Nagelamides A−H, New Dimeric Bromopyrrole Alkaloids from Marine Sponge *Agelas* Species[⊥]

Tadashi Endo,[†] Masashi Tsuda,[†] Tadashi Okada,[‡] Shinya Mitsuhashi,[‡] Hiroshi Shima,[‡] Kunimi Kikuchi,[‡] Yuzuru Mikami,[§] Jane Fromont,[||] and Jun'ichi Kobayashi*,[†]

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, Institute for Genetic Medicine, Hokkaido University, Sapporo 060-0815, Japan, Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-0856, Japan, and Western Australian Museum, Perth, WA 6000, Australia

Received December 22, 2003

Eight new dimeric bromopyrrole alkaloids, nagelamides A−H (**1**−**8**) and a monomeric one, 9,10-dihydrokeramadine (**9**), have been isolated from the Okinawan marine sponge *Agelas* sp., and the structures were elucidated from spectroscopic data. Nagelamides A−H (**1**−**8**) exhibited antibacterial activity against Gram-positive bacteria. Nagelamide G (**7**) inhibited protein phosphatase 2A activity.

Bromopyrrole alkaloids are known to be one of the most common metabolites contained in marine sponges.[1] During our search for bioactive substances from marine organisms,[2] we previously isolated several bromopyrrole alkaloids with unique cyclic skeletons from *Agelas* or *Hymeniacidon* sponges.[3,4] Recently we have investigated extracts of the Okinawan marine sponge *Agelas* sp. and isolated eight dimeric bromopyrrole alkaloids, nagelamides A−H (**1**−**8**), and a monomeric one, 9,10-dihydrokeramadine (**9**). Here we describe the isolation and structure elucidation of **1**−**9**.

Results and Discussion

The *Agelas* sponge (SS-1003) collected off Seragaki, Okinawa, was extracted with MeOH. *n*-BuOH-soluble materials of the extract were subjected to silica gel and C_{18} column chromatographies followed by C_{18} HPLC to yield nagelamides A (**1**, 0.00077%, wet weight), B (**2**, 0.00021%), C (**3**, 0.00032%), D (**4**, 0.00013%), E (**5**, 0.00062%), F (**6**, 0.00077%), G (**7**, 0.00041%), and H (**8**, 0.00032%) and 9,10-dihydrokeramadine (**9**, 0.00018%) as colorless solids together with known related alkaloids, ageliferin[5] (**10**), bromoageliferin[5] (**11**), dibromoageliferin[5] (**12**), mauritiamine[6] (**13**), keramadine[7] (**14**), oroidin,[8] stevensine,[9] tauroacidin A,[3] taurodispacamide A,[10] cycloroidin,[10] and manzacidin A.[11] The FABMS spectrum of nagelamide A (**1**) showed the pseudomolecular ion peak at *m/z* 775, 777, 779, 781, and 783 (1:4:6:4:1), indicating the presence of four bromine atoms in the molecule. Nagelamide A (**1**) was revealed to possess the molecular formula $C_{22}H_{22}O_2N_{10}Br_4$ by HRFABMS [*m/z* 774.8735 $(M + H)^+$, Δ −0.4 mmu]. The UV absorption [λ_{max}279 nm (ϵ 27 800)] was attributable to a substituted pyrrole chromophore,[8] while IR absorptions indicated the existence of OH and/or NH (3404 cm^{-1}) and amide carbonyl (1683 cm^{-1}) groups. The ^1H and ^{13}C NMR (Table 1) spectra showed signals due to two 2,3-dibromopyrrole carbonyl moieties[5a] (N-1−C-6 and N-1′−C-6′) and monosubstituted and disubstituted 1-aminoimidazole rings[5a] (C-11−C-15 and C-11′−C-15′). Detailed analyses of $^1H-^1H$ COSY, TOCSY, and HMQC disclosed the connectivities from NH-7 to H-10 and from NH-7′ to H-10 (Figure 1). *E*-Geometry of the double bond at C-9′−C-10′ was deduced from its $^1H-^1H$ coupling constant (15.9 Hz). The ROESY spectrum showed cross-peaks for H-4/NH-7 and H-4′/NH-7′, indicating that the two 2,3-dibromopyrrole carbonyl moieties were connected to NH-7 and NH-7′ through amide bonds. The HMBC spectrum revealed correlations for H-10/C-15 and H-10/C-15′, suggesting that two aminoimidazole rings were attached to C-10. The connection between C-10′ and C-11′ was deduced from the HMBC correlation observed for H-10′/C-11′. The ROESY correlation for H-10/H-10′ implied the *S-trans* diene system for C-9′−C-10′−C-11′−C-15′. Thus, the structure of nagelamide A was assigned as **1**.

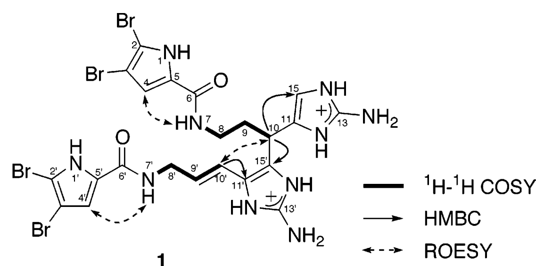


Figure 1. Selected 2D NMR correlations for nagelamide A (**1**).

The molecular formula of nagelamide B (**2**) was suggested to be $C_{22}H_{22}O_3N_{10}Br_4$ by HRFABMS [*m/z* 789.8651 $(M + H)^+$, Δ +4.1 mmu]. ^1H and ^{13}C NMR data of **2** differed from those of **1** only in the presence of an oxymethine signal (δ_H 4.15 m; δ_C 69.29 d). The gross structure of nagelamide B (**2**) was elucidated to be the 9-hydroxyl form of nagelamide A (**1**) on the basis of analyses of 2D NMR data including $^1H-^1H$ COSY, TOCSY, HMQC, HMBC, and ROESY spectra. The *threo* relationship for C-9−C-10 was elucidated by analysis of the rotation model based on a relatively small *J*(H-9,H-10) value (3.1 Hz) and ROESY correlations for H_2-8/H-10, H_2-8/H-10′, H-9/H-15, and H-10/H-10′ (Figure 2).

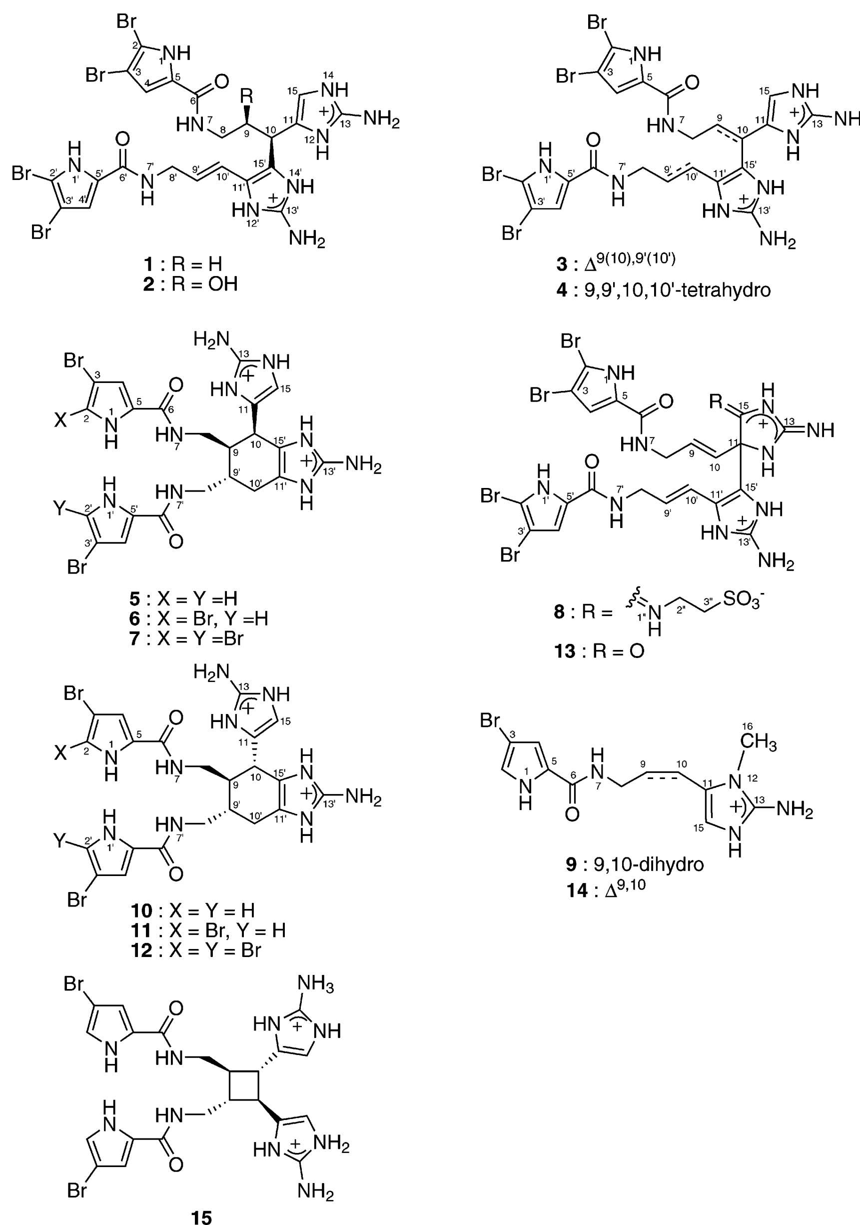
HRFABMS data [*m/z* 772.8596 $(M + H)^+$, Δ +1.6 mmu] of nagelamide C (**3**) disclosed the molecular formula $C_{22}H_{20}O_2N_{10}Br_4$, which was smaller than that of nagelamide A (**1**) by 2 amu. The ^{13}C NMR (Table 1) spectrum showed two sp^3 methylenes and 20 sp^2 carbons including six methines and 14 quaternary ones. The structure of nagelamide C (**3**) was assigned as the 9,10-dehydro form

[⊥] Dedicated to the late Dr. D. John Faulkner (Scripps) and the late Dr. Paul J. Scheuer (Hawaii) for their pioneering work on bioactive marine natural products.

* To whom correspondence should be addressed. Tel: +81 11 706 4985. Fax: +81 11 706 4989. E-mail: jkobay@pharm.hokudai.ac.jp.
[†] Graduate School of Pharmaceutical Sciences, Hokkaido University.
[‡] Institute for Genetic Medicine, Hokkaido University.
[§] Chiba University.
[||] Western Australian Museum.

10.1021/np034077n CCC: $27.50 © 2004 American Chemical Society and American Society of Pharmacognosy
Published on Web 03/30/2004

Chart 1



of **1** by detailed analyses of ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra (Figure 3). Two double bonds at C-9−C-10 and C-9′−C-10′ were suggested to have *E*- and *Z*-geometries, respectively, by the ROESY correlation for H$_2$-8/H-10′ and the *J*(H-9′,H-10′) value (15.9 Hz).

Nagelamide D (**4**) was revealed to possess the molecular formula $C_{22}H_{24}O_2N_{10}Br_4$ by HRFABMS [*m/z* 776.8735 (M + H)$^+$, Δ +0.7 mmu], which was larger than that of **1** by two hydrogen atoms. Comparison of ^{1}H and ^{13}C NMR data of **5** with those of **1** suggested that the C-1−C-15 moiety of **4** was common to that of **1**, while signals due to the C-8′−C-10′ unit of **5** differed from that of **1**. The ^{1}H−^{1}H COSY spectrum indicated the presence of a (CO)NH−(CH$_2$)$_3$− unit for N-7′−C-10′. Thus the structure of nagelamide D (**4**) was assigned as the 9,10-dihydro form of nagelamide A (**1**).

The molecular formulas of nagelamides E−G (**5**−**7**) were established to be $C_{22}H_{24}N_{10}O_2Br_2$, $C_{22}H_{23}N_{10}O_2Br_3$, and $C_{22}H_{22}N_{10}O_2Br_4$, respectively, by HRFABMS {**5**: [*m/z* 619.0517, (M + H)$^+$, Δ −1.2 mmu], **6**: [*m/z* 696.9609, (M + H)$^+$, Δ −2.4 mmu], **7**: [*m/z* 774.8726, (M + H)$^+$, Δ −1.3 mmu]. ^{1}H and ^{13}C NMR data (Table 2) of **5**−**7** were similar to one another, suggesting that nagelamides F (**6**) and G (**7**) corresponded to the monobromo and dibromo forms of nagelamide E (**5**), respectively. Furthermore, the ^{13}C NMR data of **5**−**7** were close to those of ageliferin[5] (**10**), bromoageliferin[5] (**11**), and dibromoageliferin[5] (**12**), respectively. Analyses of 2D NMR spectra implied that the gross structures of **5**−**7** were the same as those of **11**−**13**, respectively, thus indicating that nagelamides E−G (**5**−**7**) were stereoisomers at three chiral centers (C-9, C-10, and C-9′) on the cyclohexene ring of **10**−**12**, respectively. The small *J*(H-9/H-10) values for **5**−**7** suggested that the relation for H-9−H-10 differed from the diaxial relation for those of ageliferins. Further elucidation of the relative stereochemistry of each cyclohexene ring in **5**−**7** was carried out by analysis of the ROESY spectrum. The ROESY spectrum of nagelamide F (**6**) showed correlations for H-10/H$_2$-8′ and H-15/H-9′, implying two pseudo-chair conformations (**A** and **B**) for the cyclohexene ring. ROESY correlations for H$_2$-8/H-10′β (δ_H 2.74) and H-10/H$_2$-8′ suggested both 1,3-diaxial-like relations between C-8 and H-10β and between H-10 and C-8′ in the conformation **A**. On the other hand, the 1,3-diaxial-like relation between

Table 1. ^{1}H and ^{13}C NMR Data of Nagelamides A–C (**1**–**3**) in DMSO-d_6[a]

	1		**2**		**3**	
positn	δ_C[b]	δ_H (m, Hz)	δ_C[b]	δ_H (m, Hz)	δ_C[b]	δ_H (m, Hz)
1		12.72 s		12.69 s		12.71 s
1′		12.70 s		12.68 s		12.68 s
2	104.75 C		104.67 C		104.75 C	
2′	104.62 C		104.58 C		104.64 C	
3	97.90 C		97.88 C		97.83 C	
3′	97.85 C		97.88 C		97.83 C	
4	112.79 CH	6.92 brs	113.04 CH	6.95 brs	112.82 CH	6.93 brs
4′	112.68 CH	6.96 brs	112.79 CH	6.94 brs	112.74 CH	6.91 brs
5	128.05 C		128.00 C		127.88 C	
5′	127.99 C		127.95 C		127.76 C	
6	158.92 C		159.13 C		158.75 C	
6′	158.71 C		158.70 C		158.67 C	
7		8.41 t, 5.6		8.21 t, 5.3		8.46 t, 5.6
7′		8.25 t, 5.6		8.42 t, 5.5		8.45 t, 5.8
8	36.46 CH_2	3.19 m	48.57 CH_2	3.32 m	37.40 CH_2	3.96[c] m
		3.09 m		2.89 m		
8′	40.59 CH_2	3.90[c] m	40.58 CH_2	3.92[c] m	37.40 CH_2	3.86[c] m
9	31.34 CH_2	2.20 m	69.29 CH	4.15 m	129.39 CH	6.24 t, 6.7
		1.98 m				
9′	126.39 CH	6.02 dt, 15.9, 6.5	126.40 CH	6.03 dt, 15.8, 5.9	125.39 CH	6.16 dt, 15.9, 5.9
10	28.70 CH	4.17 t, 7.8	34.97 CH	4.16 m	115.85 C	
10′	116.05 CH	6.42 d, 15.9	116.22 CH	6.42 d, 15.8	116.76 CH	6.05 d, 15.9
11	126.19 C		123.40 C		123.30 C	
11′	122.22 C		122.23 C		116.54 C	
12		12.77 brs		12.24 brs		13.12 brs
12′		12.63 brs		12.78 brs		12.95 brs
13	148.03 C		147.75 C		148.21 C	
13′	147.37 C		146.99 C		148.04 C	
13-NH_2		7.74[c] s		7.53[c] s		7.87[c] s
13′-NH_2		7.50[c] s		7.49[c] s		7.73[c] s
14		12.33 brs		12.14 brs		12.79 brs
14′		12.18 brs		11.73 brs		12.52 brs
15	110.03 CH	6.75 s	110.61 CH	6.76 s	112.66 CH	6.79 s
15′	121.43 C		124.86 C		116.82 C	

[a] Signals for H-1 and H-1′, C-2 and C-2′, C-3 and C-3′, C-5 and C-5′, C-6 and C-6′, H-12 and H-12′, C-13 and C-13′, 13-NH_2 and 13′-NH_2, and H-14 and H-14′ may be interchangeable with each other. [b] In DMSO-d_6 in addition to 1% trifluoroacetic acid. [c] 2H.

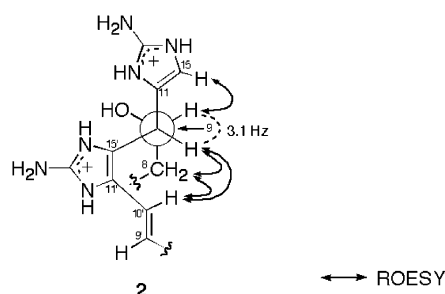


Figure 2. Rotation model for the C-9–C-10 bond of nagelamide B (**2**).

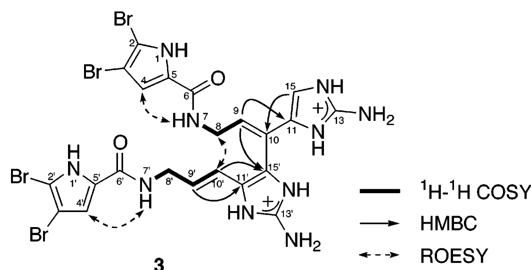


Figure 3. Selected 2D NMR correlations for nagelamide C (**3**).

C-11 and H-9′ in the conformation **B** was deduced from the ROESY correlation for H-15/H-9′. Therefore, the structures of nagelamides E–G (**5**–**7**) were concluded to be the 10-epi forms of ageliferin (**10**), bromoageliferin (**11**), and dibromoageliferin (**12**), respectively.Nagelamide H (**8**) was revealed to possess the molecular formula $C_{24}H_{24}O_5N_{11}SBr_4$ by negative-mode HRFABMS [m/z 897.8408 (M + 2 − H)$^-$, Δ +3.2 mmu]. The IR spectrum suggested the presence of OH/NH (3427 cm^{-1}), amide carbonyl (1683 cm^{-1}), and sulfonate groups (1204 and 1134 cm^{-1}). The ^{1}H and ^{13}C NMR spectra of **8** were similar to those of mauritiamine[6] (**13**), except for the presence of additional signals due to NH (δ_H 9.89) and two methylenes [C-2″: δ_H 3.56 and 3.65, δ_C 40.28; C-3″ 2.82 (2H), δ_C 48.21], which corresponded well to those of a taurine residue in tauroacidin A.[3] Furthermore, the ^{1}H–^{1}H COSY and HMQC spectra revealed a proton network from NH-1″ to C-3″, and HMBC correlations were observed for H-10/C-15 and H-1″/C-15, indicating that the taurine residue was connected to C-15. Thus, the structure of nagelamide H was assigned as **8**.

HRFABMS data [m/z 619.0517, (M + H)$^+$, Δ −1.2 mmu] of compound **9** indicated the molecular formula to be $C_{12}H_{16}ON_5Br$. ^{1}H and ^{13}C NMR data disclosed the presence of a 3-bromopyrrole carbonyl and a 1-amino-2-methylimidazole moiety in addition to three methylene carbons. The ^{1}H–^{1}H COSY spectrum showed the proton connectivity from NH-7 to H-15, while NOESY correlations for H-4/NH-7 and H_2-10/H_3-16 implied that the 3-bromopyrrole carbonyl moiety and an *N*-methyl group were attached to N-7 and N-12, respectively. Therefore, the structure of compound **9** was concluded to be the 9,10-dihydro form of keramadine[7] (**14**).

Nagelamides A–D (**1**–**4**) are a new dimeric bromopyrrole alkaloid with a connection between C-10 and C-15′, while nagelamide H (**8**) and mauritiamine[6] (**14**) possess a C-11–C-15′ bonding. On the other hand, nagelamides E–G (**5**–**7**) and ageliferins[5] (**10**–**12**) and related compounds[13] are considered to be oroidin dimers formed by [4+2]

Table 2. ^{1}H and ^{13}C NMR Data of Nagelamides E–G (**5**–**7**) in DMSO-d_6

	5[a]		**6**[b]		**7**[a]	
positn	δ_C	δ_H (m, Hz)	δ_C	δ_H (m, Hz)	δ_C	δ_H (m, Hz)
1		11.78 brs		12.66 brs		12.66 brs
1′		11.78 brs		11.78 brs		12.66 brs
2	121.25 CH	6.97 brs	104.54 C		104.66 C	
2′	121.22 CH	6.95 brs	121.23 CH	6.95 brs	104.62 C	
3	94.96 C		97.82 C		97.86 C	
3′	94.93 C		94.88 C		97.83 C	
4	111.64 CH	6.84 brs	112.76 CH	6.90 brs	112.75 CH	6.90 brs
4′	111.55 CH	6.84 brs	114.40 CH	6.84 brs	112.62 CH	6.92 brs
5	126.68 C		126.64 C		127.96 C	
5′	126.62 C		127.89 C		127.92 C	
6	159.82 C		159.76 C		159.10 C	
6′	159.81 C		159.02 C		159.06 C	
7		8.12 t, 5.9		8.14 t, 5.9		8.11 t, 5.9
7′		8.21 t, 5.9		8.22 t, 5.9		8.22 t, 5.9
8	37.52 CH$_2$	2.90 dt, 13.7, 5.9	37.53 CH$_2$	2.90 dt, 13.7, 5.9	38.61 CH$_2$	2.90 tt, 17.7, 5.9
		3.31 m		3.27 m		3.30 m
8′	40.30 CH$_2$	3.38 m	40.53 CH$_2$	3.30 m	40.36 CH$_2$	3.32 m
		3.23 m		3.20 m		3.13 m
9	39.12 CH	2.30 m	39.12 CH	2.28 m	39.20 CH	2.29 m
9′	32.99 CH	2.24 m	32.99 CH	2.23 m	32.97 CH	2.26 m
10	29.28 CH	4.22 brs	29.22 CH	4.21 brs	29.23 CH	4.21 brs
10′	19.97 CH$_2$	2.74 m	19.95 CH$_2$	2.72 m	19.71 CH$_2$	2.70 m
		2.26 m		2.25 m		2.23 m
11	123.60 C		123.52 C		123.59 C	
11′	120.52 C		120.45 C		120.49 C	
12		12.47 brs		12.46 brs		12.34 brs
12′		12.32 brs		12.33 brs		12.18 brs
13	147.50 C		147.47 C		147.37 C	
13′	147.40 C		147.38 C		147.29 C	
13-NH$_2$		7.59[c] brs		7.59[c] brs		7.53[c] brs
13′-NH$_2$		7.57[c] brs		7.57[c] brs		7.52[c] brs
14		12.32 brs		12.35 brs		12.18 brs
14′		12.18 brs		12.15 brs		12.08 brs
15	111.49 CH	6.67 s	111.48 CH	6.66 s	111.59 CH	6.67 s
15′	116.60 C		116.56 C		116.43 C	

[a] Signals for C-2 and C-2′, C-3 and C-3′, C-4 and C-4′, C-5 and C-5′, C-6 and C-6′, H-12 and H-12′, C-13 and C-13′, 13-NH$_2$ and 13′-NH$_2$, and H-14 and H-14′ may be interchangeable with each other. [b] Signals for C-6 and C-6′, H-12 and H-12′, C-13 and C-13′, 13-NH$_2$ and 13′-NH$_2$, and H-14 and H-14′ may be interchangeable with each other. [c] 2H.

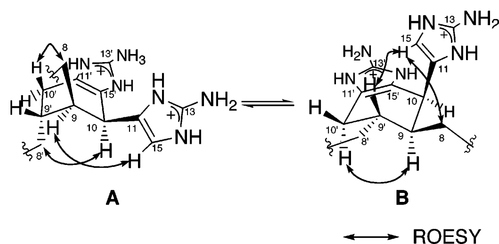


Figure 4. Two possible conformations (**A** and **B**) and relative stereochemistry of a cyclohexene ring in nagelamide F (**6**).

cycloaddition, although sceptrin[14] (**15**) and its congeners[5b,15–17] seem to be derived from [2+2] cycloaddition. Optical rotations {$[\alpha]_D$ ~0°} as well as flat CD curves between 200 and 400 nm in **1**, **2**, **4**, and **8** indicate that they are racemates. Although nagelamides E–G (**5**–**7**) are optically active, the absolute configurations remain undefined.

Dimeric bromopyrrole alkaloids nagelamides A–H (**1**–**8**), ageliferins (**10**–**12**), and sceptrin (**15**) exhibited antimicrobial activity against Gram-positive bacteria *Micrococcus luteus* and *Bacillus subtilis* and the Gram-negative bacterium *Escherichia coli* (Table 3), while 9,10-dihydrokeramadine (**9**) did not show such activity. Nagelamides A (**1**), G (**7**), and H (**8**) and sceptrin (**15**) showed inhibitory activity against protein phosphatase type 2A (IC_{50}, 48, 13, 46, and 50 μM, respectively, Table 3), a major serine/threonine protein phosphatase that appears to be critically involved in cellular growth and potentially in the development of cancer.[18]

Table 3. Antibacterial Activity and Protein Phosphatase Type 2A Inhibitory Activity of Nagelamides A–H (**1**–**8**), 9,10-Dihydrokeramamine (**9**), Ageliferins (**10**–**12**), and Sceptrin (**15**)

	antibacterial activity (MIC, μg/mL)			protein phosphatase 2A
compd	*M. luteus*	*B. subtilis*	*E. coli*	(IC_{50}, μM)
1	2.08	16.7	33.3	48
2	4.17	33.3	33.3	>50
3	4.17	33.3	33.3	>50
4	4.17	33.3	33.3	*a*
5	4.17	16.7	33.3	*a*
6	4.17	16.7	33.3	*a*
7	2.08	16.7	33.3	13
8	16.7	33.3	>33.3	46
9	>33.3	>33.3	>33.3	>50
10	4.17	8.33	33.3	>50
11	2.08	2.08	16.7	>50
12	2.08	4.16	16.7	>50
15	4.07	8.33	33.3	50

[a] Not tested.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 spectropolarimeter. IR spectra were recorded on a JASCO FT/IR-5300 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-600 spectrometer. FAB mass spectra were obtained on a JEOL HX-110 spectrometer using nitrobenzyl alcohol as a matrix. Antimicrobial activities were determined by a microbroth dilution method using BHI medium.

Sponge Description. The sponge *Agelas* sp. (order Homosclerophorida; family Plakinidae) was collected off Seragaki Beach, Okinawa, and kept frozen until used. The sponge was dark brown throughout in ethanol and flattened. The choanosome was pigmented throughout with more dense pigmentation at the surface. Spicules, which were abundant throughout the sponge, were predominantly diods with a central angulation of 65−115 × 1.5−4.5 μm in dimension. Occasional triods were present. This specimen was a reproductive female, apparently with incubating embryos. The voucher specimen (SS-1003) was deposited at the Graduate School of Pharmaceutical Sciences, Hokkaido University.

Extraction and Isolation. The sponge (1.4 kg, wet weight) was extracted with MeOH (1.2 L × 3), the extract (70.1 g) was partitioned between EtOAc (500 mL × 3) and H_2O (500 mL), and subsequently the aqueous layer was extracted with *n*-BuOH (500 mL × 3). The *n*-BuOH-soluble materials (16.0 g) were subjected to SiO_2 gel column chromatography ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1, 2 L) to give two alkaloid-containing fractions, I (373.8 mg) and II (1272.5 mg). Fraction I was separated by C_{18} column chromatography (MeOH/H_2O, 1:1) and then C_{18} HPLC (TSK-GEL ODS-80TS, TOSOH Co., Ltd., 21.6 × 300 mm; eluent, CH_3CN/H_2O/CF_3CO_2H, 40:60:0.1; flow rate, 8 mL/min; UV detection at 270 nm) to yield crude fractions Ia (17.4 mg), Ib (7.2 mg), and Ic (7.6 mg). Fraction Ia was purified by C_{18} HPLC (Wakosil-II 5C18, Wako Pure Chemical Ind., Ltd., 10 × 250 mm; eluent, MeOH/H_2O/CF_3CO_2H, 60:40:0.1; flow rate, 4 mL/min; UV detection at 270 nm) to afford nagelamides A (**1**, 10 mg, 0.00077%, wet weight, t_R 18.7 min) and C (**3**, 4.1 mg, 0.00032%, t_R 17.5 min). Fraction Ib was purified by C_{18} HPLC (Wakosil-II 5C18, 10 × 250 mm; eluent, MeOH/H_2O/CF_3CO_2H, 60:40:0.1; flow rate, 2 mL/min; UV detection at 270 nm) to give nagelamide B (**2**, 2.2 mg, 0.00021%, t_R 18.9 min). Fraction Ic was subjected to C_{18} HPLC (TSK-GEL ODS-80TS, 21.6 × 300 mm; eluent, MeOH/H_2O/CF_3CO_2H, 60:40:0.1; flow rate, 8 mL/min; UV detection at 230 nm) to yield nagelamide D (**4**, 1.8 mg, 0.00013%, t_R 44 min).

Fraction II of the first SiO_2 column was chromatographed on a C_{18} column (MeOH/H_2O, 1:1) and then C_{18} HPLC (TSK-GEL ODS-80TS, 21.6 × 300 mm; eluent, CH_3CN/H_2O/CF_3CO_2H, 40:60:0.1; flow rate, 8 mL/min; UV detection at 270 nm) to give four crude fractions, IIa (37.2 mg), IIb (20.6 mg), IIc (25.9 mg), and IId (16.7 mg), together with known alkaloids, ageliferin (**10**, 206 mg, 0.015%, t_R 13 min), bromoageliferin (**11**, 145 mg, 0.010%, t_R 18 min), and dibromoageliferin (**12**, 107 mg, 0.0076%, t_R 26 min). Fraction IIa was purified by C_{18} HPLC (TSK-GEL ODS-80TS, 21.6 × 300 mm; eluent, MeOH/H_2O/CF_3CO_2H, 50:50:0.1; flow rate, 8 mL/min; UV detection at 230 nm) to afford 9,10-dihydrokeramadine (**9**, 2.3 mg, 0.00018%, t_R 17.4 min) together with known keramadine (**14**, 2.6 mg, 0.00019%, t_R 20 min) and manzacidin A (3.4 mg, 0.00024%, t_R 24 min). Fraction IIb was separated by C_{18} HPLC (TSK-GEL ODS-80TS, 21.6 × 300 mm; eluent, MeOH/H_2O/CF_3CO_2H, 60:40:0.1; flow rate, 8 mL/min; UV detection at 230 nm) to yield nagelamide E (**5**, 8.0 mg, 0.00062%, t_R 20 min). Fraction IIc was subjected to C_{18} HPLC (TSK-GEL ODS-80TS, 21.6 × 300 mm; eluent, MeOH/H_2O/CF_3CO_2H, 70:30:0.1; flow rate, 8 mL/min; UV detection at 240 nm) to afford nagelamides F (**6**, 10.0 mg, 0.00077%, t_R 15.6 min) and H (**8**, 4.1 mg, 0.00032%, t_R 20.8 min). Fraction IId was separated by reversed-phase HPLC on 6-(phenyl)hexylsilyl (Luna Phenyl-Hexyl, Phenomenax, 10 × 250 mm; eluent, MeOH/H_2O/CF_3CO_2H, 60:40:0.1; flow rate, 3.5 mL/min; UV detection at 230 nm) to give nagelamide G (**7**, 5.3 mg, 0.00041%, t_R 38.8 min) together with known mauritiamine (**13**, 2.2 mg, 0.00016%, t_R 36.0 min). Several known alkaloids, oroidin, stevensine, tauroacidin A, taurodispacamide A, and cyclooroidin, were obtained from different fractions of the *n*-BuOH-soluble materials from those described above.

Nagelamide A (1): colorless amorphous solid; $[\alpha]^{17}_D$ ~0° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 279 (ϵ 27 800) and 202 nm (29 800); IR (KBr) ν_{max} 3404, 1683, and 1618 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (pos.) *m/z* 775, 777, 779, 781, and 783 $[(M + H)^+$, 1:4:6:4:1]; HRFABMS (pos.) *m/z* 774.8735 $[(M + H)^+$, calcd for $C_{22}H_{23}O_2N_{10}{}^{79}Br_4$, 774.8739].

Nagelamide B (2): colorless amorphous solid; $[\alpha]^{17}_D$ ~0° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 282 (ϵ 16 000) and 202 nm (19 200); IR (KBr) ν_{max} 3400 and 1683 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (pos.) *m/z* 790, 792, 794, 796, and 798 $[(M + H)^+$, 1:4:6:4:1]; HRFABMS (pos.) *m/z* 789.8651 $[(M + H)^+$, calcd for $C_{22}H_{23}O_3N_{10}{}^{79}Br_4$, 789.8610].

Nagelamide C (3): colorless amorphous solid; UV (MeOH) λ_{max} 280 (ϵ 19 700) and 202 nm (21 200); IR (KBr) ν_{max} 3434 1685, and 1618 cm^{-1}; ^1H and ^{13}C NMR (see Table 1); FABMS (pos.) *m/z* 773, 775, 777, 779, and 781 $[(M + H)^+$, 1:4:6:4:1]; HRFABMS (pos.) *m/z* 772.8596 $[(M + H)^+$, calcd for $C_{22}H_{21}O_2N_{10}{}^{79}Br_4$, 772.8582].

Nagelamide D (4): colorless amorphous solid; UV (MeOH) λ_{max} 280 (ϵ 14 900) and 202 nm (16 500); IR (KBr) ν_{max} 3425, 1683, and 1619 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.14 (1H, m, H-9′), 2.20 (2H, m, H_2-9), 2.72 (1H, m, H-9′), 2.90 (1H, m, H-8), 3.20 ~ 3.30 (5H, m, H-8, H_2-8′, and H_2-10′), 4.18 (1H, m, H-10), 6.72 (1H, s, H-15), 6.95 (1H, s, H-4). 6.96 (1H, s, H-4′), 7.50 (4H, brs, NH_2-13 and NH_2-13′), 8.13 (1H, t, *J* = 5.6 Hz, H-7), 8.21 (1H, t, *J* = 5.7 Hz, H-7′), 12.05 (1H, brs), 12.15 (2H, brs), 12.36 (1H, brs), and 12.65 (2H, brs); ^{13}C NMR (DMSO-d_6) δ 16.64 (t), 21.97 (t), 28.84 (d), 31.13 (t), 36.54 (t), 41.20 (t), 97.20 (s), 97.76 (s), 104.51 (s), 104.52 (s), 111.54 (d), 112.64 (d), 112.73 (d), 120.34 (s), 121.35 (s), 124.97 (s), 127.88 (2C, s), 147.27 (s), 147.36 (s), and 159.00 (2C, s); FABMS (pos.) *m/z* 777, 779, 781, 783, and 785 $[(M + H)^+$, 1:4:6:4:1]; HRFABMS (pos.) *m/z* 776.8746 $[(M + H)^+$, calcd for $C_{22}H_{25}O_2N_{10}{}^{79}Br_4$, 776.8739].

Nagelamide E (5): colorless amorphous solid; $[\alpha]^{17}_D$ −11.3° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 272 (ϵ 17 200), 215 (sh., 18 000), and 202 nm (32 000); IR (KBr) ν_{max} 3425 and 1684 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); FABMS (pos.) *m/z* 619, 621, and 623, $[(M + H)^+$, 1:21]; HRFABMS (pos.) *m/z* 619.0517 $[(M + H)^+$, calcd for $C_{22}H_{25}O_2N_{10}{}^{79}Br_2$, 619.0529].

Nagelamide E (6): colorless amorphous solid; $[\alpha]^{17}_D$ −14.1° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 272 (ϵ 17 700), 216 (sh., 20 100), and 202 nm (24 000); IR (KBr) ν_{max} 3421 and 1684 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); FABMS (pos.) *m/z* 697, 699, 701, and 703 $[(M + H)^+$, 1:3:3:1]; HRFABMS (pos.) *m/z* 696.9609 $[(M + H)^+$, calcd for $C_{22}H_{24}O_2N_{10}{}^{79}Br_3$, 696.9633].

Nagelamide G (7): colorless amorphous solid; $[\alpha]^{17}_D$ +6.7° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 277 (ϵ 27 400), 215 (sh., 28 900), and 202 nm (32 000); IR (KBr) ν_{max} 3441 and 1684 cm^{-1}; ^1H and ^{13}C NMR (see Table 2); FABMS (pos.) *m/z* 775, 777, 779, 781, and 783 $[(M + H)^+$, 1:4:6:4:1]; HRFABMS (pos.) *m/z* 774.8726 $[(M + H)^+$, calcd for $C_{22}H_{23}O_2N_{10}{}^{79}Br_4$, 774.8739].

Nagelamide H (8): colorless amorphous solid; $[\alpha]^{17}_D$ ~0° (*c* 1.0, MeOH); UV (MeOH) λ_{max} 279 (ϵ 28 300) and 221 nm (34 900); IR (KBr) ν_{max} 3427, 1683, 1204, and 1134 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.81 (2H, t, *J* = 7.1 Hz, H_2-3″), 3.56 (1H, m, H-2″), 3.65 (1H, m, H-2″), 3.86 (1H, m, H-8), 3.92 (2H, m, H_2-8′), 4.04 (1H, m, H-8), 5.90 (1H, d, *J* = 15.3 Hz, H-10′), 5.99 (1H, dt, *J* = 15.3 and 6.2 Hz, H-9′), 6.02 (1H, dt, *J* = 15.2 and 6.0 Hz, H-9), 6.15 (1H, d, *J* = 15.2 Hz, H-10), 6.97 (1H, s, H-4′), 7.00 (1H, s, H-4), 7.74 (2H, brs, 13′-NH_2), 8.12 (1H, t, *J* = 5.9 Hz, H-7), 8.21 (1H, t, *J* = 5.9 Hz, H-7′), 8.79 (1H, brs, 13-NH), 9.11 (1H, brs, 13-NH), 9.89 (1H, brs, H-1″), 10.19 (1H, brs, H-14), 12.60 (1H, brs, H-12′), 12.71 (1H, s, H-1), 12.72 (1H, s, H-1′), and 13.02 (1H, brs, H-14′); ^{13}C NMR (DMSO-d_6) δ 39.33 (t, C-8′), 40.09 (t, C-8), 40.28 (t, C-2″), 48.21 (t, C-3″), 69.72 (C-11′), 97.94, 97.97 (s, C-3 and C-3′), 104.76, 104.84 (s, C-2 and C-2′), 112.84 (s, C-11), 112.94, 113.04 (d, C-4 and C-4′), 115.38 (d, C-10), 123.12 (s, C-15′), 124.71 (d, C-9), 127.73, 127.87 (s, C-4 and C-4′), 129.57 (d, C-10′), 130.82 (d, C-9′), 148.27 (s, C-13′), 158.75, 158.75 (s, C-5 and C-5′), 167.31 (s, C-13), 177.43 (s, C-15); FABMS (neg.) *m/z* 893, 895, 897, 899, and 901 $[(M - H)^-$, 1:4:6:4:1]; HRFABMS (neg.) *m/z* 897.8408 $[(M - H)^-$, calcd for $C_{24}H_{25}O_5N_{11}S^{79}Br_2{}^{81}Br_2$, 897.8376].

9,10-Dihydrokeramadine (9): colorless amorphous solid; UV (MeOH) λ_{max} 280 (ϵ 19 700) and 202 nm (21 200); IR (KBr) ν_{max} 3430 and 1680 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.75 (2H, t, *J* = 7.1 Hz, H_2-9), 2.49 (2H, t, *J* = 7.1 Hz, H_2-10), 3.26 (2H, t, *J* = 6.4 Hz, H_2-8), 3.34 (3H, s, H_3-16), 6.72 (1H, brs, H-15), 6.83 (1H, brs, H-4), 6.97 (1H, s, H-2), 7.54 (1H, brs, 13-NH), 7.56 (1H, brs, 13-NH), 8.17 (1H, brt, *J* = 6.0 Hz, H-7), 11.79 (1H, brs, H-1), and 12.08 (1H, brs, H-14); ^{13}C NMR (DMSO-

d_6) δ 18.98 (t, C-10), 25.08 (t, C-9), 27.44 (q, C-16), 36.17 (t, C-8), 93.34 (s, C-3), 106.84 (d, C-4), 109.81 (d, C-4), 119.58 (d, C-2), 125.37 (s, C-11), 126.34 (s, C-5), 144.96 (s, C-13), and 158.15 (s, C-6); FABMS (pos.) *m*/*z* 326 and 328 [$(M + H)^+$, 1:1]; HRFABMS (pos.) *m*/*z* 326.0634 [$(M + H)^+$, calcd for $C_{12}H_{17}ON_5{}^{79}Br$, 326.0617].

Protein Phosphatase Activity. An aliquot of COS-7 cells (5×10^7) was frozen in liquid nitrogen and stocked at −70 °C. All of the following procedures were carried out at 4 °C. The frozen cells were lysed in 0.6 mL of hypotonic buffer containing 50 mM Tris-HCl (pH 7.5), 5 mM *threo*-1,4-dimercapto-2,3-butanediol (DTT), 0.5 mM benzamidine, 2 mM EGTA, 10 μg/mL leupeptin, and 10 μg/mL aprotinin. The cell lysate was centrifuged at 20000*g* for 10 min, and the resulting supernatants were diluted with an equal volume of glycerol and stored at −20 °C until use. The protein concentration was measured by the method of Bradford with some modifications.[19]

Phosphorylase was ^{32}P-labeled by phosphorylase kinase to 1 mol phosphate per 1 mol phosphorylase as previously described.[20] PP2A activity was measured by the method of P. Cohen with some modifications.[19] Briefly, 10 μL of cell extract was diluted with solution A (50 mM Tris-HCl (pH 7.5), 0.1 mM EGTA, and 0.1% (v/v) β-mercaptoethanol) containing 1 mg/mL BSA. Then the diluted enzymes were preincubated with 30 μL of solution A containing 0.33 mg/mL BSA and 0.02% (w/v) Brij-35 in the presence or absence of compounds for 10 min at 30 °C. The reaction was initiated with ^{32}P-labeled substrate in 20 μL of solution A containing 15 mM caffeine. In each reaction, the cell extract at the protein concentration of 5 μg/mL was used. After 10 min at 30 °C, the reaction was stopped by adding 50 μL of 10 mM H_2SO_4 acid solution containing 20 mM silicotungstic acid, and the solution was centrifuged. Subsequent procedures were essentially the same as those previously described.[19] In cell extracts, PP2A activity is defined as the phosphorylase phosphatase activity sensitive to 1 nM okadaic acid. Okadaic acid and the several compounds tested were stocked in dimethyl sulfoxide and used for assays immediately after dilution with the assay buffer. Assays were carried out in triplicate, and the mean values were presented. One unit of the enzyme was defined as the amounts of enzyme required for catalyzing the release of 1 μmol of phosphate per min.

Acknowledgment. We thank M. Kiuchi, Center for Instrumental Analysis, Hokkaido University, for FABMS measurement and Z. Nagahama for his help with collection of the sponge. This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

Note Added after ASAP: In the version of this article posted on March 30, 2004, an incorrect voucher specimen number was listed. The correct voucher specimen number is SS-1003, which appears in the version posted April 7, 2004.

References and Notes

(1) (a) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Princep, P. R. *Nat. Prod. Rep.* **2003**, *20*, 1−48. (b) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and references therein.
(2) Tsuda, M.; Endo, T.; Perpelescu, M.; Yoshida, S.; Watanabe, K.; Fromont, J.; Mikami, Y.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 1137−1141.
(3) Kobayashi, J.; Inaba, K.; Tsuda, M. *Tetrahedron* **1997**, *53*, 16679−16682.
(4) Tsuda, M.; Uemoto, H.; Kobayashi, J. *Tetrahedron Lett.* **1999**, *40*, 5709−5712.
(5) (a) Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* **1990**, *46*, 5579−5586. (b) Keifer, P. A.; Schwantz, R. E.; Koker, M. E. S.; Hugh, R. G. Jr.; Rittschof, D.; Rinehart, K. L. *J. Org. Chem.* **1991**, *56*, 2965−2975.
(6) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *J. Nat. Prod.* **1996**, *59*, 501−503.
(7) Nakamura, H.; Ohizumi, Y.; Kobayashi, J. *Tetrahedron Lett.* **1984**, *25*, 2475−2478.
(8) (a) Forenza, S.; Minale, L.; Riccio, R.; Fattorusso, E. *J. Chem. Soc., Chem. Commun.* **1971**, 1129−1130. (b) Gracia, E. E.; Benjamin, L. E.; Fryer, R. I. *J. Chem. Soc., Chem. Commun.* **1973**, 78−79.
(9) (a) Albizati, K. F.; Faulkner, D. J. *J. Org. Chem.* **1985**, *50*, 4163−4164. (b) Nanteuil, G. D.; Ahond, A.; Guilhem, J.; Poupat, C.; Dau, E. T.; Portier, P.; Pusset, M.; Pusset, J.; Laboute, P. *Tetrahedron* **1985**, *41*, 6019−6033.
(10) Fattorusso, E.; Taglialatela-Scafati, O. *Tetrahedron Lett.* **2000**, *41*, 9917−9922.
(11) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* **1991**, *56*, 4574−4576.
(12) Scott, A. I. In *Interpretation of the Ultraviolet Spectra of Natural Products*; Pergamon Press: New York, 1964; pp 165−169.
(13) Williams, D. H.; Faulkner, D. J. *Tetrahedron* **1996**, *52*, 5381−5390.
(14) Walker, R. P.; Faulkner, D. J. *J. Am. Chem. Soc.* **1981**, *103*, 6772−6773.
(15) Kobayashi, J.; Tsuda, M.; Ohizumi, Y. *Experientia* **1991**, *47*, 301−304.
(16) Shen, X.; Perry, T. L.; Dunbar, C. D.; Kelly-Borges, M.; Hamann, M. T. *J. Nat. Prod.* **1998**, *61*, 1302−1303.
(17) Eder, C.; Proksch, P.; Wary, V.; van Soest, R. W. M.; Ferdinandus, E.; Pattisina, L. A.; Sudarsono. *J. Nat. Prod.* **1999**, *62*, 1295−1297.
(18) Sontag, E. *Cell. Signalling* **2001**, *13*, 7−16.
(19) Mitsuhashi, S.; Shima, H.; Tanuma, N.; Matsuura, N.; Takekawa, M.; Urano, T.; Kataoka, T.; Ubukata, M.; Kikuchi K. *J. Biol. Chem.* **2003** *278*, 82−88.
(20) Mitsuhashi, S.; Matsuura, N.; Ubukata, M.; Oikawa, H.; Shima, H.; Kikuchi K. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 328−331.

NP034077N