Tetradehydrohalicyclamine A and 22-Hydroxyhalicyclamine A, New Cytotoxic Bis-piperidine Alkaloids from a Marine Sponge *Amphimedon* sp.[1]

Shigeki Matsunaga,[†] Yoshinari Miyata,[†] Rob W. M. van Soest,[‡] and Nobuhiro Fusetani*[,†]

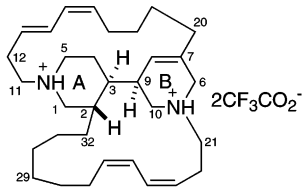
Laboratory of Aquatic Natural Products Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan, and Institute for Systematics and Ecology, University of Amsterdam, 1190 GT Amsterdam, The Netherlands

Received May 24, 2004

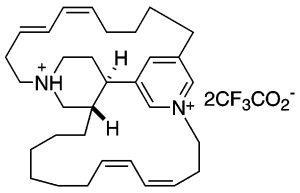
Two new 3-alkylpiperidine alkaloids, tetradehydrohalicyclamine A (**2**) and 22-hydroxyhalicyclamine A (**3**), have been isolated from a marine sponge *Amphimedon* sp. as cytotoxic constituents. Their structures were elucidated by spectroscopic analysis. Compounds **2** and **3** inhibited growth of P388 cells with IC_{50} values of 2.2 and 0.45 μg/mL, respectively.

3-Alkylpiperidine (or 3-alkylpyridine) alkaloids, which include a variety of metabolites ranging from monomeric 3-alkylpyridines to condensed bis-3-alkylpeperidines of the manzamine class, have been isolated from marine sponges of the order Haplosclerida.[2] They show a wide range of biological activities, e.g., antimicrobial,[3] antiviral,[3] cytotoxic,[3] antimalarial,[4] antifouling,[5] and ichthyotoxic.[3] In our continuous search for antitumor drug leads from Japanese marine invertebrates, we found significant cytotoxicity against P388 murine leukemia cells in the lipophilic extract of a marine sponge *Amphimedon* sp. collected in southern Japan. Bioassay-guided isolation furnished two new metabolites, tetradehydrohalicyclamine A (**2**) and 22-hydroxyhalicyclamine A (**3**), along with the known halicyclamine A (**1**).[6] We report the isolation, structure elucidation, and cytotoxicity of the new compounds.

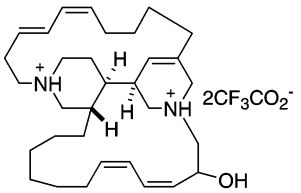
The organic extract of the sponge was subjected to solvent partitioning followed by centrifugal partition chromatography, ODS column chromatography, and ODS-HPLC to afford halicyclamine A (**1**), tetradehydrohalicyclamine A (**2**), and 22-hydroxyhalicyclamine A (**3**).



1



2



3

Compound **1** exhibited ^{1}H NMR signals significantly different from those reported for halicyclamine A, although interpretation of 2D NMR data of **1** led to the same gross structure as that of halicyclamine A. We thought that the discrepancy in ^{1}H NMR signals was due to different ionization states of the nitrogen atoms; our preparation was the bis-TFA salt, while the reported data were that of the free amine. To confirm this idea, compound **1** was passed through a silica gel column with EtOAc/Et_3N (95:5) as reported, which afforded a compound whose ^{1}H NMR spectrum was indistinguishable from that reported for halicyclamine A.[6] In the course of our structural analysis of **1**, we analyzed ROESY data (Figure 1), which was in agreement with the reported relative strereochemistry assigned partly on the basis of biosynthetic considerations.[6]

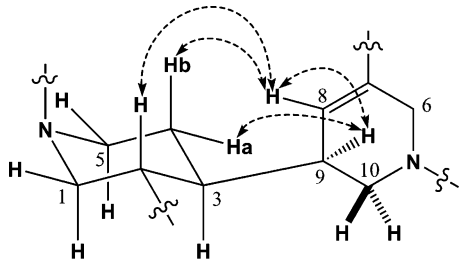


Figure 1. Relative stereochemistry of the bipiperidine system and selected ROESY correlations for halicyclamine A (**1**).

Tetradehydrohalicyclamine A (**2**) had a molecular formula of $C_{32}H_{47}N_2$ as established by HRFABMS. The high-field region of the ^{1}H NMR spectrum of **2** was similar to that of **1** (Table 1)**.** Interpretation of the COSY and HOHAHA spectra in conjunction with HSQC data allowed the assignment of ring A and two aliphatic chains identical with those in **1**; it is noteworthy that the chemical shift values of H-3, H_2-20, and H_2-21 were significantly different from those in **1**. H-2 and H-3 were assigned as *trans*-diaxial on the basis of a coupling constant of 11.9 Hz. The presence of a 1,3,5-trisubstituted pyridinium ring was evident from three aromatic protons at δ 8.98, 8.60, and 8.52 ppm with small coupling constants and the HMBC cross-peaks H-6/C-8, C-10, C-20, C-21; H-8/C-3, C-6, C-10, C-20; and H-10/C-3, C-6, C-8, C-21. Thus, the structure of tetradehydrohalicyclamine A (**2**) is as shown.

The FAB mass spectrum of 22-hydroxyhalicyclamine A (**3**) exhibited an $[M + H]^+$ ion at *m*/*z* 479, which was larger than that of **1** by 16 amu.[7] The ^{1}H NMR spectrum of **3** was similar to that of **1**, except for the presence of a signal at δ 5.07, which was attached to an oxymethine carbon at δ 61.4, indicating that **3** was an oxygenation product of **1**.

* To whom correspondence should be addressed. Tel: 81-3-5841-5299. Fax: 81-3-5841-8166. E-mail: anobu@mail.ecc.u-tokyo.ac.jp.
† The University of Tokyo.
‡ University of Amsterdam.

10.1021/np049824a CCC: $27.50
Published on Web 09/21/2004

Table 1. ^{1}H and ^{13}C NMR Spectral Data for **2** in CD_3OD

C no.	δ_H (mult, *J* in Hz)	δ_C	HMBC (# H)
1	3.05,[a] 3.44 (dd, 12.3, 3.1 Hz)	51.3	1a
2	2.11(m)	35.7	32b, 3, 4a, 1a, 1b
3	2.84 (ddd, 11.9, 6.9, 5.0 Hz)	43.6	8, 10, 1a, 1b
4	1.99,[a] 2.28[a]	26.7	3
5	3.44, 3.44[a]	51.3	
6	8.98(s)	144.6	8, 10
7		144.7	8, 20, 6
8	8.52 (s)	145.1	10, 3, 20, 6
9		144.9	8, 10, 3, 4a
10	8.60(s)	143.7	8, 2, 6
11	3.56 (dd, 13.2, 7.8 Hz), 4.17 (dd, 13.2, 9.6 Hz)	49.0	12a, 1a
12	2.52 (m), 2.73[a]	26.1	11a, 11b, 13, 14
13	5.96[a]	130.0	11a, 11b, 12a, 12b, 15
14	7.01(dd, 15.4, 11.6 Hz)	129.0	13, 15, 16
15	5.94[a]	129.1	14, 16
16	5.40 (ddd, 10.6, 5.8, 4.8 Hz)	133.0	14
17	2.01,[a] 2.79[a]	27.7	15, 16, 19a, 19b
18	0.78,[a] 1.27[a]	27.3	17a, 19b, 20
19	1.79 (m), 1.98[a]	30.5	17a, 18b, 20
20	3.07, 3.07[a]	30.3	8, 18b, 6
21	4.53,[a] 4.86[a]	62.1	10, 6
22	2.74,[a] 3.16[a]	29.9	24
23	5.46[a]	124.7	
24	6.37 (t, 11.4 Hz)	130.0	22a, 25
25	5.93[a]	123.7	24
26	5.21 (ddd, 10.2, 5.4, 5.1 Hz)	136.3	24, 28b
27	1.87,[a] 2.27[a]	25.9	29
28	1.30 1.30[a]	27.9	29
29	0.80,[a] 0.96[a]	27.1	28b
30	0.96,[a] 1.18[a]	27.8	29, 31a
31	0.72,[a] 1.38 (m)	25.9	30, 32a, 32b
32	0.81,[a] 0.96[a]	31.9	3, 1a

[a] Coupling constant was not determined due to overlapped signals.

Table 2. ^{1}H and ^{13}C NMR Spectral Data for **3** in CD_3OD

C no.	δ_H (mult, *J* in Hz)	δ_C	HMBC (# H)
1	2.83 (t, 12.9 Hz), 3.23[a]	50.9	11a
2	1.79[a]	31.0	32b
3	1.60[a]	39.5	8, 10a
4	1.72,[a] 2.16[a]	25.8	
5	3.34[a]	53.4	
6	3.44 (d, 15.6 Hz), 3.68 (d, 15.6 Hz)	52.5	8, 21a
7		132.8	6b
8	5.87 (s)	120.2	6a, 6b
9	2.71[a]	40.6	10a
10	3.27,[a] 3.52[a]	55.9	8, 21a, 6b
11	3.21,[a] 4.00 (dd, 13.2, 10.8 Hz)	49.6	1a
12	2.41 (m), 2.72[a]	26.6	
13	5.84[a]	128.5	12a
14	6.74 (dd, 15.2, 11.7 Hz)	128.4	13
15	5.93 (t, 10.8 Hz)	129.0	13, 14
16	5.46 (ddd, 10.8, 5.6, 5.2 Hz)	133.6	
17	1.80,[a] 2.60 (m)	28.2	15
18	1.14,[a] 1.28[a]	29.3	
19	1.61,[a] 1.67[a]	25.7	
20	1.97,[a] 2.36[a]	33.2	8
21	3.18 (dd, 13.2, 9.6 Hz), 3.54[a]	60.0	
22	5.07 (td, 9.6, 4.8 Hz)	61.4	21a, 21b, 24
23	5.54 (t, 10.2 Hz)	130.7	21b
24	6.54 (t, 11.2 Hz)	128.3	
25	6.45 (t, 11.4 Hz)	123.5	23
26	5.64 (ddd, 11.4, 6.4, 5.0 Hz)	138.5	24
27	2.03 (m), 2.37[a]	26.4	25
28	1.42,[a] 1.48[a]	28.6	
29	1.05, 1.05[a]	27.0	30
30	1.20, 1.20[a]	28.1	
31	0.90,[a] 1.27[a]	25.4	
32	0.92,[a] 1.27[a]	32.2	31a

[a] Coupling constant was not determined due to overlapped signals.

The elucidation of the gross structure was straightforward by interpretation of 2D NMR data (Table 2), in which C-22 of halicyclamine A was hydroxylated. The relative stereochemistry of the bicyclic portion was found to be identical with that of **1** on the basis of almost superimposable ^{1}H and ^{13}C NMR data. Attempts at preparation of MTPA esters were unsuccessful.

Compounds **1**, **2**, and **3** were cytotoxic against P388 cells with IC_{50} values of 0.45, 2.2, and 0.45 μg/mL, respectively. Tetradehydrohalicyclamine A is the first pyridine-containing halicyclamine. Interestingly, a metabolite closely related to **2** was proposed as a biosynthetic intermediate of halicyclamines.[8]

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. UV spectra were determined on a Shimadzu BioSpec-1600 DNA/protein/enzyme analyzer. NMR spectra were recorded on a JEOL alpha 600 NMR spectrometer. Chemical shifts were referenced to solvent peaks: δ_H 3.30 and δ_C 49.0 for CD_3OD. FAB mass spectra were obtained with a JEOL SX-102 mass spectrometer. Glycerol was used as the matrix.

Animal Material. The specimen was collected by hand using scuba off Iojima Island, the Satsunan Islands, southern Japan (30°47′ N; 130°17′ E). Stalked thickly flabellate sponge, 8 cm high, 5 cm in widest expansion, and 0.8 cm thick. The form appears to be derived from branches that have merged in one plane. Surface appears smooth, but provides some friction when touched. Oscules are conspicuous, slightly elevated, and occur on both sides. The sponge is compressible, but rather firm. The skeleton of the ectosome is a three-dimensional, irregular reticulation of tracts of 1−4 spicules thickness forming rounded meshes of 120−200 μm in diameter. Choanosome cavernous. The main skeleton is an anisotropic reticulation with spicule tracts occasionally entirely enfolded in sponging, but usually cemented only at the nodes. Primary tracts of 3−7 spicules thickness, lying at distances of 200−400 μm, interconnected by secondary and tertiary tracts of 1−3 spicules thickness. Spicules are exclusively curved oxeas, 90−110 × 3−5 μm. There are no matching species descriptions in the literature, but the sponge clearly belongs to the genus *Amphimedon*. The voucher was registered in the collections of the Zoological Museum of the University of Amsterdam under registration number Por.17247.

Extraction and Isolation. The sponge was frozen immediately after collection and kept frozen until extraction. The frozen sponge (500 g) was extracted with EtOH (1 L × 3) and then with $CHCl_3$/MeOH (1:1) (1 L). The combined extracts were concentrated; the residue was partitioned between water and $CHCl_3$. The organic phase was partitioned between MeOH/H_2O (9:1) and *n*-hexane. The aqueous MeOH layer was partitioned between MeOH/H_2O (6:4) and $CHCl_3$. The cytotoxic $CHCl_3$ layer was subjected to centrifugal partition chromatography (CPC-LLB, Sanki Engineering Ltd., Kyoto, Japan) with EtOAc/*n*-heptane/MeOH/H_2O (7:4:4:3), furnishing 13 fractions. The most cytotoxic fraction was fractionated by ODS flash chromatography with MeOH/H_2O (8:2) containing 0.2 M $NaClO_4$. The active fractions were purified by reversed-phase HPLC on Inertsil ODS-3 using a gradient elution of an aqueous MeOH system containing 0.2 M $NaClO_4$ followed by ODS−HPLC using a gradient elution of an aqueous MeCN system containing 0.05% TFA to yield **1** (149.6 mg), **2** (14.1 mg), and **3** (34.6 mg).

Cytotoxicity Assay. Cytotoxicity tests were performed as reported previously.[9]

Halicyclamine A (1): yellowish solid; $[\alpha]_D^{25}$ −24.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 236 nm; ^{1}H and ^{13}C NMR data, see Table S3; LRFABMS (positive) *m*/*z* 463 $[M + H]^+$; HRFABMS (positive) *m*/*z* 463.4054 (calcd for $C_{32}H_{51}N_2$, 463.4052).

Tetradehydrohalicyclamine A (2): colorless solid; $[\alpha]_D^{25}$ −14.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 232 and 273 nm; ^{1}H

and ^{13}C NMR data, see Table 1; LRFABMS (positive) *m*/*z* 459 $[M]^+$; HRFABMS (positive) *m*/*z* 459.3739 (calcd for $C_{32}H_{47}N_2$, 459.3740).

22-Hydroxyhalicyclamine A (3): colorless solid; $[\alpha]_D^{25}$ +21.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 234 and 273 nm; ^1H and ^{13}C NMR data, see Table 2; FABMS (positive) *m*/*z* 479 $[M + H]^+$.

Acknowledgment. This work was partly supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Supporting Information Available: Chemical shift assignments for compound **1** and NMR spectra for **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Part 128 of Bioactive Marine Metabolites series. Part 127: Oku, N.; Nagai, K.; Shindoh, N.; Terada, Y.; van Soest, R. W. M.; Matsunaga, S.; Fusetani, N. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2617−2620.

(2) (a) Matzanke, N.; Gregg, R. J.; Weinreb, S. M. *Org. Prep. Proc. Int.* **1998**, *30*, 3−51. (b) Tsuda, M.; Kobayashi, J. *Heterocycles* **1997**, *46*, 765−794. (c) Andersen, R. J.; Van Soest, R. W. M.; Kong, F. In *Alkaloids*: *Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon Press/Elsevier Science: New York, 1996; Vol. 10, pp 301−355.

(3) Schmitz, F. J.; Hollenbeak, K. H.; Campbell, D. C. *J. Org. Chem.* **1978**, *43*, 3916−3922.

(4) Ang, K. K. H.; Michael, J. H.; Higa, T.; Hamann, M. T.; Kara, U. A. *Antimicrob. Agents Chemother.* **2000**, *44*, 1645−1649.

(5) Faimali, M.; Sepcic, K.; Turk, T.; Geraci, S. *Biofouling* **2003**, *19*, 47−56.

(6) Jaspers, M.; Pasupathy, V.; Crews, P. *J. Org. Chem.* **1994**, *59*, 3253−3255.

(7) We were not able to measure the HRFABMS and ROESY data due to the degradation of the compound.

(8) Kaiser, A.; Billot, X.; Gateau-Olesker, A.; Marazano, C.; Das, B. C. *J. Am. Chem. Soc.* **1998**, *120*, 8026−8034.

(9) Fukuzawa, S.; Matsunaga, S.; Fusetani, N. *J. Org. Chem.* **1995**, *60*, 608−614.

NP049824A