New Alkaloids from the Papua New Guinean Sponge *Agelas nakamurai*

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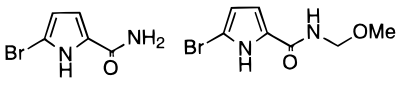
Two new bromopyrroles and a new diterpene possessing a 9-methyladenium moiety have been isolated from the Papua New Guinean sponge *Agelas nakamurai* Hoshino.

Sponges belonging to the genus *Agelas* have proved to be rich sources of purinoditerpenes and bromopyrrole derivatives, exhibiting interesting bioactivities such as antimicrobial, Na, K-ATPase inhibitory, and antiserotonergic.[1,2] During our investigation of bioactive products from marine organisms, we have isolated two new bromopyrrole alkaloids and a new diterpene possessing a 9-methyladenium moiety from the Papua New Guinean sponge *Agelas nakamurai* Hoshino.

The methanolic extract of *A. nakamurai* was partitioned between water and dichloromethane, and the aqueous solution was extracted with *n*-butanol. The *n*-butanol extract was chromatographed on silica gel and reversed-phase HPLC to give new compounds **1**−**3**, together with agelasine B (**4**).[3] In this paper, we describe the isolation and structural elucidation of these compounds.



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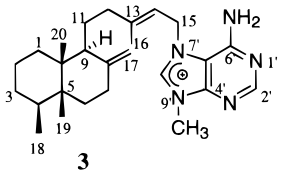

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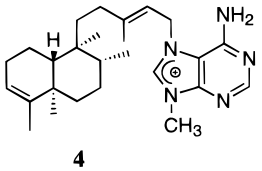

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The mass spectrum of **1**, needles, mp 137−138 °C, gave molecular ions at *m*/*z* 188 and 190 in a ratio of ca. 1:1, suggesting that **1** was a monobromo compound. The molecular formula was determined as $C_5H_5N_2OBr$ by HRFABMS. The IR spectrum indicated absorption band due to an amide carbonyl and an aromatic ring at 1662 and 1589 cm^{-1}, respectively. In the ^{13}C NMR spectrum, resonances due to a disubstituted pyrrole at δ 104.4 (s), 112.2 (d), 114.5 (d), and 126.2 (s) were observed as well as an amide carbonyl at δ 164.7 (s). The ^1H NMR spectrum indicated resonances due to 2,5-disubstituted pyrrole protons at δ 6.20 (1H, d, J = 3.8 Hz, H-4), 6.53 (1H, d, J = 3.8 Hz, H-3), and 9.76 (1H, br s, NH) as well as those due to a $CONH_2$ moiety at δ 5.63 (2H, br s). The positions of the $CONH_2$ moiety at C-2 and the bromine atom at C-5 were assigned by comparing the chemical shifts with literature data.[4,5] On the basis of the above results, compound **1** was concluded to be 5-bromopyrrole-2-carboamide, which had been synthesized previously.[6] This is the first isolation of **1** as a natural product.

The ^1H NMR spectrum of compound **2**, white powder, $C_7H_9N_2O_2Br$, was similar to that of **1**, except that additional resonances due to methoxy protons at δ 3.40 (3H, s) and methylene protons at δ 4.88 (2H, d, J = 7.0 Hz) were observed, and one of the two protons in the carboxamide group (δ 6.55, 1H, overlapped) was missing. The methylene protons were confirmed to be attached to oxygen and nitrogen atoms since the protons were coupled to an amide proton and the chemical shift [δ_C 71.4 (t)] in the ^{13}C NMR spectrum was deshielded. This implied the presence of a methoxymethyl group attached to the amide nitrogen atom. Thus, compound **2** was assigned as 5-bromopyrrole-2-(*N*-methoxymethyl)carboxamide.

Compound **3**, $C_{26}H_{39}N_5$, was isomeric with agelasine B, which was also isolated from the same sponge. The presence of a 9-methyladenine moiety was readily assigned by comparing the ^1H NMR spectrum with that of agelasine B: NMe (δ 4.09, 3H, s), NH_2 (δ 6.80, 2H, br s), and two aromatic protons (δ 8.49, 1H, s, H-2′, 10.9, 1H, br s, H-8′). This was also supported by the ^{13}C NMR spectral data (see the Experimental Section). The molecular formula suggested that the remaining portion except for the adenine moiety consisted of a diterpene with four degrees of unsaturation. Resonances due to four olefinic carbons at δ 106.4 (t), 116.0 (d), 147.5 (s), and 149.3 (s) in the ^{13}C NMR spectrum were observed, suggesting that the diterpene moiety was bicyclic. The ^1H NMR spectrum showed resonances due to four methyl protons at δ 0.60 (3H, s), 0.72 (3H, s), 0.77 (3H, d, J = 7.0 Hz), and 1.87 (3H, br s) as well as those of three olefinic protons due to terminal methylenes at δ 4.41 and 4.82 (1H each, br s), and a trisubstituted proton at δ 5.44 (1H, br t, J = 6.8 Hz) suggested a labdane-related carbon skeleton. The gross structure was determined by analysis of $^1H-^1H$ COSY, $^{13}C-^1H$
COSY, and COLOC spectra, which were all measured in CD_3OD for solubility reasons. The connectivity of C-12 to C-15 was suggested by inspection of the cross-peaks in the COLOC spectrum: H-16 (3H, δ 1.90, br s)/C-12 (δ 39.8, t), C-13 (δ 149.7, s), and C-14 (δ 115.8, d), H-15 (2H, δ 5.24, d, J = 7.6 Hz)/C-13 and C-14. That C-15 was attached to N-7 of the 9-methyladenine moiety was evident from comparing chemical shifts of H-15 (δ 5.71, br d, J = 6.8 Hz) and C-15 (δ 48.6, t) in $CDCl_3$ with those of agelasines A−F.[3] The connectivity of C-2 to C-11, including three methyl groups, except for C-1 to C-3 and C-8 to C-9, was confirmed by observation of the cross-peaks of H-20 (3H, δ 0.66, s) to C-1 (δ 33.0, t), C-5 (δ 40.7, s), C-9 (δ 43.6, d),

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Table 1. Antimicrobial Activity (IC_{50} μg/mL) of **1** and **4**

compd	*S. epidermidis* 13889	*E. faecalis* 12964	*E. faecium* 12367	*S. aureus* 12732	MRSAa	*C. albicans* IFO-1269	*C. neoformans* TIMM-0354
1	3.13	6.25	3.13	0.78	3.13	6.25	3.13
4	1.56	6.25	3.13	0.78	3.13	>12.5	3.13

[a] MRSA: methicillin-resistant *S. aureus*.

and C-10 (δ 43.3, s); H-18 (3H, δ 0.80, d, $J = 7.0$ Hz) to C-3 (δ 32.3, t) and C-4 (δ 31.8, d); H-19 (3H, δ 0.78, s) to C-4, C-5, C-6 (δ 35.1, t), and C-10; H-6 (1H, δ ca. 1.60, overlapped) to C-8 (δ 150.7, s); and H-17 (1H each, δ 4.53 and 4.88, br s) to C-6 (δ 34.1) in the COLOC spectrum. The H-17 was allylically coupled to one of H-7 (1H, δ 2.18, m) and H-9 (δ 2.67, 1H, br d, $J = 9.9$ Hz), which in turn was coupled to H-11 (δ ca. 1.60, 2H, overlapped) in the ^{1}H−^{1}H COSY spectrum, indicating the connectivity of C-7 to C-11. The *E* configuration of the olefinic bond at C-14 was confirmed by the chemical shift of C-16 (δ 17.2). The relative stereochemisty was assigned on the basis of NOE experiments. The *cis* ring junction resulted from the NOE enhancement between H-19 and H-20. Irradiation of H-4 resulted in enhancements of H-7 and H-9, implying that these protons occurred on the same face of the ring system (α), and hence, the methyl group at C-4 was on the opposite face to the protons (β) as well as the methyl groups at C-5 and C-10. Therefore, the structure of **3** was concluded to possess the unusual thelepogane skeleton. Thelepogine, isolated from the terrestrial grass *Thelepogon elegansas*,[7] and nakamurol A, isolated from the sponge *A. nakamurai*, collected at Okinawa, Japan,[8] are the only known thelepogane-type diterpenes, This is the second isolation of thelepogan-type diterpene from a sponge.

Compounds **1**−**3** showed no inhibitory activity against HIV-1 integrase at a concentration of 10 μg/mL and no antimicrobial activity against the Gram-negative bacterium *Escherichia coli* and *Pseudomonas seruginosa* at up to 12.5 μg/mL. However, compounds **1** and agelasine B (**4**) exhibited antimicrobial activity against Gram-positive bacteria and fungi (Table 1).

Experimental Section

General Experimental Procedures. Melting points were uncorrected. UV and IR spectra were recorded on a UV-210 and a MASCO FT/IR 5300. NMR spectra were recorded with a 400 MHz JEOL NMR instruments using TMS as internal standard and $CDCl_3$ or CD_3OD as solvents. MS spectra were obtained with a JEOL JMS DX-300 or a JEOL JMS D-300.

Animal Material. The sponge *A. nakamurai* Hoshino (collection no. 170) was collected by using scuba at −15 m at Fly Islands, Papua New Guinea, and was frozen immediately after collection. The sponge forms an irregular thick-walled flabellate mass with an irregular surface. The skeleton is a fine-meshed reticulation of spongin fibers of which the primary ones are cored with one to four spicules in cross section, and the connecting fibers are uncored. All fibers are lightly echinated. The spicules are the usual verticillated acanthostyles 180−285 × 12−20 μm with 17−26 whorls of spines. The specimen was compared to the type of specimen described by Hoshino from the Ryukyus, and they conformed in all aspects. A voucher specimen has been deposited at Faculty of Science, Kagoshima University, and also in the Zoological Museum of Amsterdam, reg. no. ZMA POR. 11477.

Extraction and Isolation. The organism (wet weight 4.7 kg) was chopped into small pieces and extracted with MeOH (30 L). The MeOH extract was suspended in H_2O and extracted with CH_2Cl_2. The aqueous solution was further extracted with *n*-BuOH. A portion (6.1 g) of the *n*-BuOH extract (99.1 g) was absorbed on silica gel and subjected to chromatography on silica gel (40 g) packed in hexane, and fractions (100 mL) were collected as follows: 1−2 (MeOH−CH_2Cl_2, 1:19), 3−5 (MeOH−CH_2Cl_2, 1:9), 6−7 (MeOH−CH_2Cl_2, 1:4), 8−10 (MeOH−CH_2Cl_2, 8:3), 11−14 (MeOH−CH_2Cl_2, 13:7), 15−16 (MeOH), and 17−18 (MeOH). Fractions 1−3 (776 mg) were chromatographed on silica gel using MeOH and CH_2Cl_2, increasing the proportion of MeOH to elute the fractions from the column. The fractions eluted with MeOH−CH_2Cl_2 (1:99) gave a residue (505 mg), which was again chromatographed on silica gel using MeOH−CH_2Cl_2 (1:49−1:24) and applied to HPLC (ODS) with MeOH−H_2O (2:3) to give **1** (21.8 mg) and **2** (2.7 mg) and agelasine B (64.9 mg) with MeOH−H_2O (1:4). Fractions 4−11 (2.8 g) was subjected to HPLC (ODS) with with MeOH−H_2O (1:4) to afford **3** (48.9 mg).

Compound 1: needles; mp 137−139 °C (lit.[6] mp 136−138 °C); UV (MeOH) λ_{max} (log ε) 213 (3.47), 268 (3.93) nm; IR (KBr) ν_{max} 3241, 3177, 1662, 1632, 1589, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.63 (2H, br s, $CONH_2$), 6.20 (1H, d, $J = 3.8$ Hz, H-4), 6.53 (1H, $J = 3.8$ Hz, H-3), 9.76 (1H, br s, NH); ^{13}C NMR ($CDCl_3$) δ 104.4 (s, C-5), 111.9 (d, C-3), 112.2 (d, C-4), 126.2 (s, C-2), 161.6 (s, $CONH_2$); LREIMS *m/z* 190, 188, 173, 171, 146, 144; HREIMS *m/z* 187.9577 (M^+, calcd for $C_5H_5N_2O^{79}Br$, 187.9584).

Compound 2: amorphous white solid; UV (MeOH) λ_{max} (log ε) 214 (3.60), 272 (4.05) nm; IR (KBr) ν_{max} 3308, 3194, 1653, 1559, 1528, 1414 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.40 (3H, s, OMe), 4.88 (2H, d, $J = 7.0$ Hz, H-8), 6.20 (1H, d, $J = 3.9$ Hz, H-4), 6.55 (1H, br d, $J = 3.9$ Hz, H-3), 6.55 (1H, overlapped, H-7), 9.92 (1H, br s, H-1), ^{13}C NMR ($CDCl_3$) δ 56.1 (s, OMe), 71.4 (t, H-8), 104.5 (s, C-5), 111.3 (d, C-3), 112.2 (d, C-4), 126.5 (s, C-2), 160.5 (s, CONH); LREIMS *m/z* 234, 232, 202, 200, 174, 172, 146, 144; HREIMS *m/z* 231.9814 (M^+, calcd for $C_7H_9N_2O_2^{79}$-Br, 231.8946).

Compound 3: amorphous white solid; $[\alpha]_D$ +12.9° (*c* 0.18, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.24), 272 (ε 8950) nm; R (KBr) ν_{max} 3061, 1669, 1614, 1587 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.60 (3H, s, H-20), 0.72 (3H, s, H-19), 0.77 (3H, d, $J = 7.0$ Hz, H-18), 1.87 (3H, br s, H-16), 2.11 (1H, dt, $J = 4.2$, 13.6 Hz, H-7), 2.53 (1H, br d, $J = 10.3$ Hz, H-9), 4.09 (3H, s, $N_{9'}$-Me), 4.41 and 4.82 (1H each, br s, H-17), 5.44 (1H, br t, $J = 6.8$ Hz, H-14), 5.71 (2H, br d, $J = 6.8$ Hz, H-15), 6.80 (2H, br s, NH_2), 8.49 (1H, s, H-2′), 10.9 (1H, br s, H-8′); ^{13}C NMR ($CDCl_3$) δ 16.3 (q, C-18 or C-19), 16.4 (q, C-19 or C-18), 17.5 (q, C-16), 18.4 (q, C-20), 21.4 (t, C-2), 22.4 (t, C-11), 30.5 (d, C-4), 31.1 (t, C-3), 31.9 (t, C-1), 32.0 ($N_{9'}$-Me), 33.0 (t, C-7), 33.9 (t, C-6), 38.8 (t, C-12), 39.5 (s, C-5), 42.3 (s, C-10), 42.5 (d, C-9), 48.6 (t, C-15), 106.4 (t, C-17), 109.9 (s, C-5′), 116.0 (d, C-14), 142.1 (d, C-8′), 147.5 (s, C-13), 149.3 (s, C-8), 149.6 (s, C-4′), 152.4 (s, C-6′), 156.2 (d, C-2′); ^{1}H NMR (CD_3OD) δ 0.66 (3H, s, H-20), 0.78 (3H, s, H-19), 0.80 (3H, d, $J = 7.0$ Hz, H-18), 1.28−1.46 (6H, overlapped, H-1, H-2 × 2, H-3 × 3, H-6), 1.54−1.66 (4H, overlapped, H-1, H-6, H-11 × 2), 1.90 (3H, br s, H-16), 2.04−2.12 (2H, overlapped, H-7, H-12), 2.18 (1H, dt, $J = 4.4$, 13.6 Hz, H-7), ca. 2.30 (1H, m, H-4), 2.36−2.43 (1H, m, H-12), 2.67 (1H, br d, $J = 9.9$ Hz, H-9), 4.90 (3H, $N_{9'}$-Me), 4.53 and 4.88 (1H each, br s, H-17), 5.24 (1H, d, $J = 6.7$ Hz, H-15), 5.57 (1H, br t, $J = 6.7$ Hz), 8.46 (1H, s, H′-2); ^{13}C NMR (CD_3OD) δ 16.7 (q, C-4), 16.9 (q, C-5), 17.2 (q, C-13), 18.9 (q, C-10), 22.5 (t, C-2), 23.4 (t, C-11), 31.8 (d, C-4), 32.1 (q, $N_{9'}$-Me), 32.3 (t, C-3), 33.0 (t, C-1), 34.1 (t, C-7), 35.1 (t, C-6), 39.8 (t, C-12), 40.7 (s, C-5), 43.3 (s, C-10), 43.6 (d, C-9), 48.7 (t, C-15), 107.1 (t, C-17), 111.1 (s, C-5′), 115.8 (d, C-14), 144.2 (d, C-8′), 149.7 (s, C-13), 150.7 (s, C-8), 150.9 (s, C-4′), 154.2 (s, C-6′), 157.2 (d, C-2′); LREIMS *m/z* 421 (M^+).

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