*Tetrahedron* Vol. 47, No. 33, pp. 6617-6622, 1991
Printed in Great Britain

0040-4020/91 $3.00+.00


# PUREALIDINS B AND C, NEW BROMOTYROSINE ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *PSAMMAPLYSILLA PUREA*

Jun'ichi Kobayashi*, Masashi Tsuda, Kaori Agemi, Hideyuki Shigemori, Masami Ishibashi, Takuma Sasaki[a], and Yuzuru Mikami[b]

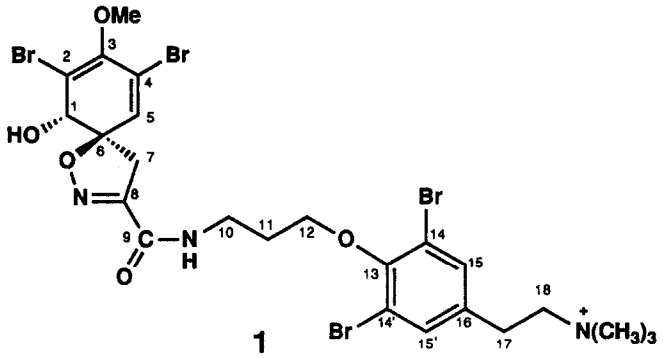
Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan, [a]Cancer Research Institute, Kanazawa University, Kanazawa 920, Japan, and [b]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 280, Japan

(*Received in Japan* 25 *March* 1991)

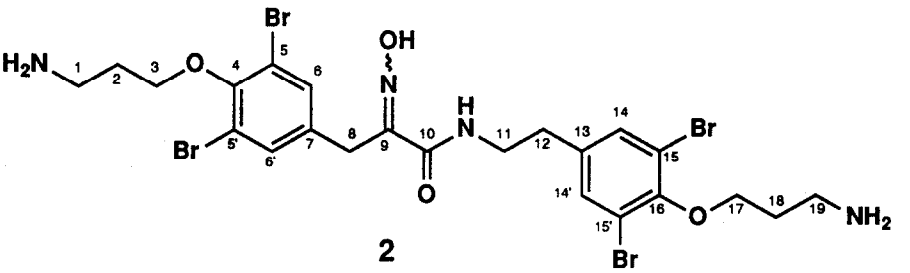
**Abstract**: New bromotyrosine-derived alkaloids, purealidins B (**1**) and C (**2**), have been isolated from the Okinawan marine sponge *Psammaplysilla purea* and their structures elucidated on the basis of spectroscopic data. Purealidin B (**1**) showed antibacterial activity, while purealidin C (**2**) exhibited antifungal and antineoplastic activities.

Recently several bromotyrosine-derived alkaloids have been isolated from marine sponges, especially from the family Verongidae,[1] and we have also isolated purealin[2], lipopurealins A ~ C[3], and purealidin A[4] from the Okinawan marine sponge *Psammaplysilla purea.* Purealin and lipopurealins were shown to exhibit unique bioactivities such as activation of myosin K,EDTA-ATPase or inhibition of Na,K-ATPase. These alkaloids, especially purealin, proved to be useful tools for studying regulatory mechanisms of these enzymes.[5] During our survey of bioactive substances from Okinawan marine organisms,[6] we further examined extracts of the sponge *P. purea* to obtain other bromotyrosine-derived compounds exhibiting interesting bioactivities. In this paper we describe the isolation and structural elucidation of new bromotyrosine-derived alkaloids, named purealidins B (**1**) and C (**2**), possessing antimicrobial and/or antineoplastic activity.

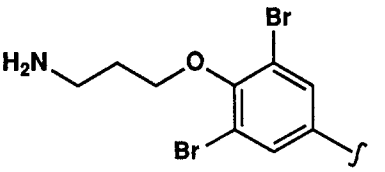
The sponge *P. purea* was collected off Kerama Islands, Okinawa, by SCUBA and kept frozen until used. The methanol extract was partitioned between ethyl acetate and water and the aqueous layer was subsequently extracted with *n*-butanol. The ethyl acetate soluble fraction was subjected to silica gel column chromatography with $CHCl_3$/*n*-BuOH/AcOH/$H_2O$ (1.5:6:1:1) to afford purealidin B (**1**, 0.02%, wet weight) together with known aplysamine-2[7] (0.013%). The *n*-butanol soluble fraction was also separated by a silica gel column with $CHCl_3$/*n*-BuOH/AcOH/$H_2O$ (1.5:6:1:1) followed by $C_{18}$ medium pressure liquid chromatography with $CH_3CN/H_2O/CF_3CO_2H$ (25:75:0.2 to 35:65:0.2) and $C_{18}$ HPLC ($CH_3CN/H_2O/CF_3CO_2H$, 35:65:0.2) to give purealidin C (**2**, 0.005%) along with known purealidin A (0.024%).[4]




1




2




A

Purealidin B (**1**) showed $M^+$ ions in the ratio of about 1:4:6:4:1 at *m/z* 756, 758, 760, 762, and 764 in the FABMS spectrum, indicating the presence of four bromine atoms. The molecular formula of **1**, $C_{24}H_{30}N_3O_5Br_4$ (as a cation), was established by HRFABMS (*m/z* 759.8868, $M^+$, $\Delta$ +1.5 mmu). The presence of amide carbonyl was indicated by IR absorption at 1690 $cm^{-1}$. The UV spectrum of **1** was closely similar to that of purealin[2] that possesses a spiroisoxazole ring. The $^1H$ NMR spectrum of **1** in DMSO-$d_6$ showed $D_2O$-exchangeable protons at $\delta$ 8.57 (1H, t) and 6.36 (1H, br s), which were attributable to amide and hydroxy protons. Signals for primary amino protons, usually found in the purealin-related compounds, were not observed, which was consistent with the fact that **1**

was ninhydrin-negative. The $^1$H-$^1$H COSY spectrum of **1** revealed the proton connectivities from the amide proton (δ 8.57, 1H, t, deuterium-exchangeable) to three methylene unit [$H_2$-10 (δ 3.38, 2H, m), $H_2$-11 (δ 1.99, 2H, m), and $H_2$-12 (δ 3.95, 2H, t)]. In the COSY spectrum the methylene protons bearing nitrogen (δ 3.50, 2H, m; $H_2$-18) were coupled to benzyl methylene protons (δ 3.05, 2H, m; $H_2$-17). The COSY spectrum also revealed the presence of isolated methylene protons (δ 3.62 and 3.20, each 1H d; $H_2$-7). The $^{13}$C NMR data including DEPT experiment (Table 1) of **1** showed two methyl signals, six $sp^3$ methylene signals, one of which bore oxygen atom, one $sp^3$ methine, one $sp^3$ quaternary carbon, two $sp^2$ methine signals, six $sp^2$ quaternary carbon signals other than an amide carbonyl. Considering the intensities of the $^{13}$C signals, the two $sp^2$ carbons at δ 117.5 (s) and 133.4 (d) were ascribed to two carbons on a symmetrical benzene ring, respectively. The protonated carbons were all assigned by heteronuclear multiple quantum coherence (HMQC)[8] experiment. The intense $^{13}$C signal at δ 52.3 was assigned to trimethyl ammonium group. The $^{13}$C chemical shifts of trimethyl ammonium group (δ 52.3) and the methylene (δ 64.2; C-18) bearing ammonium nitrogen of **1** were similar to those of choline (δ 54.8 and 68.3).[9] Nine protons were estimated by the integration value for the *N*-methyl signal at δ 3.09 (s) in the $^1$H NMR of **1**, thus elucidating the presence of an $NMe_3^+$ group connected on C-18. Further evidence for the structure of purealidin B (**1**) was provided by

Table 1. $^1$H and $^{13}$C NMR Data of Purealidins B (**1**) and C (**2**)[a]

| Position | Purealidin B (**1**) $^{13}$C | $^1$H | *J* (Hz) | Position | Purealidin C (**2**) $^{13}$C | $^1$H | *J* (Hz) |
|---|---|---|---|---|---|---|---|
| 1 | 73.5 d | 3.91 s | | 1 | 36.5 t | 3.04 m | |
| 2 | 120.8 s | | | 2 | 27.7 t | 2.07 m | |
| 3 | 147.1 s | | | 3 | 70.4 t | 3.97 t | 6.0 |
| 4 | 113.0 s | | | 4 | 150.5 s | | |
| 5 | 131.2 d | 6.58 s | | 5, 5' | 117.2 s | | |
| 6 | 90.2 s | | | 6, 6' | 132.9 d | 7.48 s | |
| 7 | 39.1 t | 3.20 d | 18.1 | 7 | 136.4 s | | |
| | | 3.62 d | 18.1 | 8 | 27.7 t | 3.74 s | |
| 8 | 154.4 s | | | 9 | 150.8 s | | |
| 9 | 158.9 s | | | 10 | 163.0 s | | |
| 10 | 36.1 t | 3.38 m | | 11 | 49.7 t | 3.34 m | |
| 11 | 29.3 t | 1.99 m | | 12 | 33.3 t | 2.27 t | 6.6 |
| 12 | 71.3 t | 3.95 t | 6.3 | 13 | 139.1 s | | |
| 13 | 151.3 s | | | 14,14' | 132.9 d | 7.45 s | |
| 14,14' | 117.5 s | | | 15,15' | 117.1 s | | |
| 15,15' | 132.9 d | 7.68 s | | 16 | 150.4 s | | |
| 16 | 135.8 s | | | 17 | 70.3 t | 3.97 t | 6.0 |
| 17 | 26.9 t | 3.05 m | | 18 | 27.7 t | 2.07 m | |
| 18 | 64.2 t | 3.50 m | | 19 | 36.5 t | 3.04 m | |
| 1-OH | | 6.36 m | | 9-NOH | | 12.05 s | |
| 3-OMe | 59.5 q | 3.64 s | | 10-NH | | 8.12 t | 5.8 |
| 9-NH | | 8.57 t | 5.8 | 1,19-$NH_2$ | | 7.86 br s | |
| 18-$NMe_3$ | 52.3 q | 3.09 s | | | | | |

a)Spectra recorded on a JEOL JMN GX-270 spectrometer in DMSO-$d_6$ as TFA salts.

comparison of spectral data with those of purealin, previously isolated from the same sponge.[2] The $^{13}C$ chemical shifts of C-1 ~ C-16 moiety of **1** including the spiroisoxazole ring corresponded well to those of purealin. The absolute configuration of **1** was deduced to be the same as that of purealin on the basis of CD data ($[\theta]_{252}$ -8100 and $[\theta]_{290}$ -8000). Thus the structure of purealidin B was established to be **1**.

Purealidin C (**2**), which was optically inactive, showed quasi-molecular ion peaks at *m/z* 741, 743, 745, 747, and 749 (1:4:6:4:1), indicating the presence of four bromine atoms. The HRFABMS data revealed the molecular formula to be $C_{23}H_{28}N_4O_4Br_4$ [*m/z* 744.8868, $(M+H)^+$, Δ -1.3 mmu]. The IR absorptions at 3400 and 1690 $cm^{-1}$ implied the presence of NH/OH and amide carbonyl group, respectively. The $^1H$ NMR spectrum showed three deuterium-exchangeable signals at δ 12.1 (1H, s), 8.12 (1H, t), and 7.86 (4H, br.s), which were assignable to an oxime, an amide, and two primary amine protons, respectively. The $^{13}C$ NMR spectrum of **2** showed signals due to six $sp^3$ methylene, one $sp^2$ methine, and seven $sp^2$ quaternary carbons other than an amide carbonyl. The presence of partial structure **A** was suggested by comparison of the $^{13}C$ chemical shifts of **2** with those of purealidin A[4] as well as the $^1H$-$^1H$ COSY cross-peaks ($NH_2/H_2$-1, $H_2$-1/$H_2$-2, and $H_2$-2/$H_2$-3).[10] Two sets of carbon signals due to segment **A** were observed in the $^{13}C$ NMR spectrum of **2**, indicating that **2** contains two sets of segment **A**. The structure of the rest of the molecule (C-8 ~ C-12) other than two of segment **A** was revealed by the $^1H$-$^{13}C$ long-range correlations obtained in the $^1H$-detected heteronuclear multiple-bond correlation (HMBC) experiment.[11] The methylene protons at δ 3.74 ($H_2$-8) showed the cross-peaks to C-6, C-6', C-7, C-9, and C-10. The $^{13}C$ chemical shifts of C-8, C-9, and C-10 were corresponding well to the -$CH_2$-C(=N-OH)-C(=O)- part of purealin[2] or purealidin A.[4] The methylene protons at δ 3.34 ($H_2$-11), which were coupled to an amide proton (δ 8.12) and methylene protons (δ 2.73; $H_2$-12) in the $^1H$-$^1H$ COSY spectrum, were correlated to C-10 (δ 163.0) and C-13 (δ 139.1) in the HMBC spectrum. Thus the structure of purealidin C was concluded to be **2**.

Purealidin C (**2**) showed cytotoxicity against human epidermoid carcinoma KB cells and murine lymphoma L1210 cells with $IC_{50}$ values of 3.2 and 2.4 μg/mL, respectively, while purealidin B (**1**) exhibited almost no cytotoxicity. Purealidin C (**2**) also showed modest antifungal (*Candida albicans, Cryptococcus neoformans,* and *Paecilomyces variotii*) and antibacterial (*Staphylococcus aureus, Sarcina lutea,* and *Bacillus subtilis*) activities, while purealidin B (**1**) was active only against *S. aureus* and *S. lutea* among them.[12]

## EXPERIMENTAL

***General methods.*** Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV, IR and CD spectra were taken on a Shimadzu UV-220 spectrometer, a JASCO A-120 spectrometer, and a JASCO J-500A spectropolarimeter, respectively. $^1H$ and $^{13}C$ NMR spectra were recorded on a JEOL JMN GX-270 and an EX-400 spectrometers in DMSO-$d_6$. The resonances of residual DMSO at $\delta_H$ 2.49 and $\delta_C$ 40.0 were used as internal

references for $^{1}H$ and $^{13}C$ NMR spectra, respectively. FAB mass spectra were obtained on a JOEL HX-110 spectrometer by using glycerol as a matrix.

***Isolation***. The brown-colored sponge *Psammaplysilla purea* was collected off Kerama Islands, Okinawa, and kept frozen until used. The sponge (1 kg, wet weight) was extracted with methanol (1.3 L x 2). After evaporation under reduced pressure, the residue (44.1 g) was partitioned between ethyl acetate (400 mL x 3) and 1M NaCl aqueous solution and the aqueous layer was subsequently extracted with *n*-butanol (400 mL x 3). The ethyl acetate and *n*-butanol soluble fractions were evaporated under reduced pressure to give crude residues (2.68 and 5.78 g, respectively). A portion (0.91 g) of the ethyl acetate soluble fraction was subjected to a silica gel column (Wako gel C-300, Wako Pure Chemical, 28 x 425 mm) with chloroform/*n*-butanol/acetic acid/water (1.5:6:1:1) to give aplysamine-2[7] (0.013% yield, wet weight, 320 ~ 400 mL) and purealidin B (**1**, 0.02%, 520-800 mL). A part (1.01 g) of *n*-butanol soluble portion was chromatographed on a silica gel column (Wako gel C-300, 28 x 420 mm) with chloroform/*n*-butanol/acetic acid/water (1.5:6:1:1). A fraction (360 mL ~ 460 mL) was rechromatographed on a reversed-phase column (Develosil Lop ODS 24S, Nomura Chemical, 30 x 300 mm) with acetonitrile/water (25:75 to 35:65) containing 0.2% trifluoroacetic acid, followed by HPLC separation (Develosil ODS-5, Nomura Chemical, 10 x 250 mm; flow rate, 2.5 mL/min; UV detection at 254 nm) with acetonitrile/water/trifluoroacetic acid (35:65:0.2) to afford purealidin C (**2**, 0.0001%, Rt 10.4 min).

***Purealidin B*** (**1**). a colorless amorphous solid; $[\alpha]_D^{18}$ -4.5° (*c* 1.3, MeOH); UV (MeOH) $\lambda_{max}$ 220 (ε 10000) and 284 nm (1000); IR (KBr) $\nu_{max}$ 3450, 2980, 2880, 1690, 1470, 1400, 1220, and 1150 $cm^{-1}$; CD (MeOH) $[\theta]_{252}$ -8100 and $[\theta]_{290}$ -8000; $^{1}H$ and $^{13}C$ NMR (Table 1); FABMS *m/z* 764, 762, 760, 758, 756 ($M^+$), 748, 746, 744, 742, 740, 684, 682, 680, 678, 602, 600, and 658; HRFABMS *m/z* 759.8892 ($M^+$, calcd for $C_{24}H_{30}O_5N_3{}^{79}Br_2{}^{81}Br_2$, 759.8877).

***Purealidin C*** (**2**). a colorless amorphous solid; UV (MeOH) $\lambda_{max}$ 210 (ε 23000) and 285 nm (1600); IR (KBr) $\nu_{max}$ 3400, 3100, 1680, 1400, 1200, and 1130 $cm^{-1}$; $^{1}H$ and $^{13}C$ NMR (Table 1); FABMS *m/z* 771, 769, 767, 765, 763 $(M+Na)^+$, 749, 747, 745, 743, 741 $(M+H)^+$, 669, 667, 665, and 663; HRFABMS *m/z* 744.8868 (M+H, calcd for $C_{23}H_{29}O_4N_4{}^{79}Br_2{}^{81}Br_2$, 744.8881).

**Acknowledgement:** This study was partly supported by Cooperative Research Program ('90-01) of Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan.

## REFERENCES AND NOTES

1. (a) Arabshahi, L.; Schmitz, F. J, *J. Org. Chem.* **1987**, *52*, 3584. (b) Rodoriguez, A. D.; Akee, R. K.; Scheuer, P. J., *Tetrahedron Lett.* **1987**, *28*, 4989. (c) Gunasekera, M.; Gunasekera, S. P., *J. Nat. Prod.* **1989**, *52*, 753. (d) Morris, S. A.; Anderson, R. J., *Can. J. Chem.* **1989**, *67*, 667. (e) Kernan, M. R.; Cambie, R. C.; Bergquist, P. R., *J.*

*Nat. Prod.* **1990**, *53*, 615. (f) Kernan, M. R.; Cambie, R. C.; Bergquist, P. R. *J. Nat. Prod.* **1990**, *53*, 720.

2. Nakamura, H.; Wu, H.; Kobayashi, J.; Nakamura, Y.; Ohizumi, Y.; Hirata, Y. *Tetrahedron Lett.* **1985**, *26*, 4517.
3. Wu, H; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Hirata, Y. *Experientia* **1986**, *42*, 855.
4. Ishibashi, M.; Tsuda, M.; Ohizumi, Y.; Sasaki, T.; Kobayashi, J. *Experientia* **1991**, *47*, 299.
5. (a) Takito, J.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y.; Ebisawa, K.; Nonomura, Y. *J. Biol. Chem.* **1986**, *261*, 13861. (b) Nakamura, Y.; Kobayashi, M.; Nakamura, H.; Wu, H.; Kobayashi, J.; Ohizumi, Y. *Eur. J. Biochem.* **1987**, *167*, 1. (c) Takito, J.; Ohizumi, Y.; Nakamura, H.; Kobayashi, J.; Ebisawa, K.; Nonomura, Y., *Eur. J. Pharmacol.* **1987**, *142*, 189. (d) Takito, J.; Nakamura, H.; Kobayashi, J.; Ohizumi, Y. *Biochem. Biophys. Acta.* **1987**, *912*, 404.
6. (a) Kobayashi, J.; Tsuda, M.; Murayama, T.; Nakamura, H.; Ohizumi, Y.; Ishibashi, M.; Iwamura, M.; Ohta, T.; Nozoe, S. *Tetrahedron* **1990**, *46*, 5579. (b) Kobayashi, J.; Cheng, J.-F.; Kikuchi, Y.; Ishibashi, M.; Yamamura, S.; Ohizumi, Y.; Ohta, T.; Nozoe, S. *Tetrahedron Lett.* **1990**, *31*, 4617. (c) Kobayashi, J.; Murayama, T.; Ishibashi, M.; Kosuge, S.; Takamatsu, M.; Ohizumi, Y.; Kobayashi, H.; Ohta, T.; Nozoe, S.; Sasaki, T. *Tetrahedron* **1990**, *46*, 7699. (d) Kobayashi, J.; Murayama, T.; Kosuge, S.; Kanda, F.; Ishibashi, M.; Kobayashi, H.; Ohizumi, Y.; Ohta, T.; Nozoe, S.; Sasaki, T. *J. Chem. Soc., Perkin Trans. 1* **1990**, 3301. (e) Kikuchi, Y.; Ishibashi, M.; Sasaki, T.; Kobayashi, J. *Tetrahedron Lett.* **1991**, *32*, 797. (f) Kobayashi, J.; Cheng, J.-F.; Yamamura, S.; Ishibashi, M. *Tetrahedron Lett.* **1991**, *32*, 1227. (g) Kobayashi, J.; Kanda, F.; Ishibashi, M.; Shigemori, H. *J. Org. Chem.* in press.
7. Xynas, R.; Capon, R. J. *Aust. J. Chem.* **1990**, *42*, 1427.
8. Bax, A.; Subramanian, S. *J. Mag. Reson.* **1986**, *67*, 565.
9. Kalinowski, H.-O.; Berger, S.; Braun, S. In *Carbon-13 NMR Spectroscopy* ; John Wiley & Sons: Chichester, 1988; p. 226.
10. In the $^1$H NMR spectrum of **2**, the signals for $H_2$-1, $H_2$-2, and $H_2$-3 were overlapped with $H_2$-19, $H_2$-18, and $H_2$-17, respectively.
11. Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108* , 2093.
12. Minimum inhibitory concentrations (MIC, μg/mL) of **1** and **2** were followed: *C. albicans*, 31.3 for **1**; *C. neoformans*, 15.6 for **1**; *P. variotii*, 31.3 for **1**; *S. aureus*, 62.5 for **1** and **2**; *S. lutea*, 3.9 and 15.6 for **1** and **2**, respectively; *B. subtilis*, 15.6 for **1**.