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PUREALIDINS E–G, NEW BROMOTYROSINE ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *PSAMMAPLYSILLA PUREA*

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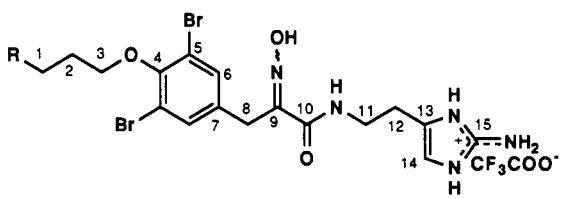
ABSTRACT.—Three new bromotyrosine alkaloids, purealidins E [**1**], F [**2**], and G [**3**], have been isolated from the Okinawan marine sponge *Psammaplysilla purea* and the structures elucidated on the basis of spectroscopic data.

A number of bromotyrosine-derived alkaloids have been isolated from marine sponges, many of which belong to the family Verongidae (1). We have isolated several bioactive bromotyrosine alkaloids such as purealin (2), lipopurealins A–C (3), and purealidins A–D (4–6) from the Okinawan marine sponge *Psammaplysilla purea* Carter. Purealin in particular proved to be a useful tool for studying regulatory mechanisms of myosin K^+,EDTA-ATPase or Na^+,K^+-ATPase (7). During our continuing investigations on bioactive substances from Okinawan marine organisms (8–11), extracts of *P. purea* were further examined to obtain three other biogenetically related compounds, purealidins E [**1**], F [**2**], and G [**3**]. In this paper we describe the isolation and structural elucidation of **1–3**.

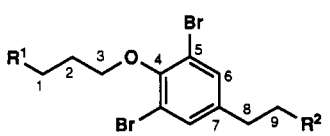
The sponge *P. purea* was collected off Kerama Islands, Okinawa, and kept frozen until used. The *n*-BuOH-soluble fraction of the MeOH extract was subjected to a Si gel column using $CHCl_3$–*n*-BuOH–HOAc–H_2O (1.5:6:1:1) and then *n*-BuOH–HOAc–H_2O (2:1:1). The fraction eluted by *n*-BuOH–HOAc–H_2O (2:1:1) was purified by Sephadex LH-20 and reversed-phase hplc with MeCN-H_2O-TFA (30:70:0.3) to afford purealidin E [**1**] (0.002%, wet wt) and a crude mixture of purealidins F and G. The mixture was rechromatographed on reversed-phase hplc with MeOH-H_2O-TFA (30:70:0.1) to give purealidins F [**2**] (0.002%) and G [**3**] (0.002%).

Purealidin E [**1**] was optically inactive and showed $[M]^+$ ions at *m/z* 559, 561, and 563 in the ratio of 1:2:1. The molecular formula, $C_{20}H_{29}N_6O_3Br_2$, of **1** was confirmed by hrfabms. The ^{1}H-nmr spectrum of **1** was similar to that of purealidin A [**4**] (4), except for the chemical shift of H_2-1 [δ 3.61 (t, 2H)] and appearance of *N*-methyl protons at δ 3.12 (s, 9H). The ^{13}C-nmr chemical shifts (Table 1) of C-1 (δ 63.0) and *N*-methyl group (δ 52.3) were similar to those of purealidin B (5) (δ 64.2 and 52.3) and those of choline (δ 68.3 and 54.8) (12), suggesting that a trimethyl ammonium group is present at C-1. Thus the structure of purealidin E [**1**]



1 R=$NMe_3^+CF_3OO^-$

4 R=$NH_3^+CF_3COO^-$



2 R^1=$NH_2Me^+Cl^-$ R^2=$NHMe_2^+Cl^-$

3 R^1=$NHMe_2^+Cl^-$ R^2=$NH_2Me^+Cl^-$

5 R^1=$NHMe_2^+Cl^-$ R^2=$NHMe_2^+Cl^-$

TABLE 1. ^{13}C-nmr Data of Purealidins E [1] and A [4] in DMSO-d_6.

Carbon	Compound 1 δ	1 m[b]	4[a] δ	4[a] m[b]
C-1	63.0	t	36.5	t
C-2	23.4	t	27.9	t
C-3	70.0	t	70.4	t
C-4	150.3	s	150.5	s
C-5	117.1	s	117.1	s
C-6	132.9	d	132.9	d
C-7	136.6	s	136.4	s
C-8	27.9	t	27.7	t
C-9	150.8	s	150.9	s
C-10	163.1	s	163.1	s
C-11	37.3	t	37.3	t
C-12	24.4	t	24.4	t
C-13	124.3	s	124.3	s
C-14	109.2	d	109.2	d
C-15	147.0	s	146.9	s
NMe_3	42.3	q	—	

[a]Data for this compound are from Ishibashi *et al.* (4).

[b]Multiplicity in DEPT.

was assigned as the 1-trimethylammonium form of purealidin A [4].

Purealidins F [2] and G [3] showed the same $[M]^+$ ions at *m/z* 392, 394, and 396 in the ratio of ca. 1:2:1 in the eims. The molecular formula, $C_{14}H_{22}N_2OBr_2$, of **2** and **3** was confirmed by hreims. The ^{1}H-nmr spectra of **2** and **3** as HCl salts, being similar to each other, corresponded well to that of aplysamine 1 [5] (13). The differences were found in the terminal *N*-methyl groups: aplysamine 1 [5] possessed two *N,N*-dimethyl terminal groups [δ 2.91 (6H, s) and 2.96 (6H, s)], while the ^{1}H-nmr spectra of **2** and **3** showed one *N*-methyl [δ 2.77 (3H, s) and 2.72 (3H, s), respectively] and one *N,N*-dimethyl signal [δ 2.93 (6H, s) and 2.96 (6H, s), respectively]. The ^{1}H-nmr signals due to H_2-1 (δ 3.34) for **2** and H_2-9 (δ 2.94) for **3** resonated at a higher field than those of aplysamine 1 [5] [δ 3.50 (H_2-1) and 3.22 (H_2-9)], indicating that **2** possessed an *N*-methyl group in place of an *N,N*-dimethyl group at C-1 of aplysamine 1 [5], and that **3** possessed an *N*-methyl group at C-9 in place of the *N,N*-dimethyl group of aplysamine 1 [5]. We concluded that in Xynas and Capon (13) the ^{1}H-nmr signals due to H_2-1 and H_2-9 for aplysamine 1 [5] were inversely assigned, on the basis of detailed comparison of the ^{1}H-nmr data of related bromotyrosine derivatives such as aplysamine 2 (δ 3.46, H_2-20) (13) and anomoian A (δ 3.20, H_2-16) (14).

EXPERIMENTAL

GENERAL METHODS.—Uv and ir spectra were measured on Shimadzu uv-220 and JASCO ir Report-100 spectrometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL GX-270 and EX-400 spectrometers. Fab and ei mass spectra were obtained on JEOL HX-110 and DX-303 spectrometers, respectively.

ISOLATION.—The brown-colored sponge *P. purea* was collected by scuba off Kerama Islands, Okinawa, and kept frozen until used. A voucher specimen was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University. The sponge (1 kg, wet wt) was extracted with MeOH (1.3 liters × 2). After evaporation under reduced pressure, the residue (44 g) was partitioned between EtOAc (400 ml × 3) and 1M NaCl aqueous solution, and the aqueous solution was subsequently extracted with *n*-BuOH (400 ml × 3). The *n*-BuOH-soluble fraction (5.8 g) was subjected to a Si gel column (Wako C-300, Wako Pure Chemical, 50 × 430 mm) with $CHCl_3$–*n*-BuOH–HOAc–H_2O (1.5:6:1:1) (2.6 liters) and continuously eluted with *n*-BuOH–HOAc–H_2O (2:1:1) (1.5 liters). The fraction (1.3 g) eluted with *n*-BuOH/HOAc/H_2O was rechromatographed using gel filtration on a Sephadex LH-20 column (Pharmacia Fine Chemicals, 20 × 1000 mm) followed by separation by hplc [YMC Pack AM-323 ODS, Yamamura Chemical, 10 × 250 mm; eluent MeCN-H_2O-TFA (30:70:0.3); flow rate 2.5 ml/min; uv detection at 254 nm] to afford purealidins D (6) (0.0025%, Rt 7.8 min) and E [1] (0.002%, Rt 7.2 min) and a mixture of purealidins F [2] and G [3]. The mixture was subjected to reversed-phase hplc [YMC Pack AM-323 ODS, 10 × 250 mm; eluent MeOH-H_2O-TFA (30:70:0.1); flow rate 2.5 ml/min; uv detection at 254 nm] to give purealidins F [2] (0.002%, Rt 10.8 min) and G [3] (0.002%, Rt 11.3 min).

Purealidin E [1].—Colorless amorphous solid: uv (MeOH) λ max 287 (ε 1000), 218 nm (13600); ir (KBr) ν max 3400, 1680, 1350, 1200

cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.24 (br s, 1H, NH-14),m 12.07 (s, 1H, NOH-9), 11.86 (br s, 1H, NH-13), 8.17 (t, 1H, $J=5.9$ Hz, NH-10), 7.47 (s, 2H, H-6), 7.46 (s, 2H, NH_2-15), 6.58 (br s, 1H, H-14), 4.00 (t, $J=5.4$ Hz, 2H, H_2-3), 3.76 (s, 2H, H_2-8), 3.61 (t, $J=7.3$ Hz, 2H, H_2-1), 3.37 (dt, $J=5.9$, 6.8 Hz, 2H, H_2-11), 3.12 (s, 9H, NMe_3), 2.61 (t, $J=6.8$ Hz, H_2-12), 2.24 (tt, $J=5.4$ and 7.3 Hz, H_2-2); ^{13}C nmr see Table 1; fabms *m/z* $[M]^+$ 563, 561, 559 (1:2:1), 481, 479 (1:1); hrfabms found *m/z* 559.0682, calcd for $C_{20}H_{29}N_6O_3Br_2$ $[M]^+$ 559.0668.

Purealidin F [**2**].—Colorless oil: uv (MeOH, HCl salt) λ max 285 (ε 1000), 274 (800), 218 nm (12000); ir (KBr, HCl salt) ν max 3420, 2950, 1635, 1455, 1260, 1035 cm^{-1}; ^1H nmr (CD_3OD, HCl salt) δ 7.62 (s, 2H, H_2-6), 4.13 (t, 2H, $J=5.5$ Hz, H_2-3), 3.34 (t, 2H, $J=8.4$ Hz, H_2-1), 3.25 (t, 2H, $J=8.1$ Hz, H_2-9), 3.01 (t, 2H, $J=8.1$ Hz, H_2-8), 2.93 (s, 6H, NMe_2), 2.77 (s, 3H, NMe), 2.24 (tt, 2H, $J=5.5$, 8.4 Hz, H_2-2); eims *m/z* $[M]^+$ 396, 394, 392 (1:2:1), 352, 349, 347 (1:2:1), 324, 322, 320 (1:2:1), 95, 89, 69, 53, 51, 44; hreims found *m/z* 392.0081, calcd for $C_{14}H_{22}N_2OBr_2$ $[M]^+$ 392.0098.

Purealidin G [**3**].—Colorless oil; uv (MeOH, HCl salt) λ max 288 (ε 1100), 276 (800), 214 nm (14000); ir (KBr, HCl salt) ν max 3420, 2950, 1635, 1455, 1260, 1035 cm^{-1}; ^1H nmr (CD_3OD, HCl salt) δ 7.59 (s, 2H, H_2-6), 4.12 (t, 2H, $J=5.5$ Hz, H_2-3), 3.52 (t, 2H, $J=8.4$ Hz, H_2-1), 3.23 (t, 2H, $J=8.1$ Hz, H_2-8), 2.96 (s, 6H, NMe_2), 2.94 (t, 2H, $J=8.1$ Hz, H_2-9), 2.72 (s, 3H, NMe), 2.30 (tt, 2H, $J=5.5$, 8.4 Hz, H_2-2); eims *m/z* $[M]^+$ 396, 394, 392 (1:2:1), 353, 351, 349 (1:2:1), 315, 313 (1:1), 87, 69, 58, 44; hreims found *m/z* 394.0053, calcd for $C_{14}H_{22}N_2OBr_2$ $[M]^+$ 394.0079.

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LITERATURE CITED

1. J. Kobayashi and M. Ishibashi, in: "The Alkaloids." Ed. by A. Brossi and G.A. Cordell, Academic Press, San Diego, 1992, Vol. 41, p. 41.
2. H. Nakamura, H. Wu, J. Kobayashi, Y. Nakamura, Y. Ohizumi, and Y. Hirata, *Tetrahedron Lett.*, **26**, 4517 (1985).
3. H. Wu, H. Nakamura, J. Kobayashi, Y. Ohizumi, and Y. Hirata, *Experientia*, **42**, 855 (1986).
4. M. Ishibashi, M. Tsuda, Y. Ohizumi, T. Sasaki, and J. Kobayashi, *Experientia*, **47**, 299 (1991).
5. J. Kobayashi, M. Tsuda, K. Agemi, H. Shigemori, M. Ishibashi, T. Sasaki, and Y. Mikami, *Tetrahedron*, **33**, 6617 (1991).
6. M. Tsuda, H. Shigemori, M. Ishibashi, and J. Kobayashi, *Tetrahedron Lett.*, **33**, 2597 (1992).
7. J. Takito, H. Nakamura, J. Kobayashi, Y. Ohizumi, K. Ebisawa, and Y. Nonomura, *J. Biol. Chem.*, **261**, 13861 (1986).
8. J. Kobayashi, F. Itagaki, H. Shigemori, M. Ishibashi, K. Takahashi, M. Ogura, S. Nagasawa, T. Nakamura, H. Hirota, T. Ohta, and S. Nozoe, *J. Am. Chem. Soc.*, **113**, 7812 (1991).
9. J. Kobayashi, M. Tsuda, M. Ishibashi, H. Shigemori, T. Yamasu, H. Hirota, and T. Sasaki, *J. Antibiot.*, **44**, 1259 (1991).
10. J. Kobayashi, M.Tsuda, A. Tanabe, M. Ishibashi, J.-F. Cheng, S. Yamamura, and T. Sasaki, *J. Nat. Prod.*, **54**, 1634 (1991).
11. M. Tsuda, M. Ishibashi, K. Agemi, T. Sasaki, and J. Kobayashi, *Tetrahedron*, **47**, 2181 (1991).
12. H.-O. Kalinowski, S. Berger, and S. Braun, "Carbon-13 NMR Spectroscopy," John Wiley & Sons, Chichester, 1988, p. 226.
13. R. Xynas and R.J. Capon, *Aust. J. Chem.*, **42**, 1427 (1989).
14. M.R. Kernan, R.C. Cambie, and P.R. Bergquist, *J. Nat. Prod.*, **53**, 720 (1990).

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