employ of secretion has been claimed for the carabid *Pterostichus lucublandus*[19].

Little is known about the chemical basis of sexual behavior in carabid beetles. The possibility that the defensive secretion plays a role in the courtship of some species is worth investigating. With respect to *O. americanus*, it would clearly have been desirable to have had a larger number of individuals for analysis, to determine whether factors such as age or reproductive status also affect secretory composition.

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Purealidin A, a new cytotoxic bromotyrosine-derived alkaloid from the Okinawan marine sponge *Psammaplysilla purea*

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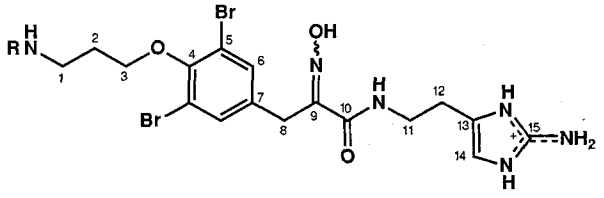
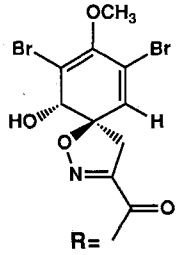
Summary. A new bromotyrosine-derived alkaloid with antileukemic activity, purealidin A (**5**), has been isolated from the Okinawan marine sponge *Psammaplysilla purea* and its chemical structure elucidated on the basis of the spectroscopic data.
Key words. Antileukemic activity; purealidin A; bromotyrosine-derived alkaloid; sponge; *Psammaplysilla purea.*

Recently, several bromotyrosine-derived alkaloids have been isolated from marine sponges[1-7], especially from the family Verongidae, and we have also reported isolation of purealin (**1**)[8] and lipopurealins A ~ C (**2** ~ **4**)[9] from the Okinawan marine sponge *Psammaplysilla purea*. Purealin (**1**) was shown to activate myosin K,EDTA-ATPase, while purealin (**1**) and lipopurealins (**2** ~ **4**) exhibited inhibitory activity against Na,K-ATPase. Purealin and its related alkaloids, therefore, have been shown to have properties which are at present unique, which could make them valuable as a biochemical tool for regulating enzyme activity[10-13]. During our studies on bioactive substances from Okinawan marine organisms[14-17], we further investigated the sponge *P. purea* to see whether we could obtain other purealin-related compounds which might show similar unusual biological activity. In this paper we describe the isolation and structure elucidation of a new purealin-related alkaloid, named purealidin A (**5**), which exhibits potent antileukemic activity.

The sponge *P. purea* was collected at Minna Island, 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 *n*-butanol-soluble material was subjected to silica gel flash column chromatography; it was eluted with $CHCl_3$/*n*-BuOH/AcOH/H_2O (1.5:6:1:1) followed by a Sephadex LH-20 column with $CHCl_3$/MeOH (1:1). The fraction containing purealin-related compounds (detectable by ninhydrin test on TLC) was further purified by reversed-phase HPLC [ODS, CH_3CN/H_2O (25:75) with 0.1% trifluoroacetic acid] to furnish purealidin A (**5**, 0.0003% yield, wet weight).

Purealidin A (**5**)[18] showed intense M + H ions in the ratio of about 1:2:1 at *m/z* 517, 519, and 521 in the positive FABMS spectrum, which indicated the presence





1 R=

2 R= $\overset{O}{\overset{\|}{C}}(CH_2)_{12}CH_3$

3 R= $\overset{O}{\overset{\|}{C}}(CH_2)_{11}\overset{CH_3}{\overset{|}{C}}HCH_3$

4 R= $\overset{O}{\overset{\|}{C}}(CH_2)_{14}CH_3$

5 R= H

of two bromine atoms in the molecule. The UV spectrum of **5** showed analogous absorptions (λ_{max} 277 nm) to those of lipopurealins (**2** ~ **4**)[9], implying the presence of a common chromophore. The molecular formula of purealidin A (**5**) was determined to be $C_{17}H_{22}N_5O_3Br_2$ by high resolution FABMS (*m/z* 517.0241, M + H, Δ + 4.3 nm). This composition corresponds to the amine part that is commonly present in purealin (**1**) and lipopurealins (**2** ~ **4**), in which acyl groups are connected to the amino group on C-1 to construct the amide bond. In the ^{1}H NMR of **5** in DMSO-d_6 six signals disappeared on addition of D_2O and they were ascribed to NH's or OH's. The two-dimensional ^{1}H-^{1}H COSY spectrum of **5** revealed the proton connectivities from NH_2 on C-1 (δ 7.83, 2H br s, exchangeable) to three methylene unit [H_2-1 (δ 3.06, 2H m), H_2-2 (δ 2.02, 2H m), and H_2-3 (δ 3.98, 2H t)]. The COSY spectrum also showed that the aromatic protons [H-6 and 6′ (δ 7.45, 2H s)] were coupled to benzyl protons (H_2-8: δ 3.75, 2H s) and the NH-10 (δ 8.15, 1H t, exchangeable) was connected to two methylene units [H_2-11 (δ 3.36, 2H dt) and H_2-12 (δ 2.59, 2H t)].

Both of the two exchangeable broad singlets (NH-14 and NH-13) resonating in the fairly low field (δ 12.16 and 11.77) were coupled to H-14 (δ 6.57), suggesting the presence of imidazole ring. The ^{13}C NMR of **5** exhibited six sp^3 methylenes, among which one bore oxygen atom (δ 70.40) and two bore nitrogens (δ 37.28 and 36.46), two sp^2 methines, and seven sp^2 quarternary carbons. The two signals at δ 117.14 (s) and 132.88 (d) were quite tall and were attributed to two carbons, due to the symmetrical benzene ring. These ^{1}H and ^{13}C NMR data described above were fully consistent with the structure of each amine part of **1** ~ **4**. The detailed interpretation of these NMR data enabled complete assignment of the ^{1}H and ^{13}C NMR signals for **5**, which was supported by comparison with the NMR data for purealin (**1**)[8] and lipopurealins (**2** ~ **4**)[9], thus establishing the structure of purealidin A to be **5**.

Purealidin A (**5**) showed weak inhibitory activity (22% inhibition at 10^{-4} M) on Na,K-ATPase, but had no effect on myosin K,EDTA-ATPase. These results suggest that the acyl part of purealin (**1**), lacking in **5**, is important for activation of those ATPases. Purealidin A (**5**) exhibited some cytotoxicity against L1210 murine leukemia cells in vitro with the IC_{50} value of 1.1 μg/ml.

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18 **5:** Colorless amorphous solids; UV (MeOH) λ_{max} 277 nm (ε 520); IR (film) 3400, 1680, 1540, 1435, 1200, and 1140 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 12.16 (1H, br s; NH-14), 12.03 (1H, s; NOH-9), 11.77 (1H, br s; NH-13), 8.15 (1H, t, J = 6 Hz; NH-10), 7.83 (2H, br s; NH_2-1), 7.45 (2H, s; H-6,6′), 6.57 (1H, s; H-14), 3.98 (2H, t, J = 6 Hz; H_2-3), 3.75 (2H, s; H_2-8), 3.36 (2H, dt, J = 6 Hz; H_2-11), 3.06 (2H, m; H_2-1), 2.59 (2H, t, J = 6 Hz; H_2-12), and 2.02 (2H, m; H_2-2); ^{1}H-^{1}H COSY correlations (H/H): NH_2-1/H_2-1, H_2-1/H_2-2, H_2-2/H_2-3, H-6,6′/H_2-8, NH-10/H_2-11, H_2-11/H_2-12, H-14/NH-13, and H-14/NH-14; ^{13}C NMR (DMSO-d_6) δ 163.10 s (C-10), 150.86 s (C-9), 150.46 s (C-4), 146.93 s (C-15), 136.44 s (C-7), 132.88 d (C-6,6′), 124.30 s (C-13), 117.14 s (C-5,5′), 109.22 d (C-14), 70.40 t (C-3), 37.28 t (C-11), 36.46 t (C-1), 27.90 t (C-2), 27.69 t (C-8), and 24.37 t (C-12); FABMS (positive ion, glycerol matrix) *m/z* 559, 557, 555 [ca. 1:2:1, $(M + K)^+$], 521, 519, 517 [ca. 1:2:1, $(M + H)^+$], 505, 503, 501 [ca. 1:2:1, $(M+H-NH_2)^+$], 441, and 439 [ca. 1:1, $(M-Br)^+$]; HRFABMS (positive) found *m/z* 517.0241, calcd for $C_{17}H_{23}O_3N_6Br_2$ (M + H) 517.0198.

0014-4754/91/030299-02$1.50 + 0.20/0