JOC*Note*

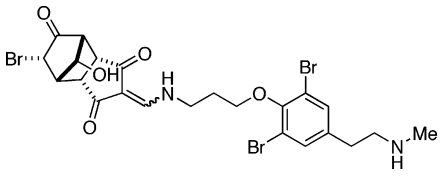
Novel Bromotyrosine Derivatives That Inhibit Growth of the Fish Pathogenic Bacterium *Aeromonas hydrophila*, from a Marine Sponge *Hexadella* sp.[1]

Shigeki Matsunaga,[†] Hirotsugu Kobayashi,[†] 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, 1090 GT Amsterdam, The Netherlands

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

Received October 12, 2004

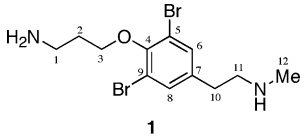


kuchinoenamine (**3**)

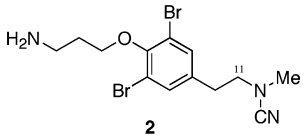
Three new bromotyrosine derivatives, 11-*N*-methylmoloka′iamine (**1**), 11-*N*-cyano-11-*N*-methylmoloka′iamine (**2**), and kuchinoenamine (**3**), were isolated as antibacterial constituents from a marine sponge *Hexadella* sp. Their structures were elucidated on the basis of spectral and chemical methods. They exhibited moderate antibacterial activity against the fish pathogenic bacterium *Aeromonas hydrophila*.

Marine sponges of the order Verongida have proved to be a rich source of bromotyrosine-derived metabolites[2] which show a wide range of biological activities including anti-HIV,[3−5] antibacterial,[6,7] antifouling,[6,7] cytotoxicity,[6−10] toxicity to the crab,[11] Na,K-ATPase inhibitory,[12] and histamine H_3 antagonistic.[13] In our search for biologically active compounds from Japanese marine invertebrates, we found that the hydrophilic extract of a marine sponge *Hexadella* sp. collected in southern Japan exhibited moderate antibacterial activity against the bacterium *Aeromonas hydrophila,* which causes hemorrhagic septicemia in fish.[14] Bioassay-guided fractionation afforded three new bromotyrosine derivatives, 11-*N*-methylmoloka′iamine (**1**), 11-*N*-cyano-11-*N*-methylmoloka′iamine (**2**), and kuchinoenamine (**3**). This paper describes the isolation, structure elucidation, and antimicrobial activities of these compounds.

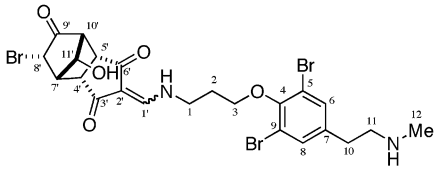
The MeOH extract of the frozen sponges (1.0 kg) were partitioned between Et_2O and H_2O. The aqueous layer was further partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was sequencially separated by ODS flash chromatography, gel filtration, centrifugal partition chromatography, and reversed-phase HPLC to afford 11-*N*-methylmoloka′iamine (**1**), 11-*N*-cyano-11-*N*-methylmoloka′iamine (**2**), and kuchinoenamine (**3**) in yields of 638.2, 1.0, and 13.2 mg, respectively.



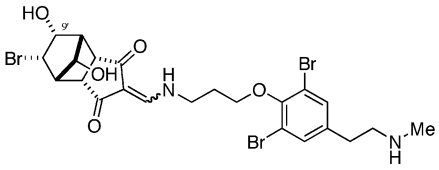
1



2



3



6

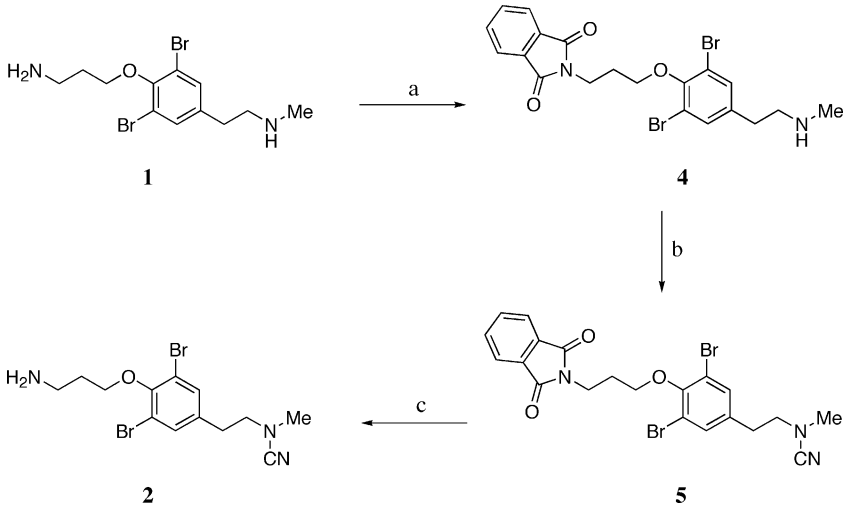
11-*N*-Methylmoloka′iamine (**1**) had a molecular formula of $C_{12}H_{18}N_2OBr_2$ as analyzed by HRFABMS. Interpretation of the ^{1}H NMR spectrum together with HMQC data led to the presence of an *N*-methyl (δ_H 2.67, δ_C 33.7), two unfunctionalized methylenes (δ_H 2.16, δ_C 29.2; δ_H 2.90, δ_C 31.8), two nitrogenous methylenes (δ_H 3.26, δ_C 38.8; δ_H 3.19, δ_C 50.7), an oxygenated methylene (δ_H 4.08, δ_C 71.7), and an aromatic signal [δ_H 7.53 (2H), δ_C134.5 (2C)]. In addition, four nonprotonated sp^2 carbon signals [δ_C 119.3 (2C), 153.1, and 137.4] were observed in the ^{13}C NMR spectrum. The COSY spectrum revealed spin systems for N−CH_2−CH_2−CH_2−O and CH_2−CH_2−N units, thereby indicating **1** to be an *N*-methyl derivative of moloka′iamine (**7**).[15] 11-*N*-Methylation was deduced on

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

(1) Bioactive Marine Metabolites. 130. Part 129: Hamada, T.; Matsunaga, S.; Yano, G.; Fusetani, N. *J. Am. Chem. Soc.*, **2005**, *127*, 110−118.
(2) Blunt, J. D.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48 and the previous reviews in this series.
(3) Jurek, J.; Yoshida, W. Y.; Scheuer, P. J. *J. Nat. Prod.* **1993**, *56*, 1609−1612.
(4) Ichiba, T.; Scheuer, P. J. *J. Org. Chem.* **1993**, *58*, 4149−4150.
(5) Ross, S. A.; Weete, J. D.; Schinazi, R. F.; Wirtz, S. S.; Tharnish, P.; Scheuer, P. J.; Hamann, M. T. *J. Nat. Prod.* **2000**, *63*, 501−503.
(6) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Org. Chem.* **1996**, *61*, 2936−2937.
(7) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 8181−8186.
(8) Ishibashi, M.; Tsuda, M.; Ohizumi, Y.; Sasaki, T.; Kobayashi, J. *Experimentia* **1990**, *47*, 299−300.
(9) Kobayashi, J.; Honma, K.; Sasaki, T.; Tsuda, M. *Chem. Pharm. Bull.* **1995**, *43*, 403−407.
(10) Tabudravu, J. N.; Jaspars, M. *J. Nat. Prod.* **2002**, *65*, 1798−1801.
(11) Fusetani, N.; Masuda, Y.; Nakao, Y.; Matsunaga, S.; van Soest, R. W. M. *Tetrahedron* **2001**, *57*, 7507−7511.
(12) Nakamura, H.; Wu, H.; Kobayashi, J.; Nakamura, Y.; Ohizumi, Y. *Tetrahedron Lett.* **1985**, *37*, 4517−4520.
(13) Mierzwa, R.; King, A.; Conover, M. A.; Tozzi, S.; Puar, M. S.; Patel, M.; Coval, S. J. *J. Nat. Prod.* **1994**, *57*, 175−177.

10.1021/jo048203j CCC: $30.25
Published on Web 01/28/2005

SCHEME 1 [a]



[a] Reagents and conditions: (a) *N*-carbethoxyphthalimide, Na_2CO_3, THF−H_2O (8:2), rt, 30 min; (b) CNBr, $NaHCO_3$, EtOH, rt, 12 h; (c) $H_2NNH_2{\cdot}H_2O$, EtOH, rt, 15 h.

the basis of an HMBC correlation between the *N*-methyl proton and C-11.

11-*N*-Cyano-11-*N*-methylmoloka′iamine (**2**) had a molecular formula of $C_{13}H_{17}N_3OBr_2$ as established by HRFABMS, indicating that a hydrogen in **1** was replaced by a CN unit. In fact, the ^{1}H NMR spectrum measured in CD_3OD exhibited the same sets of signals as observed in **1**. HMBC cross-peaks were observed between *N*-Me and C-11 and between *N*-Me and a carbon at δ 118.8 which was assigned as a cyano group. This was in agreement with an IR band at 2210 cm^{-1}. Other NMR data were superimposable on those of **1**. Therefore, **2** was assigned to the 11-*N* cyanamide of **1**. To confirm this assignment an 11-*N* cyano derivative of **1** was prepared as outlined in Scheme 1. Compound **1** was converted to the phthalimide **4**,[16] which was treated with CNBr to give the cyanamide **5**.[17] Removal of the phthalimide group of **5** with hydrazine[18] afforded **2** which was indistinguishable from the natural product in the ^{1}H NMR and FAB mass spectra. To the best of our knowledge, **2** is the first marine natural product encompassing a cyanamide moiety.[2] As to natural products containing a cyanamide moiety, only one compound was reported from a mushroom.[19]

Kuchinoenamine (**3**) exhibited a cluster of ion peaks in a ratio of 1:3:3:1 at *m/z* 647, 649, 651, and 653 in FABMS, indicating the presence of three bromine atoms. The molecular formula of $C_{23}H_{25}O_5N_2Br_3$ was determined on the basis of HRFABMS and NMR data. The ^{1}H and ^{13}C NMR spectra (Table 1) readily indicated the presence

TABLE 1. ^{1}H and ^{13}C NMR Spectral Data in CD_3OD at 600/150 MHz for Kuchinoenamine (3)

position	δ_H (mult, *J* in Hz) **3**	δ_C **3**	HMBC
1	3.86 (m, br)	49.4	C2, 3, 1′
2	2.19 (s, br)	31.3	
3	4.06 (m, br)	71.3	
4		153.3	
5, 9		119.5	
6, 8	7.55 (s)	134.4	C5, 6, 7, 8, 9, 10
7		137.1	
10	2.95 (t, 6.9)	31.8	C6, 7, 8, 11
11	3.24 (t, 6.9)	50.9	C7, 10, 12
12	2.72 (s)	33.7	C11
1′	7.89 (s)	157.6	C1, 2′ 3′
2′		111.4	
3′		205.0	
4′	3.38 (dd, 5.8, 8.9)	48.7	C2′, 3′, 5′, 6′, 7′, 8′
5′	3.49 (dd, 4.8, 8.9)	51.1	C3′, 4′, 6′, 7′, 9′, 10′
6′		201.6	
7′	3.12 (s, br)	51.1	
8′	4.64 (d, 4.2)	50.7	C4′, 7′, 9′
9′		205.3	
10′	3.10 (d, 4.2)	59.5	C4′, 5′, 7′, 9′, 11′
11′	4.53 (s)	79.1	C4′, 7′, 10′

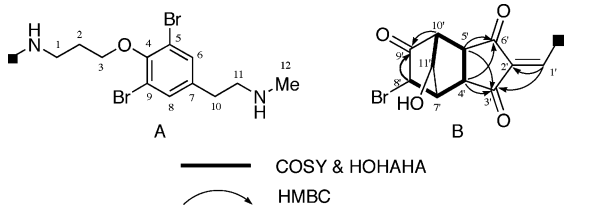
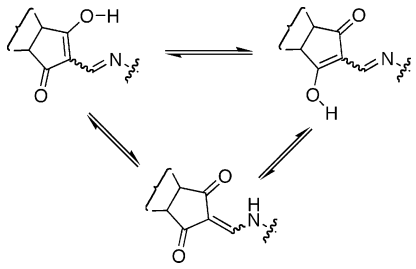


FIGURE 1. Structures of units A and B of **3**.

of fragment A in **3** (Figure 1). The remaining elements of $C_{11}H_8O_4Br$ were composed of a polarized trisubstituted olefin (δ_H 7.89, δ_C 157.6; δ_C 111.4), six methines (δ_H 3.10, δ_C 59.5; δ_H 3.12, δ_C 51.1; δ_H 3.38, δ_C 48.7; δ_H 3.49, δ_C 51.1; δ_H 4.53, δ_C 79.1; δ_H 4.64, δ_C 50.7), and three ketones (δ_C 201.6, 205.0, and 205.3), thereby suggesting the presence of three rings. Some ^{1}H and ^{13}C NMR signals were either broadened or doubled, indicating the presence of conformational equilibrium in the molecule. Interpretation of

(14) Roberts, R. J. In *Bacterial Disease of Fish*; Inglis, V., Roberts, R. J., Bromage, N. R., Eds.; Blackwell Sci. Publ.: Oxford, 1993; pp 143−155.
(15) Hamann, M. T.; Scheuer, P. J. *J. Org. Chem.* **1993**, *58*, 6565−6569.
(16) McArthur, C. R.; Worster, P. M.; Okon, A. U. *Synth. Commun.* **1983**, *13*, 311−318.
(17) Snider, B. B.; O'Hare, S. M. *Tetrahedron Lett.* **2001**, *42*, 2455−2358.
(18) Sasaki, T.; Minamoto, K.; Itoh, H. *J. Org. Chem.* **1978**, *43*, 2320−2325.
(19) Gasco, A.; Serafino, A.; Mortarini, V.; Menziani, E.; Bianco, M. A.; Scurti, J. C. *Tetrahedron Lett.* **1974**, *38*, 3431−3432.

SCHEME 2



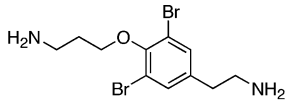
the COSY and HOHAHA spectra led to the six continuous methine system (C-8′, C-7′, C-4′, C-5′, C-10′, and C-11′). A slow exchange of H-8′ signal in CD_3OD solution allowed to link a ketone (C-9′) to C-8′ which must be brominated as judged from its chemical shift values (δ_H 4.64, δ_C 50.7). HMBC cross-peaks, H-5′/C-9′ and H-10′/C-9′, connected C-9′ and C-10′, while an HMBC cross-peak H-11′/C-4′ led to the connectivity of C-11′ and C-7′. HMBC cross-peaks H-4′/C-3′, H-4′/C-6′, H-5′/C-3′, and H-5′/C-6′ were consistent with that C-4′ and C-5′ were adjacent to a keto group, respectively. An insertion of the polarized trisubstituted olefin between the keto groups at C-3′ and C-6′ was implied by an HMBC cross-peak H-1′/C-3′, thereby completing the gross structure of the unit B. Units A and B were connected between 1-N and C-1′ on the basis of an HMBC cross-peak H_2-1/C-1′.

To confirm this structure, we attempted to reduce the C-9′ keto group. Due to its alkaline-labile property, **3** was decomposed upon treatment with $NaBH_4$. However, a clean reduction proceeded when treated with $Na(OAc)_3BH$ in MeOH to afford a single isomer of diol **6**. COSY cross-peaks between H-8′ and H-9′ placed an oxymethine between C-8′ and C-10′ in **6**. The doubled NMR signals were accounted for by the equilibria as shown in Scheme 2. We were not able to infer which one dominates.

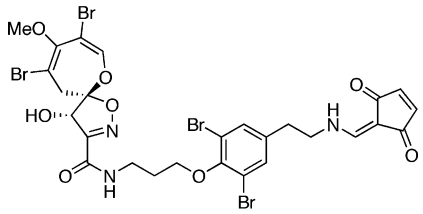
The relative stereochemistry of the tricyclic portion was derived from the $^1H-^1H$ coupling constants and NOESY data. A coupling constant of 8.9 Hz between H-4′ and H-5′ suggested that they were both *exo*, which was supported by coupling constants $J_{H-4',\ H-7'} = 4.8$ Hz and $J_{H-5',\ H-10'} = 5.8$ Hz. A coupling constant of 4.2 Hz between H-7′ and H-8′ revealed that H-8′ was *exo*.[20] A NOESY correlation between H-8′ and H-11′ showed the orientation of the C-11′ hydroxyl group. As to the C-9′ stereochemistry in **6**, a large coupling constant between H-8′ and H-9′ indicated that H-9′ was also *exo* (Figure 2).

Compounds **1**−**3** showed antibacterial activity in disk agar diffusion assay against the fish pathogenic bacterium *Aeromonas hydrophila* (100 μg/ diameter 6.5 mm disk; **1**, 7.5 mm zone of inhibition; **2**, 8 mm; **3**, 7 mm).

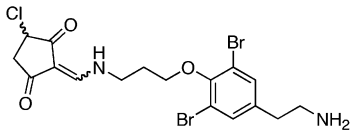
Two bromotyrosine derivatives related to aerothionin and bis-indole alkaloids have so far been reported from marine sponges of the genus *Hexadella*.[22,23] Compounds **1** and **2** are *N*-substituted derivatives of moloka′iamine (**7**) from an unidentified genus of verongid sponge, while kuchinoenamine (**3**) had a unique tricyclo[5.2.1.$0^{2,6}$]-decane skeleton attached to **1** via an enamine linkage and is biogenetically related to psammaplysin E (**8**)[4] and waiana′enamines A (**9**), and B (**10**);[21] the tricyclo[5.2.1.$0^{2,6}$]-decane moiety may be formed by a [4 + 2] cycloaddition of a cyclopenta-2,4-dienol and an N-substituted 2-aminomethylenecyclopent-4-ene-1,3-dione followed by bromohydrin formation and oxidation.



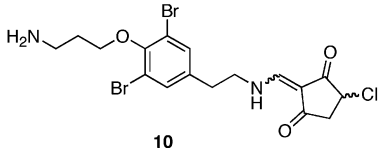
7



8



9



10

Experimental Section

Animal Material. The sponge specimens were collected by hand using scuba at a depth of 10 m off Kuchinoerabu-jima Island in the Satsunan Islands (30° 27.22′ N; 130° 11.43′ E). They were immediately frozen and preserved at −20 °C until extraction. The sponge was identified as *Hexadella* sp. (family Ianthellidae, order Verongida): a massive sponge with irregular surface, which is in places heavily encrusted by other organisms, and shows some irregularly distributed openings. Color is shiny gray, turning dark brown in alcohol. In cross section the sponge is shown to be a thin sheet of 3−5 mm thickness consolidating thick masses of debris, causing the irregular surface. At the microscopic level, the surface shows characteristic striation patterns known for *Hexadella*, and the debris is seen to be enveloped in collagen, without forming fibers, showing that the sponge is using the debris for support. This phenomenon is atypical for *Hexadella*, but has also been described for the North Atlantic deep-water species *Hexadella detritifera*. Currently we do not consider these sponges conspecific on account of large differences in geographic distance and habitat. No *Hexadella* species have been described from Japan. The voucher is incorporated in the Zoological Museum of Amsterdam under reg. no. 17063

Extraction and Isolation. The frozen sponges (1.0 kg wet weight) were extracted three times with MeOH, and the combined extracts were concentrated and partitioned between water and Et_2O. The aqueous layer was further extracted with *n*-BuOH. The *n*-BuOH fraction was separated by ODS flash chromatography with aqueous MeOH. The fraction eluted with

(20) Stevens, V. R.; Gaeta, F. C. A.; Lawrence, D. S. *J. Am. Chem. Soc.* **1983**, *105*, 7713−7719.
(21) Lacy, C.; Scheuer, P. J. *J. Nat. Prod.* **2000**, *63*, 119−121.
(22) Morris, S. A.; Andersen, R. J. *Can. J. Chem.* **1989**, *67*, 677−681.
(23) Morris, S. A.; Andersen, R. J. *Tetrahedron* **1990**, *46*, 715−720.

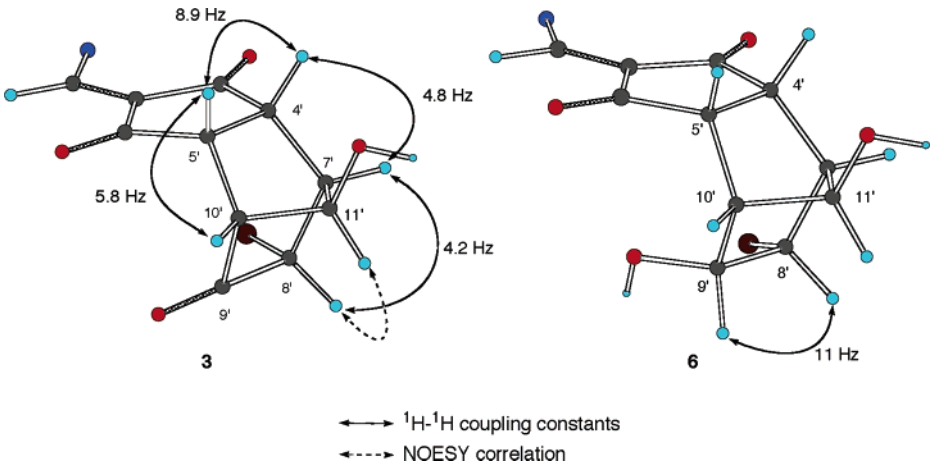


FIGURE 2. Relative stereochemistry of **3** and **6**.

H_2O was gel-filtered on Sephadex G-10 with H_2O. The active fraction was further separated by two rounds of HPLC on a phenylhexyl column, first using a gradient elution of 0−50% MeCN containing 0.05% TFA and then isocratic elution with 9% MeCN containing 0.05% TFA to afford compound **1** (638.2 mg). The fractions eluted with 20% and 50% MeOH from ODS flash chromatography were combined and gel-filtered on Sephadex LH-20 with MeOH/H_2O (50:50). The active fractions were separated by centrifugal partition chromatography [rotation, 1700 rpm; mobile phase, *n*-BuOH/MeOH/H_2O (4:1:5); flow rate, 3 mL/min; ascending mode] to afford 35 fractions (15 mL each) and additional 16 fractions by a reversed elution. The active fractions eluted between 135 and 210 mL were separated by ODS HPLC (gradient elution of 0−80% MeOH containing 0.05% TFA) followed by reversed-phase HPLC on a C_{30} column (23% MeCN containing 0.05% TFA) to afford compound **3** (13.2 mg). Another fraction from the second ODS HPLC was finally purified by reversed-phase HPLC on a phenylhexyl column (25% MeCN containing 0.05% TFA) to furnish compound **2** (1.0 mg).

11-*N*-Methylmoloka′iamine (1): white powder; UV (MeOH) λ_{max} 277 (ϵ 340), 284 nm (330); IR ν_{max} (film) 3850, 3732, 3356, 2943, 2832, 1682, 1455, 1117, 1033 cm^{-1}; HRFABMS *m/z* 366.9831 $(M + H)^+$ (calcd for $C_{12}H_{19}ON_2{}^{79}Br^{81}Br$, Δ −1.3 mmu); ^{1}H NMR (600 MHz, CD_3OD) δ 7.53 (s, 2H, H-6, 8), δ 4.08 (t, J = 5.8 Hz, 2H, H-3), δ 3.26 (t, J = 7.5 Hz, 2H, H-1), δ 3.19 (t, J = 7.7 Hz, 2H, H-11), δ 2.90 (t, J = 7.7 Hz, 2H, H-10), δ 2.67 (s, 3H, H-12), δ 2.16 (quint, J = 5.8, 7.5 Hz, 2H, H-2); ^{13}C NMR (150 MHz, CD_3OD) δ 153.1 (C-4), δ 137.4 (C-7), δ 134.5 (C-6, 8), δ 119.3 (C-5, 9), δ 71.7 (C-3), δ 50.7 (C-11), δ 38.8 (C-1), δ 33.7 (C-12), δ 31.8 (C-10), δ 29.2 (C-2).

11-*N*-Cyano-11-*N*-methylmoloka′iamine (2): colorless solid; UV (MeOH) λ_{max} 277 (ϵ 440), 284 nm (440); IR ν_{max} (film) 3418, 2210, 1652, 1457, 1203, 1140 cm^{-1}; HRFABMS *m/z* 391.9788 $(M + H)^+$ (calcd for $C_{13}H_{18}ON_3{}^{79}Br^{81}Br$, Δ −0.8 mmu); ^{1}H NMR (600 MHz, CD_3OD) δ 7.56 (s, 2H, H-6, 8), δ 4.13 (t, J = 5.8 Hz, 2H, H-3), δ 3.31 (t, J = 7.1 Hz, 2H, H-1), δ 3.27 (t, J = 6.9 Hz, 2H, H-11), δ 2.89 (t, J = 6.9 Hz, 2H, H-10), δ 2.86 (s, 3H, H-12), δ 2.20 (quint, J = 5.8, 7.1 Hz, 2H, H-2); ^{13}C NMR (150 MHz, CD_3OD) δ 152.6 (C-4), δ 139.2 (C-7), δ 134.5 (C-6, 8), δ 119.0 (C-5, 9), δ 118.8 (N-*C*N), δ 71.6 (C-3), δ 54.6 (C-11), δ 39.1 (C-12), δ 38.9 (C-1), δ 33.1 (C-10), δ 29.0 (C-2).

Kuchinoenamine (3): colorless solid; $[\alpha]^{21}{}_D$ +21 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 244 (ϵ 8620), 310 nm (12730); IR ν_{max} (film) 3440, 1757, 1730, 1682, 1614, 1556, 1471, 1257, 1203, 1136, 721 cm^{-1}; HRFABMS *m/z* 650.9357 $(M + H)^+$ (calcd for $C_{23}H_{26}O_5N_2{}^{79}Br^{81}Br_2$, Δ +0.6 mmu); ^{1}H and ^{13}C NMR data, see Table 1.

Preparation of Phthalimide 4. A solution of *N*-carboethoxyphthalimide (65.7 mg, 0.3 mmol) in THF/H_2O (4:1, 2.0 mL) was added to a solution of compound **1** (59.6 mg, 0.1 mmol) and Na_2CO_3 (63.6 mg, 0.6 mmol) in THF/H_2O (4:1, 2.0 mL) at rt. The mixture was stirred at rt for 30 min to afford phtahlimide **4**. The product was used in the next reaction without purification. **4**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.85 (dd, J = 5.0, 3.1 Hz, 2H), δ 7.81 (dd, J = 5.0, 3.1 Hz, 2H), δ 7.53 (s, 2H), δ 4.07 (t, J = 6.2 Hz, 2H), δ 3.95 (t, J = 7.3 Hz, 2H), δ 3.22 (t, J = 7.7 Hz, 2H), δ 2.92 (t, J = 7.7 Hz, 2H), δ 2.70 (s, 3H), δ 2.25 (quint, J = 6.2, 7.3 Hz, 2H).

Preparation of Cyanamide 5. To a mixture of **4** prepared as described above and $NaHCO_3$ (42.0 mg, 0.5 mmol) in EtOH (2.5 mL) was added dropwise a solution of CNBr (53.0 mg, 0.5 mmol) in EtOH (2.5 mL) at rt. After stirring for 12 h at rt, the reaction mixture was evaporated, suspended in H_2O, and extracted with EtOAc to give **5**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.85 (dd, J = 5.4, 3.1 Hz, 2H), δ 7.80 (dd, J = 5.4, 3.1 Hz, 2H), δ 7.51 (s, 2H), δ 4.07 (t, J = 6.2 Hz, 2H), δ 3.96 (t, J = 7.3 Hz, 2H), δ 3.26 (t, J = 7.1 Hz, 2H), δ 2.86 (t, J = 7.1 Hz, 2H), δ 2.84 (s, 3H), δ 2.24 (tt, J = 6.2, 7.3 Hz, 2H).

Preparation of 2. A solution of hydrazine monohydrate (32.0 mg, 1.0 mmol) in EtOH (2.0 mL) was added dropwisely to a solution of **5** in EtOH (5.0 mL) at rt. The reaction mixture was stirred for 15 h at rt and evaporated. The residue was purified by reversed-phase HPLC on a phenylhexyl column (25% MeCN containing 0.05% TFA) to afford **2** (19.5 mg, 31.4%) whose ^{1}H NMR and FAB mass spectra were indistinguishable from those of the natural product.

Preparation of the Diol 6. A solution of **3** (5.0 mg, 7.7 μmol) in MeOH (1 mL) was added to $Na(OAc)_3BH$ (31 mg, 146 μmol) at rt. The mixture was stirred for 1 h at rt and evaporated. The residue was purified by ODS HPLC (gradient elution of 5−35% MeCN containing 0.05% TFA) to afford **6** (1.5 mg, 30.0%) and unreacted **3** (3.5 mg). **6**: ^{1}H NMR (600 MHz, CD_3OD) δ 7.86 (br, 1H), δ 7.53 (s, 2H), δ 4.42 (br, 1H), δ 4.20 (s, 1H), δ 4.11 (br, 1H), δ 4.07 (br, 2H), δ 3.85 (br, 2H), δ 3.22 (t, 6.9 Hz, 2H), δ 3.22 (overlapped, 1H), δ 3.12 (br, 1H), δ 2.92 (t, 6.9 Hz, 2H), δ 2.84 (br, 1H), δ 2.80 (br, 1H), δ 2.70 (s, 3H), δ 2.20 (br, 2H); ^{13}C NMR (HSQC, CD_3OD) δ 156.0 (C-1′), δ 133.0 (C-6, 8), δ 78.5 (C-11′), δ 69.9 (C-3), δ 67.3 (C-9′), δ 52.5 (C-7′), δ 52.2 (C-8′), δ 51.5 (C-10′), δ 49.2 (C-11), δ 48.2 (C-4′), δ 48.0 (C-5′), δ 47.7 (C-1), δ 33.2 (C-12), δ 30.6 (C-10), δ 30.1 (C-2).

Acknowledgment. Thanks are due to the crew of R/V Toyoshio-maru for assistance in collecting the sponge. This work was partly supported by a Grant-in-Aid from the Japan Society for the Promotion of Science.

Supporting Information Available: General experimental procedure; ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and HMQC spectra of **1**−**3** in CD_3OD; HOHAHA spectrum of **3** in CD_3OD; NOESY spectrum of **3** in CD_3OH. This material is available free of charge via the Internet at http://pubs.acs.org.

JO048203J