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LIPOPUREALINS D AND E AND PUREALIDIN H, NEW BROMOTYROSINE ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *PSAMMAPLYSILLA PUREA*

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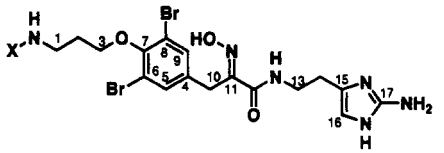
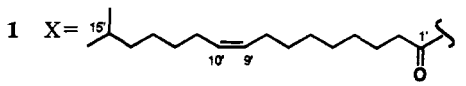
ABSTRACT.—Three new bromotyrosine alkaloids, lipopurealins D and E, and purealidin H, have been isolated from the Okinawan marine sponge *Psammaplysilla purea* and the structures elucidated on the basis of 2D nmr and fabms/ms data.

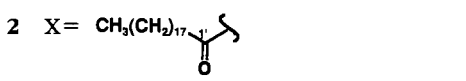
Marine sponges belonging to the order Verongidae have proven to be a rich source of bromotyrosine alkaloids (1), among which lipopurealins A–C, isolated from the Okinawan marine sponge *Psammaplysilla purea* Carter (Aplysinellidae) (2) are unique metabolites with a long acyl chain. In our search for bioactive substances from Okinawan marine organisms (3–5), extracts of this sponge were further examined to obtain two new lipopurealin congeners, lipopurealins D [**1**] and E [**2**], and a new bromotyrosine derivative, purealidin H [**3**]. We describe herein the isolation and structure elucidation of **1–3**.

The EtOAc-soluble fraction of the MeOH extract of this sponge collected off the Kerama Islands, Okinawa, Japan, was subjected to Si gel and Sephadex LH-20 cc and C_{18} hplc to afford lipopurealins D [**1**] (0.0004%, wet wt) and E [**2**] (0.0007%), and purealidin H [**3**] (0.0004%), together with the known related compounds, lipopurealin B (2) and purealin (6).

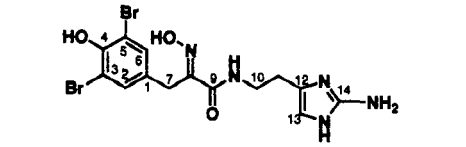




1 X=



2 X= $CH_3(CH_2)_{17}$



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Hrfabms data of compounds **1** and **2** established the molecular formulas $C_{34}H_{52}N_6O_4Br_2$ (*m/z* 767.2540, M^++H, Δ +4.5 mmu) and $C_{36}H_{58}N_6O_4Br_2$ (*m/z* 797.2956, M^++H, Δ −0.8 mmu), respectively. Ir absorptions at 3400 and 1675 cm^{-1} were attributed to NH/OH and amide carbonyl groups, respectively. The uv spectra of **1** and **2** showed the characteristic absorption (λ max 284 nm) of substituted aromatic ring(s). The ^{1}H- and ^{13}C-nmr features of **1** and **2** suggested that these compounds contain a common bromotyrosine unit (C-1–C-17) with an aminohistamine moiety, corresponding to $C_{17}H_{21}N_6O_3Br_2$, and a long acyl chain, $C_{17}H_{31}O$ and $C_{19}H_{37}O$, respectively. The ^{13}C-nmr chemical shifts of C-11 (δ_C 152.9) suggested that C-11 was assignable to the carbon of an α-ketoxime (7). The *E*- geometry of the oxime at C-11 of **1** and **2** was deduced from the chemical shift of C-10 (δ_C 28.9) (7).

The ^{1}H-nmr spectrum of **1** revealed signals due to a doublet methyl (δ_H 0.86, 6H), a multiplet methine (δ_H 1.51), and a disubstituted olefin (δ_H 5.33, 2H), im-

plying the presence of an unsaturated long acyl chain with a branched terminus. The position of the double bond was revealed from daughter ion peaks observed in the collisionally activated dissociation (cad) (8) mass spectrum for a fragment ion (*m/z* 308), corresponding to **A** (Figure 1). The intense fragment ions characteristic for two allyl positions at *m/z* 168 and 222 and a homoallyl position at *m/z* 236 indicated that the double bond was located at C-9′ (9). *Z*-Geometry of the olefin was deduced from the chemical shift (δ_C 27.9) of the allylic carbons (C-8′ and C-11) (10). Thus, the structure of lipopurealin D was elucidated as **1**.

The ^{1}H-nmr spectrum of lipopurealin E [**2**], containing signals due to a triplet methyl [δ_H 0.88, 3H] and a long acyl chain (30H, δ_H 1.33–1.26), indicated the presence of a *n*-nonadecanoyl group at C-1. Thus, the structure of lipopurealin E was assigned as **2**.

The molecular formula, $C_{14}H_{15}N_5O_3Br_2$, of purealidin H [**3**] was established by hrfabms (*m/z* 459.9631, M^++H, Δ +1.1 mmu). The ^{1}H- and ^{13}C-nmr spectra of **3** revealed all the resonances corresponding to C-4 through C-17 of lipopurealins D [**1**] and E [**2**] except for a phenolic hydroxy proton signal (δ_H 9.77). The structure of purealidin H was thus determined as **3**.

Lipopurealins D [**1**] and E [**2**] isolated from the sponge *Psammaplysilla purea* are new lipopurealin congeners with a long acyl chain. Similar bromotyrosine alkaloids with a long acyl chain have been reported in marine sponges as follows: lipopurealins A–C (2) from *P. purea*, araplysillin 2 (11) from *P. arabica*, psammaplysin D (12) from *Aplysinella* sp., and aplysamine 5 (13) from *P. purpurea*. Lipopurealins D [**1**] and E [**2**] and purealidin H [**3**] showed no cytotoxicity (>10 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Uv and ir spectra were taken on Jasco Ubest-35 and Jasco ir Report-100 spectrometers, respectively. ^{1}H-

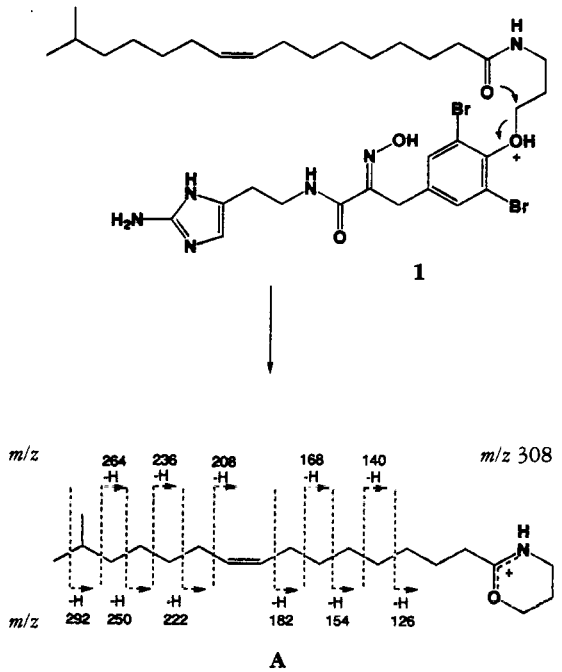

FIGURE 1. Fabms/ms fragmentations of *m/z* 308 from lipopurealin D [**1**].

and ^{13}C-nmr spectra were conducted with JEOL EX-400 and GSX-270 spectrometers. Fabms and fabms/ms spectra were recorded employing JEOL HX-110 and VG70-4SE spectrometers, respectively.

ANIMAL MATERIAL.—The dark-brown sponge, *Psammaplysilla purea* Carter, was collected off the Kerama Islands, Okinawa, Japan, and kept frozen until used. A voucher specimen is deposited at the Mukaishima Marine Biological Station.

EXTRACTION AND ISOLATION.—The sponge (1.0 kg, wet wt) was extracted with MeOH (1.3 liters×2). After evaporation under reduced pressure, the residue (44.1 g) was partitioned between EtOAc (400 ml×3) and 1 M aqueous NaCl. A portion (1.07 g) of the EtOAc-soluble material was subjected to Si gel cc [$CHCl_3$-*n*-BuOH-HOAc-H_2O (1.5:6:1:1)] and then passage over a Sephadex LH-20 column [$CHCl_3$-MeOH (1:1)] to give purealin (0.018%, wet wt), crude purealidin H, and a mixture of lipopurealins. The mixture of lipopurealins was purified by C_{18} hplc [YMC Pack AM-323 ODS, 10×250 mm; flow rate, 2.0 ml/min; uv detection at 230 nm] with MeOH-H_2O (9:1) containing 0.2 M aqueous NaCl to afford lipopurealins D [**1**] (0.0004%, wet wt, R_t 31 min), E [**2**] (0.0007%, R_t 76 min), and B (0.0014%, R_t 28.5 min). Purealidin H [**3**] (0.0004%, R_t 6.5 min) was obtained by C_{18} hplc [YMC Pack AM-323 ODS, 10×250 mm; eluent CH_3CN-H_2O-TFA (35:65:0.1); flow rate, 2.0 ml/min; uv detection at 230 nm].

Lipopurealin D [**1**].—Colorless amorphous solid: uv (MeOH) λ max 284 (ε 1100), 274 nm (1400); ir (KBr) ν max 3400, 2910, 1675, 1625, 1535, 1450, 1250, 1120 cm^{-1}; ^{1}H nmr (CD_3OD, 400 MHz) δ 7.46 (2H, s, H-5 and H-9), 6.41 (1H, s, H-16), 5.33 (2H, t, J=5.4 Hz, H-9′ and H-10′), 4.00 (2H, t, J=6.1 Hz, H_2-3), 3.82 (2H, s, H_2-10), 3.45 (2H, t, J=6.8 Hz, H_2-13), 3.43 (2H, t, J=7.3 Hz, H_2-1), 2.66 (2H, t, J=6.8 Hz, H_2-14), 2.18 (2H, t, J=7.3 Hz, H_2-2′), 2.03 (6H, m, H_2-2), H_2-8′, and H_2-11′), 1.60 (2H, m, H_2-3′), 1.51 (1H, m, J=6.8 Hz, H-15′), 1.35–1.27 (14H, m, CH_2), 0.86 (6H, d, J=6.8 Hz, H_3-16′, and H_3-17′); ^{13}C nmr (CD_3OD, 100 MHz) δ 177.2 (s, C-1′), 166.3 (s, C-12), 153.7 (s, C-7), 152.9 (s, C-11), 148.3 (s, C-17), 138.2 (s, C-4), 135.3 (2C, d, C-5 and C-9), 131.7 (d, C-9′), 131.6 (d, C-10′), 126.2 (s, C-15), 119.6 (2C, s, C-6 and C-8), 112.0 (d, C-16), 73.1 (t, C-3), 40.2 (t, C-13), 38.5 (t, C-1), 38.0 (t, C-2′), 31.9–31.1 (8C, t), 31.1 (t, C-2), 30.0 (d, C-15′), 28.9 (t, C-10), 27.9 (t, 2C, C-8′ and C-11′), 27.4 (t, C-3′), 24.5 (t, C-14), 23.8 (2C, q, C-16′ and C-17′); fabms *m/z* $[M+H]^+$ 767, 769, 771 (1:2:1); hrfabms found *m/z* 767.2540 calcd for $C_{34}H_{53}N_6O_4Br_2$ $[M+H]^+$ 767.2495.

Lipopuralin E [**2**].—Colorless amorphous solid: uv (MeOH) λ max 284 (ε 710), 274 nm (1000); ir (KBr) ν max 3400, 2840, 1675, 1620, 1535, 1450, 1200, 1130 cm^{-1}; ^{1}H nmr (CD_3OD, 400 MHz) δ 7.46 (2H, s, H-5 and H-9), 6.46 (1H, s, H-16), 4.00 (2H, t, J=6.1 Hz, H_2-3), 3.82 (2H, s, H_2-10), 3.45 (2H, t, J=6.8 Hz, H_2-13), 3.43 (2H, t, J=6.8 Hz, H_2-1), 2.67 (2H, t, J=6.8 Hz, H_2-14), 2.18 (2H, t, J=7.6 Hz, H_2-2′), 2.03 (2H, tt, J=6.1 and 6.8 Hz, H_2-2), 1.60 (2H, m, H_2-3′), 1.33–1.26 (30H, m, CH_2), 0.89 (3H, t, J=6.8 Hz, H_3-19′); ^{13}C nmr (CD_3OD, 100 MHz) δ 177.2 (s, C-1′), 166.4 (s, C-12), 153.6 (s, C-7), 152.9 (s, C-11), 148.5 (s, C-17), 138.1 (s, C-4), 135.3 (2C, d, C-5 and C-9), 126.4 (s, C-15), 119.6 (2C, s, C-6 and C-8), 111.7 (d, C-16), 73.1 (t, C-3), 39.9 (t, C-13), 39.0 (t, C-1), 38.0 (t, C-2′), 34.7–31.2 (14C, t), 31.1 (t, C-2), 29.6 (t, C-17′), 28.9 (t, C-10), 27.9 (t, C-18′), 24.5 (t, C-14), 15.2 (q, C-19′); fabms $[M+H]^+$ *m/z* 797, 799, 801 (1:2:1); hrfabms found *m/z* 797.2956, calcd for $C_{36}H_{59}N_6O_4Br_2$ $[M+H]^+$ 797.2964.

Purealidin H [**3**].—Colorless oil; uv (MeOH) λ max 284 (ε 1400), 277 nm (1700); ir (KBr) ν max 3400, 2920, 2845, 1680, 1520, 1470, 1200, 1135, 1120 cm^{-1}; ^{1}H nmr (DMSO-d_6, 400 MHz) δ 11.98 (1H, br s, 13-NH), 11.93 (1H, s, 8-NOH), 11.56 (1H, br s, 12-NH), 9.77 (1H, br s, 4-OH), 8.13 (1H, t, J=5.9 Hz, NH-9), 7.33 (2H, s, 14-NH_2), 7.32 (2H, s, H-2 and H-6), 6.57 (1H, s, H-13), 3.69 (2H, s, H_2-7), 3.36 (2H, dt, J=5.9 and 6.8 Hz, H_2-10), 2.59 (2H, t, J=6.8 Hz, H_2-11); ^{13}C nmr (DMSO-d_6, 100 MHz) δ 163.4 (s, C-9), 151.5 (s, C-8), 149.2 (s, C-4), 147.0 (s, C-14), 132.5 (2C, d, C-2 and C-6), 131.4 (s, C-1), 124.5 (s, C-12), 111.9 (2C, s, C-3 and C-5), 109.4 (d, C-13), 37.5 (t, C-10), 27.7 (t, C-7), 24.6 (t, C-11); fabms $[M+H]^+$ *m/z* 460, 462, 464 (1:2:1); hrfabms found *m/z* 459.9631, calcd for $C_{14}H_{15}N_6O_3Br_2$ $[M+H]^+$ 459.9620.

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