Hachijodines A−G: Seven New Cytotoxic 3-Alkylpyridine Alkaloids from Two Marine Sponges of the Genera *Xestospongia* and *Amphimedon*[1]

Sachiko Tsukamoto,[†] Motohiko Takahashi,[†] Shigeki Matsunaga,[†] Nobuhiro Fusetani,*[,†] and Rob W. M. van Soest[‡]

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 (Zoological Museum), University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands

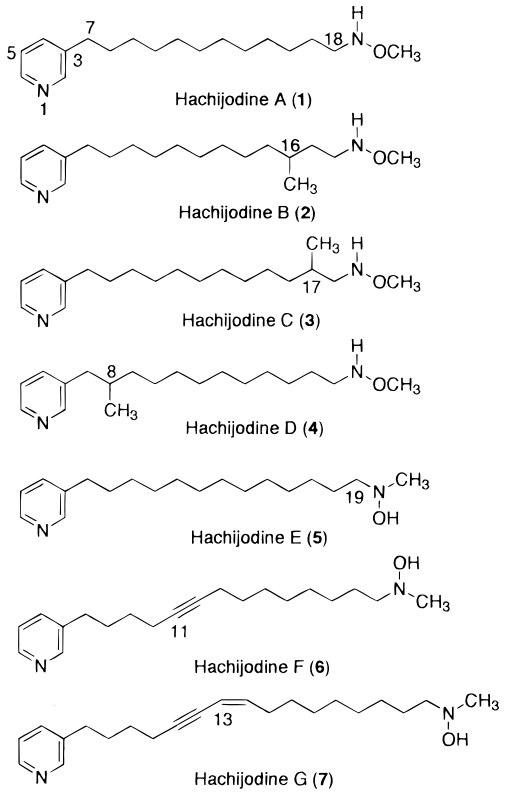
Received November 17, 1999

Seven cytotoxic 3-alkylpyridine alkaloids, hachijodines A−G, have been isolated from two marine sponges of the genera *Xestospongia* and *Amphimedon*. Their structures were determined on the basis of spectral data. These alkaloids are moderately cytotoxic against P388 murine leukemia cells with IC_{50} values of 1.0−2.3 μg/mL.

3-Alkylpiperidine alkaloids, which include monomers (e.g., niphatyne) and their oligomers (e.g., halitoxin), bis-3-alkylpiperidine macrocycles (e.g., cyclostellettamine), bis-quinolizadine (e.g., petrosine) and bis-1-oxaquinolizadine (e.g., xestospongin) macrocycles, and condensed bis-3-alkylpiperidines (e.g., manzamine), are often found in marine sponges of the families Callyspongiidae, Niphatidae, Chalinidae, Phloeodictydae, and Petrosiidae in the order Haplosclerida.[2] Therefore, they can be used as chemical markers for sponges in these families. More than 30 simple 3-alkylpyridines have been reported from marine sponges; the 3-alkyl component terminates in a primary amine, methylamine, methoxy amine, methoxy methylamine, imine oxide, or oxime methyl ether functionality. These pyridines show cytotoxic, antimicrobial, and other biological activitites. In our continuing search for biologically active metabolites from Japanese marine invertebrates, we found that the lipophilic extracts of two sponges of the genera *Xestospongia* (family Petrosiidae) and *Amphimedon* (family Niphatidae) collected off Hachijo-jima Island were cytotoxic against P388 murine leukemia cells. Bioassay-guided purification led to the isolation of seven new cytotoxic 3-alkylpyridine alkaloids, which were named hachijodines A−G. We report here isolation, structure determination, and cytotoxicity of these compounds.

The frozen specimens of *Xestospongia* sp. (250 g) were extracted with MeOH. The ether-soluble portion was fractionated by silica gel column chromatography (MeOH/CH_2Cl_2) followed by HPLC on ODS and phenyl-bonded silica gel to furnish four new cytotoxic compounds, hachijodines A (**1**, 16.0 mg), B (**2**, 1.6 mg), C (**3**, 2.1 mg), and D (**4**, 2.1 mg). The frozen samples of *Amphimedon* sp. (1 kg) were extracted with MeOH and $CHCl_3$/MeOH (1:1). The combined extracts were similarly processed to afford hachijodines E (**5**, 18.2 mg), F (**6**, 10.2 mg), and G (**7**, 30.2 mg).

Hachijodine A (**1**) had a molecular formula of $C_{18}H_{32}N_2O$ as established by HRFABMS. The ^{1}H NMR spectrum of **1** showed four aromatic protons [δ 7.96 (1H, br s, H-5), 8.46 (1H, d, J = 8.0 Hz, H-4), 8.68 (1H, br s, H-6), and 8.92 (1H, br s, H-2)], reminiscent of a 3-substituted pyridine, which was supported by COSY data. The presence of a C_{12} methylene chain was straightforward from NMR data; this



chain was linked to the pyridine ring at the position 3 and terminated in a methoxy amino group [δ 3.25 (2H, t, J = 8.0 Hz, H_2-18)/δ_C 50.2 (C-18); δ_H 3.88 (3H, s, OMe), δ_C 62.0 (OMe)]. These NMR data were similar to those reported for niphatesine H,[3] a metabolite of a marine sponge of the genus *Niphates*. Thus, hachijodine A (**1**) is 3-[12-(*N*-methoxyamino)dodecanyl]pyridine.

Hachijodines B (**2**), C (**3**), and D (**4**) possessed the same molecular formula of $C_{19}H_{34}N_2O$, a CH_2 unit larger than **1**. Their ^{1}H NMR spectra were almost superimposable on that of **1** except for the presence of a secondary methyl and a multiplet methine signal. Analysis of the COSY spectra placed the methyl group on C-16 [δ 0.97 (3H, d, J = 6.0 Hz, 16-Me) and 1.56 (1H, m, H-16)], C-17 [δ 1.02 (3H, d, J = 7.0 Hz, 17-Me) and 1.92 (1H, m, H-17)], and C-8 [δ 0.88 (3H, d, J = 6.5 Hz, 8-Me) and 1.83 (1H, m, H-8)] for **2**−**4**,

* 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/np9905766 CCC: $19.00
Published on Web 03/30/2000

respectively. Therefore, hachijodines B−D (**2**−**4**) are 16-methyl-, 17-methyl-, and 8-methylhachijodine A, respectively.

The FAB mass spectrum of hachijodine E (**5**) showed a pseudomolecular ion peak at *m*/*z* 306 $(M + H)^+$ corresponding to a molecular formula of $C_{19}H_{34}N_2O$.[4] The ^1H NMR spectrum of **5** was very similar to that of hachijodine A (**1**), suggesting that **5** was a 3-alkylpyridine in which an aliphatic chain was composed of 13 methylenes [δ 1.2−1.35 (18H, m, H_2-9−H_2-17), 1.65 (4H, m, H_2-8 and H_2-18), 2.75 (2H, t, J = 7.8 Hz, H_2-7), and 3.18 (2H, t, J = 8.7 Hz, H_2-19)]. Besides these similarities, the chemical shift values of a singlet methyl group were distinct between the two compounds (δ_H 3.03/δ_C 45.9 in **5** vs δ_H 3.88/δ_C 62.0 in **1**). The upfield shifts of the *N*-methyl signal in **5** were consistent with an *N*-hydroxy-*N*-methylamino group, which was supported by the low-field shift of a methylene carbon linked to a nitrogen atom [δ_C 60.4 (C-19) in **5** vs δ_C 50.2 (C-18) in **1**].[5] Hence, hachijodine E (**5**) is 3-[13-(*N*-hydroxy-*N*-methylamino)tridecanyl]pyridine.

Hachijodine F (**6**) had a molecular formula of $C_{20}H_{32}N_2O$, which was established by a pseudomolecular ion peak at *m*/*z* 317 $(M + H)^+$ and NMR data.[4] The ^1H NMR spectrum of **6** was again superimposable on that of **5** except for the presence of two triplet methylene signals at δ 2.11 (2H, t, J = 7.0 Hz, H_2-13) and 2.21 (2H, t, J = 6.7 Hz, H_2-10). Interpretation of the COSY spectrum led to a $(CH_2)_4$ unit [δ 1.55 (2H, m, H_2-9), 1.82 (2H, m, H_2-8), 2.21 (2H, t, J = 6.7 Hz, H_2-10), and 2.85 (2H, t, J = 7.8 Hz, H_2-7)] and a $(CH_2)_8$ unit [δ 1.25−1.3 (6H, m, H_2-16−H_2-18), 1.34 (2H, m, H_2-15), 1.44 (2H, m, H_2-14), 1.71 (1H, m, H-19), 1.79 (1H, m, H-19), 2.11 (2H, t, J = 7.0 Hz, H_2-13), and 3.22 (2H, t, J = 8.7 Hz, H_2-20)]. These subunits were connected through an acetylene unit (δ 79.0 and 81.1), which was substantiated by long-range coupling between H_2-10 and H_2-13. Therefore, the structure of hachijodine F (**6**) is as shown.

Hachijodine G (**7**) was larger than **6** by a C_2H_2 unit.[4] The ^1H and ^{13}C NMR spectra of **7** exhibited signals for a *cis*-vinyl group [δ_H 5.41 (d, J = 10.7 Hz, H-13)/δ_C 109.1 (C-13) and δ_H 5.81 (dt, J = 10.7 and 7.2 Hz, H-14)/δ_C 142.3 (C-14)], in addition to signals observed in **6**. In fact, interpretation of the COSY spectrum resulted in a $(CH_2)_8$ unit (C-15 to C-22) and a $(CH_2)_4$ unit (C-7 to C-10). A long-range coupling between H_2-10 and H-13 and an upfield shift of the C-13 carbon (δ 109.1) could place an enyne unit between the two alkyl chains, thereby completing the structure of **7**.

Although 3-alkylpyridine alkaloids with side chains terminating in an aldehyde group have been reported from opisthobranch mollusks,[6,7] more than 30 alkylpyridines having side chains terminating in nitrogenous functionalities have so far been isolated from marine sponges: theonelladins A and B (primary amine and methylamine, respectively) from *Theonella swinhoei*;[8] niphatyne A (methoxyamine) from *Niphates* sp.;[9] xestamine A (methoxy methylamine) from *Xestospongia wiedenmayeri*;[10] cribrochalinamine oxide A (imine oxide) from *Cribrochalina* sp.;[11] and ikimine A (oxime methyl ether) from an unidentified sponge.[12] Hachijodines E−G are the first example of 3-alkylpyridines having a hydroxy methylamino terminus in the side chain.

Hachijodines A−G (**1**−**7**) were cytotoxic against P388 murine leukemia cells: **1**, 2.2 μg/mL; **2**, 2.2 μg/mL; **3**, 2.2 μg/mL; **4**, 2.2 μg/mL; **5**, 2.3 μg/mL; **6**, 1.0 μg/mL; **7**, 1.0 μg/mL. 3-Alkylpyridines with an amino terminus in their side chains have been reported to be cytotoxic except for xestamines A−C bearing a methoxy methylamino functionality;[10] it is puzzling why xestamines are inactive.

Experimental Section

Animal Material. The sponge *Xestospongia* sp. was collected using scuba off Hachijo-jima Island (33°07′ N, 139°48′ E). The sponge is a thickly encrusting mass, about 10 × 8 × 3 cm, with numerous oscular chimneys. The surface is optically smooth and slightly rough to the touch. The color is light beige. The ectosomal skeleton grades into the choanosomal skeleton. It is basically a tangential halichondrioid crust of intercrossing spicules making vaguely delimited meshes of about 100 μm. The choanosomal skeleton is alveolar, and spicules are arranged around vague meshes of 100−200 μm, with 3−5 spicules in the surrounding tracts and many loose interstitial spicules. Superimposed on this skeleton is a system of longitudinal tracts, 5−7 spicules in cross section, which together with the alveolar meshes make a vague anisotropic reticulation. Spicules are short, curved, and sharply pointed oxeas, 175−228 × 4−11 μm. The specimen is assigned to *Xestospongia*, subgenus *Neopetrosia* (order Haplosclerida, family Petrosiidae) because of the fine-grained surface, the short spicules, and the longitudinal tracts. It is closely related to the common Indo-Pacific dark brown sponge *Xestospongia exigua*, differing from it mostly in color. A voucher is incorporated in the Zoological Museum of Amsterdam under reg. no. 13014.

The sponge *Amphimedon* sp. was also collected off Hachijo-jima Island (33°07′ N, 139°44′ E). The sponge is a globular mass, 7 × 5 × 2−3 cm, with dozens of prominent oscules, 2−5 μm in diameter, flush with the surface or slightly elevated. The surface is more or less smooth but uneven, with inconspicuous shallow grooves and pits. The color is red-brown outside with beige interior in life and beige in alcohol. The skeleton of the surface is a tangential reticulation of spicule tracts with little binding spongin, in places very dense and confused, with irregular meshes 70−250 μm in diameter. In contrast, the choanosomal skeleton is a regular tight-meshed system of spongin-enforced main spicule tracts, 20−50 μm in diameter with 3−7 spicules in cross section, connected by secondary tracts, 10−20 μm in diameter, 2−4 spicules in cross section. Mesh size is 120−220 μm. Spicules are short, robust, sharply pointed oxeas, 115−150 × 5−9 μm. The specimen is assigned to *Amphimedon* (order Haplosclerida, family Niphatidae) because of the combination of a relatively smooth surface, ectosomal tangential skeleton of spicule tracts, and a choanosomal anisotropic skeleton of spicule tracts. There are no matching descriptions in the literature. A voucher is incorporated in the Zoological Museum of Amsterdam under reg. no. 14404.

Isolation of Hachijodines A−D (1−4). The frozen sample of *Xestospongia* sp. (250 g) was extracted with MeOH (500 mL × 3). The extract was concentrated under reduced pressure and extracted with ether; the ether layer (3.50 g) was fractionated on Si gel with 3% MeOH/CH_2Cl_2. A portion (0.53 g) of a cytotoxic fraction (2.33 g) was purified by ODS HPLC with 50% MeOH/H_2O containing 0.1% TFA to afford hachijodine A (**1**, 16.0 mg, 2.8 × 10^{-2} % wet weight), a fraction (39.9 mg) containing hachijodine B, and a mixture of hachijodines C and D (16.9 mg). The latter two fractions were separately purified by HPLC on a phenyl/hexyl-bonded column with 30% CH_3CN/H_2O containing 0.1% TFA to yield hachijodines B (**2**, 1.6 mg, 2.8 × 10^{-3} %), C (**3**, 2.1 mg, 3.7 × 10^{-3} %), and D (**4**, 2.1 mg, 3.7 × 10^{-3} %).

Hachijodine A (1): UV λ_{max} (MeOH) 260 nm (ϵ 3300); ^1H NMR (CD_3OD) δ 1.30−1.37 (16H, m, H_2-9−H_2-16), 1.68 (2H, m, H_2-17), 1.71 (2H, m, H_2-8), 2.86 (2H, t, J = 8.0 Hz, H_2-7), 3.25 (2H, t, J = 8.0 Hz, H_2-18), 3.88 (3H, s, OMe), 7.96 (1H, br s, H-5), 8.46 (1H, d, J = 8.0 Hz, H-4), 8.68 (1H, br s, H-6), and 8.92 (1H, br s, H-2); FABMS (positive, glycerol matrix) *m*/*z* 293 $(M + H)^+$; HRFABMS (positive, PEG400 in NBA matrix) *m*/*z* 293.2595 (calcd for $C_{18}H_{32}N_2O$, 293.2593).

Hachijodine B (2): UV λ_{max} (MeOH) 260 nm (ϵ 3300); ^1H NMR (CD_3OD) δ 0.97 (3H, d, J = 6.0 Hz, 16-Me), 1.20 (1H, m,

H-15), 1.3−1.4 (10H, m, H_2-10−H_2-14), 1.40 (1H, m, H-15), 1.41 (2H, m, H_2-9), 1.56 (1H, m, H-16), 1.73 (2H, m, H_2-17), 1.75 (2H, m, H_2-8), 2.90 (2H, t, J = 9.0 Hz, H_2-7), 3.30 (1H, dd, J = 12.0, 9.6 Hz, H-18), 3.34 (1H, dd, J = 12.0, 7.2 Hz, H-18), 3.94 (3H, s, OMe), 8.03 (1H, br s, H-5), 8.52 (1H, d, J = 8.0 Hz, H-4), 8.73 (1H, br s, H-6), and 8.78 (1H, br s, H-2); FABMS (positive, glycerol matrix) m/z 307 $(M + H)^+$; HRFABMS (positive, PEG400 in NBA matrix) m/z 307.2750 (calcd for $C_{19}H_{34}N_2O$, 307.2749).

Hachijodine C (3): UV λ_{max} (MeOH) 260 nm (ϵ 3200); ^{1}H NMR (CD_3OD) δ 1.02 (3H, d, J = 7.0 Hz, 17-Me), 1.24 (1H, m, H-16), 1.3−1.4 (12H, m, H_2-10−H_2-15), 1.40 (1H, m, H-16), 1.71 (2H, m, H_2-8), 1.92 (1H, m, H-17), 2.88 (2H, t, J = 9.6 Hz, H_2-7), 3.08 (1H, dd, J = 15.6, 9.6 Hz, H-18), 3.23 (1H, dd, J = 15.6, 7.2 Hz, H-18), 3.90 (3H, s, OMe), 1.37 (2H, m, H_2-9), 8.05 (1H, br s, H-5), 8.51 (1H, d, J = 8.0 Hz, H-4), 8.75 (1H, br s, H-6), and 8.75 (1H, br s, H-2); FABMS (positive, glycerol matrix) m/z 307 $(M + H)^+$; HRFABMS (positive, PEG400 in NBA matrix) m/z 307.2748 (calcd for $C_{19}H_{34}N_2O$, 307.2749).

Hachijodine D (4): UV λ_{max} (MeOH) 260 nm (ϵ 3200); ^{1}H NMR (CD_3OD) δ 0.88 (3H, d, J = 6.5 Hz, 8-Me), 1.24 (1H, m, H-9), 1.40 (1H, m, H-9), 1.3−1.4 (12H, m, H_2-10−H_2-15), 1.35 (2H, m, H_2-9), 1.68 (1H, m, H_2-17), 2.66 (1H, dd, J = 15.0, 10.8 Hz, H-7), 2.92 (1H, dd, J = 15.0, 7.2 Hz, H-7), 3.26 (2H, t, J = 9.6 Hz, H_2-18), 3.90 (3H, s, OMe), 8.05 (1H, br s, H-5), 8.49 (1H, d, J = 8.0 Hz, H-4), 8.75 (1H, br s, H-6), and 8.75 (1H, br s, H-2); FABMS (positive, glycerol matrix) m/z 307 $(M + H)^+$; HRFABMS (positive, PEG400 in NBA matrix) m/z 307.2747 (calcd for $C_{19}H_{34}N_2O$, 307.2749).

Isolation of Hachijodines E−G (5−7). The frozen sponge of the genus *Amphimedon* (1 kg) was extracted with MeOH (3 L × 3) and $CHCl_3$/MeOH (1:1, 2 L × 2). The combined extracts were concentrated under reduced pressure and extracted with ether; the ether layer (12.3 g) was separated on Si gel with MeOH/$CHCl_3$. A portion (969 mg) of active fractions (5.5 g) eluted with $CHCl_3$ and 10% MeOH/$CHCl_3$ was fractionated by ODS flash column chromatography with MeOH/H_2O. A 70% MeOH/H_2O fraction (491.1 mg) was purified by ODS HPLC with 50% MeOH/H_2O containing 0.05% TFA to afford hachijodines E (**5**, 18.2 mg, 1.0×10^{-2} %), F (**6**, 10.2 mg, 5.8×10^{-3} %), and G (**7**, 30.2 mg, 1.7×10^{-2} %).

Hachijodine E (5): ^{1}H NMR (CD_3OD) δ 1.2−1.35 (18H, m, H_2-9−H_2-17), 1.65 (4H, m, H_2-8 and H_2-18), 2.75 (2H, t, J = 7.8 Hz, H_2-7), 3.03 (3H, s, NMe), 3.18 (2H, t, J = 8.7 Hz, H_2-19), 7.98 (1H, d, J = 7.0 Hz, H-4), 7.60 (1H, dd, J = 6.7, 7.0 Hz, H-5), 8.58 (1H, d, J = 6.7 Hz, H-6), and 8.60 (1H, s, H-2); FABMS (positive, glycerol matrix) m/z 307 $(M + H)^+$.

Hachijodine F (6): ^{1}H NMR (CD_3OD) δ 1.25−1.3 (6H, m, H_2-16−H_2-18), 1.34 (2H, m, H_2-15), 1.44 (2H, m, H_2-14), 1.55 (2H, m, H_2-9), 1.71 (1H, m, H-19), 1.79 (1H, m, H-19), 1.82 (2H, m, H_2-8), 2.11 (2H, t, J = 7.0 Hz, H_2-13), 2.21 (2H, t, J = 6.7 Hz, H_2-10), 2.85 (2H, t, J = 7.8 Hz, H_2-7), 3.06 (3H, s, NMe), 3.22 (2H, t, J = 8.7 Hz, H_2-20), 7.81 (1H, t, J = 6.7 Hz, H-5), 8.19 (1H, d, J = 6.7 Hz, H-4), 8.72 (1H, s, H-2), and 8.74 (1H, d, J = 6.7 Hz, H-6); FABMS (positive, glycerol matrix) m/z 317 $(M + H)^+$.

Hachijodine G (7): ^{1}H NMR (CD_3OD) δ 1.25−1.3 (8H, m, H_2-17−H_2-20), 1.35 (2H, m, H_2-16), 1.62 (2H, m, H_2-9), 1.72 (1H, m, H-21), 1.78 (1H, m, H-21), 1.85 (2H, m, H_2-8), 2.23 (2H, dt, J = 7.2, 7.4 Hz, H-15), 2.41 (2H, t, J = 6.8 Hz, H_2-10), 2.86 (2H, t, J = 7.7 Hz, H_2-7), 3.06 (3H, s, NMe), 3.22 (2H, t, J = 7.5 Hz, H_2-22), 5.41 (2H, d, J = 10.7 Hz, H-13), 5.81 (2H, dt, J = 10.7, 7.2 Hz, H-14), 7.80 (1H, t, J = 6.7 Hz, H-5), 8.18 (1H, d, J = 6.7 Hz, H-4), and 8.73 (2H, s, H-2 and H-6); FABMS (positive, glycerol matrix) m/z 343 $(M + H)^+$.

Acknowledgment. We thank Prof. P. J. Scheuer of the University of Hawaii for reading the manuscript. This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan and the Japan Society for the promoting of Science, "Research for the Future Program" (JSPS-RFTF96I100301).

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NP9905766