that they were all substituted by a nitrogen atom.[7] Considering the molecular formula, which showed the absence of oxygen atom, and the chemical shift of the remaining sp^2 carbon (δ 150.6), the four methine carbons were linked to a guanidyl group to form the hexahydro-5,6,6a-triazaacenaphthalene ring system as observed in ptilomycalin and batzelladine class of sponge metabolites.[8] The NMR data within the ring system of **1** agreed well with those in the literature. To satisfy the molecular formula the remaining portion was an *n*-hexyl group (unit d), which should be attached to C-1″ (Figure 1).

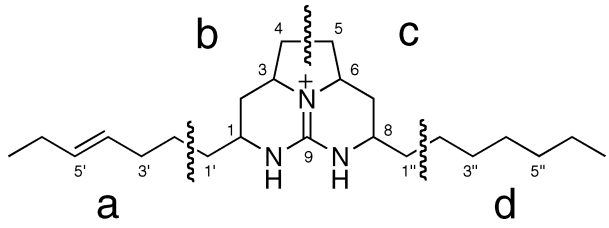


Figure 1. Partial structures in merobatzelladine A (**1**).

The relative stereochemistry of the tricyclic portion was assigned by analysis of NMR data. The ROESY cross peak between H-1 and H-3 showed that they were *syn*, whereas ROESY cross peaks between H-6 and H_2-1″ suggested that H-6 and H-8 were *anti*. The assignment of the relationship between the angular hydrogen atoms (H-3 and H-6) was not possible by analysis of NMR data due to the degeneracy of ^1H and ^{13}C signals. Then we turned our attention to compare carbon chemical shifts of **1** with those of synthetic compounds with defined stereochemistry: the data for the isomer with 1,3-*syn*, 3,6-*syn*, 6,8-*syn*-stereochemistry (plane-symmetric isomer) and those with 1,3- *syn*, 3,6-*anti*, 6,8-*syn*-stereochemistry (C_2-symmetric isomer) had been reported (Figure 2).[9] Carbon chemical shifts of C-1, C-2, and C-3 in **1** coincided well with those of the plane-symmetric isomer within errors less than 0.1 ppm, but differed from those of the C_2-symmetric isomer by between 0.4 and 1.6 ppm.[9a] As expected the chemical shifts for the C-6 to C-8 portion in **1** differed from both of the model

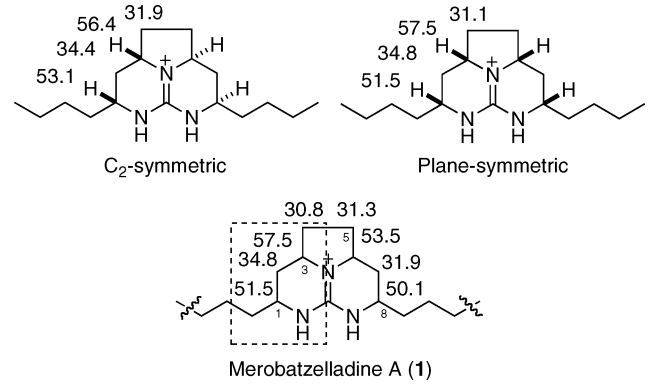


Figure 2. Carbon chemical shifts of the tricyclic portion of **1** and model compounds.[9]

(4) Ueoka, R.; Ito, A.; Izumikawa, M.; Maeda, S.; Takagi, M.; Shin-ya, K.; Yoshida, M.; van Soest, R. W. M.; Matsunaga, S. *Toxicon* **2009**, *53*, 680–684.

(5) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38*, 178–179.

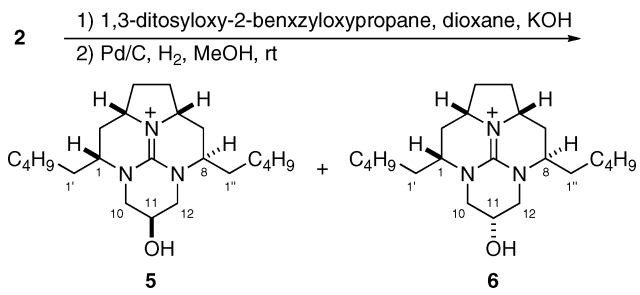
(6) Breitmaier, E.; Voelter, W. *Carbon-13 NMR Spectroscopy*; VCH: Weinheim, 1990; pp 192−194.

compounds, because **1** had 6,8-*anti* stereochemistry. In related compounds with the same tricyclic skeleton 3,6-*syn*- and 3,6-*anti*-isomers gave diagnostic carbon chemical shift values corresponding to each skeleton and long-distance substituent effects were hardly observed.[10] Therefore, we assigned 3,6-*syn* stereochemistry for **1**.

Merobatzelladine B (**2**) had a molecular formula of $C_{19}H_{36}N_3$ as the free base, which was analyzed by HRESIMS. The ^1H and ^{13}C NMR data of **2** coincided well with those of **1** except for the absence of the olefinic carbons. Interpretation of the COSY and HSQC data and comparison of the NMR data with those of **1** demonstrated that **2** had the tricyclic portion identical with that of **1**: the identity of the relative stereochemistry was implied by the superimposable NMR data and confirmed by the ROESY data. The molecular formula and the NMR data suggested that **2** had two saturated linear side chains composed of 10 carbons in total. The lengths of the side chains were determined by interpretation of ^{13}C NMR data. ^{13}C chemical shifts of three carbons from the terminus of the side chains were assigned by the HMBC data as δ 14.3 (2C; C-5′ and C-5″), 23.6 (2C; C-4′ and C-4″), and 32.8/32.9 (C-3′ and C-3″), whereas HSQC spectrum allowed the assignment of C-1′/C-1″ and C-2′/C-2″ signals, leaving no unassigned carbon signals. Therefore, both side chains are *n*-pentyl group.

Then, we sought to determine the absolute stereochemistry of merobatzelladine B (**2**). It was reported that related cyclic guanidines could be converted to the triamines by reduction of the guanidyl group followed by acidic hydrolysis.[11] The resulting triamine would be a good substrate for the modified Mosher analysis. However, our attempts to reduce **1** or **2** with $NaBH_4$ were unsuccessful. Then we turned our attention to use two of the nitrogen atoms as a scaffold to introduce a fused six-membered ring in which a secondary hydroxyl group was preinstalled (Scheme 1).[11] Even though **1** decomposed under

Scheme 1. Introduction of the Fourth Ring System in Merobatzelladine B (**2**)



the reported reaction condition to conjugate with 1,3-ditosyloxy-2-benxyloxypropane, **2** was stable enough to be converted to a mixture of diastereomeric tetracyclic derivatives **3** and **4**. They were separated by HPLC and the benzyl group was removed by catalytic hydrogenation to afford **5** and **6** whose relative stereochemistry was assigned by interpretation of the NMR data in DMSO-d_6 as described below.

In **6** H-10a and H-12a were coupled to H-11 by 8.5 Hz indicating that H-11 was axial, suggesting that **6** could be represented by either **6a** or **6b** (Figure 3). Structure **6a**

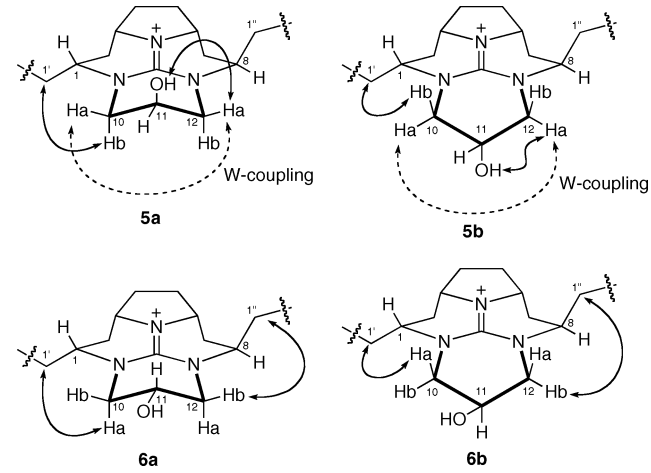


Figure 3. Possible structures of **5** (**5a** and **5b**) and **6** (**6a** and **6b**) with NOESY correlations shown by solid arrows.

accounted for all the observed NOESY cross peaks. However, NOESY data were not consistent with **6b**: the distances between H_2-1′ and H-10b are shorter than those between H_2-1′ and H-10b, but cross peaks not between the former pairs but between the latter pairs were observed; the same was true of the NOESY cross peaks and the distances between protons on C-1″ and C-12. Therefore, **6** should be represented by structure **6a**. With the structure of **6** established, **5** should be the diastereomer of **6** differing in the stereochemistry at C-11. H-11 in **5** was in the equatorial position because all the C-10 and C-12 methylene protons appeared as a broad doublet. Therefore, compound **5** was represented by structure **5a**, because structure **5b** was a conformational isomer of **6a**.

The absolute stereochemistry of **6** was determined by application of the modified Mosher's method.[12] Compound **6** was converted to the (*S*)- and (*R*)-MTPA esters (**7** and **8**, respectively). In both **7** and **8**, the OMTPA group occupied the axial position as demonstrated by the ^1H NMR data. Even though the distribution of the signs of chemical shift differences in axial esters are not completely uniform, previous studies suggested that this methodology could be applicable to the axial esters.[13,14] Analysis of the

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(8) Heys, L.; Moore, C. G.; Murphy, P. J. *Chem. Soc. Rev.* **2000**, *29*, 57–67.

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$\Delta\delta$ values allowed us to assign the 11*S*-stereochemistry for **6** (Figure 4). From this result the absolute stereochemistry of merobatzelladine B (**2**) was assigned as 1*R*,3*S*,6*R*,8*R*.

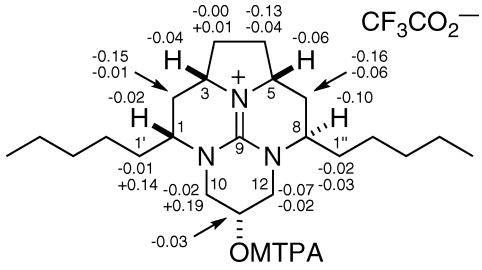

Figure 4. Modified Mosher analysis of **6**.

Merobatzelladines exhibit moderate antimicrobial activity against *Vibrio anguillarum*: inhibitory zones of 9−10 mm on application of 50 μg of a sample to a paper disk of 6 mm diameter. Merobatzelladines A and B also inhibit *Tripanosoma brucei brucei* (GUT at 3.1) with IC_{50} value of 0.24 μg/mL each.[15] They display moderate inhibitory activity against the K1 strain of *Plasmodium falciparum* with IC_{50} values of 0.48 μg/mL and 0.97 μg/mL, respectively.[16]

Merobatzelladines are new members of the batzelladines/ptilomycalin class of metabolites which contain one hexahydro-5,6,6a-triazaacenaphthalene ring system or two. A wide range of biological activities have been reported for this class of metabolites.[17] It is interesting to speculate whether merobatzelladines inhibit protein−protein interactions relevant to the infection of AIDS virus.[18] Although merobatzelladine A or B may be considered as a fragment of batzelladines, one side-chain in merobatzelladines emanates in the *syn*-direction to the angular hydrogen within the six-membered ring system: in all previously isolated congeners with this ring system, the relevant portion has *anti*-relationship.[17] This deviation in the stereochemistry distinguishes merobatzelladines from a fragment of batzelladines such as batzelladine K.[19] This feature is interesting in light of the recently proposed biogenesis of sponge-derived cyclic guanidines.[20] The assignment of the stereochemistry of the unfunctionalized hexahydro-5,6,6a-triazaacenaphthalene ring system had only been accomplished by total synthesis.[21] We have overcome the difficulty in the structure elucidation by devising a method using a derivatization reaction for this ring system followed by the modified Mosher analysis. Even though the assignment of the relative stereochemistry of the newly introduced hydroxyl group was not simple, we were able to assign the absolute stereochemistry of merobatzelladine B (**2**).

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Supporting Information Available: Experimental details, ^{1}H and ^{13}C NMR assignments of **5**-**8**, and NMR spectra of **1**, **2**, and **5**-**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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