Pergamon

Tetrahedron Vol. 51, No. 13, pp. 3745-3748, 1995

0040-4020/95 $9.50+0.00

0040-4020(95)00121-2

Aplysillamides A and B, New Antimicrobial Guanidine Alkaloids from the Okinawan Marine Sponge *Psammaplysilla purea*

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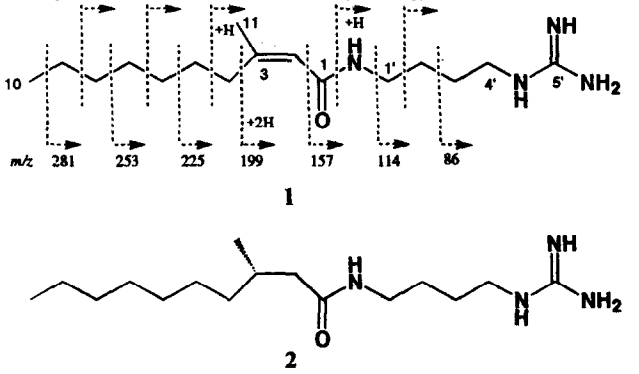
Abstract: Two new guanidine alkaloids, aplysillamides A (**1**) and B (**2**), with antimicrobial activity have been isolated from the Okinawan marine sponge *Psammaplysilla purea* and the structures elucidated on the basis of spectroscopic data. The absolute stereochemistry at C-3 of **2** was established as *S* by synthesis of **2**.

A series of bioactive bromotyrosine-derived alkaloids has been isolated from the Okinawan marine sponge *Psammaplysilla purea*[1-4]. Further investigation of extracts of this sponge led to isolation of two new guanidine alkaloids, aplysillamides A (**1**) and B (**2**) with anitifungal and antibacterial activities. This paper describes the isolation, structure elucidation, and antimicrobial activity of **1** and **2**. The absolute configuration at C-3 of **2** was established by synthesis of **2**.

The EtOAc-soluble fraction of methanolic extract of this sponge, collected off Ishigaki Island, Okinawa, was subjected to a silica gel column ($CHCl_3$/*n*-BuOH/AcOH/H_2O, 1.5:6:1:1) followed by a C_{18} column (MeCN/H_2O/CF_3CO_2H, 35:65:0.1) and reversed-phase HPLC (MeCN/H_2O/CF_3CO_2H, 42:58:0.1) to afford aplysillamides A (**1**, 0.002 %, wet weight) and B (**2**, 0.002 %).

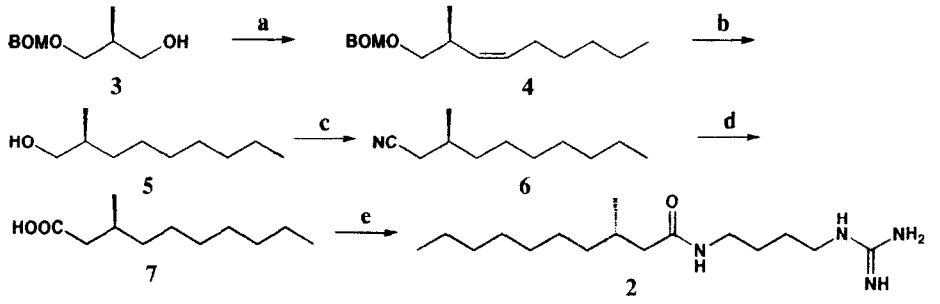
HRFABMS data of aplysillamide A (**1**) provided the molecular formula, $C_{16}H_{32}N_4O$ [*m/z* 297.2670, $(M+H)^+$, Δ +1.6 mmu]. The presence of a guanidine moiety was elucidated by positive coloration to the Sakaguchi test as well as a quaternary carbon signal (δ_C 156.7) in the ^{13}C NMR spectrum. The UV absorption {225 nm (ε 10000)}, IR band (1680 cm^{-1}), and carbon resonance at δ_C 165.7 were attributed to an α,β-unsaturated amide carbonyl group. The ^1H NMR spectrum of **1** showed proton signals due to two



1

2

Fig. 1. Structures of Aplysillamides A (1) and B (2) and EIMS Fragmentations of 1



Scheme 1. Synthesis of Aplysillamide B (2)

(a) 1) DMSO, $(COCl)_2$, CH_2Cl_2, -78 °C, 30 min, then Et_3N; 2) $Ph_3PC_6H_{13}Br$, *n*-BuLi, THF, rt, 12 h; (b) Raney-Ni, H_2, EtOH, rt, 66 h; (c) 1) TsCl, Et_3N, DMAP, CH_2Cl_2, rt, 2 h; 2) NaCN, DMSO, 70 °C, 1 h; (d) NaOH, H_2O_2, EtOH, reflux, 22 h; (e) 1) HOSu, DCC, Dioxane, 4 °C, 20 h; 2) Agmatine Sulfate, THF-H_2O (1:1), rt, 44 h

NH (δ 7.75 and 7.57), an olefin (δ 5.60), a methyl (δ 1.75, 3H) on a double bond, a doublet methyl (δ 0.85, 3H), and ten methylenes [δ 3.07 (2H), 3.06 (2H), 2.58 (2H), 1.5 ~ 1.4 (6H), and 1.3 ~ 1.15 (8H)]. ^1H-^1H COSY cross-peaks, HMBC correlations, and EIMS fragmentations (Fig. 1) revealed the presence of a 4-(aminobutyl)guanidine (agmatine) moiety and a 3-methyl-2-decenoyl group. The HMBC correlation for H_2-1'/C-1 indicated that the agmatine moiety was attached at C-1 through an amide bond. *E*-Geometry of the double bond was ascertained by the NOESY cross-peak for H-2/H_3-11. Thus the structure of aplysillamide A was assigned to be **1**.

Aplysillamide B (**2**) was optically active {$[\alpha]_D^{21}$ -2.4° (*c* 0.1, MeOH)}. HRFABMS data of **2** established the molecular formula, $C_{16}H_{34}N_4O$ [*m/z* 299.2799, $(M+H)^+$, Δ -1.2 mmu]. The ^1H and ^{13}C NMR data implied that **2** was 2,3-dihydro form of aplysillamide A (**1**). In order to determine the absolute configuration at C-3 of aplysillamide B (**2**), **2** was synthesized as shown in Scheme 1. The alcohol (**3**; BOMO- = benzyloxymethoxy-) with *S*-configuration, which was prepared from methyl (2*R*)-3-hydroxy-2-methylpropionate, was applied to Swern oxidation and then Wittig reaction with hexylidene-triphenylphosphorane to afford the *E*-olefin (**4**). Reduction and deprotection of the olefin (**4**) with Raney-Ni in H_2 atmosphere gave the alcohol (**5**), which was converted into the cyanide (**6**) by tosylation followed by treatment with sodium cyanide in DMSO, and alkaline hydrolysis of **6** afforded the corresponding carboxylic acid (**7**). After esterification of **7** with *N*-hydroxysuccinimide (HOSu),[5,6] the succinimidyl ester of **7** was condensed with agmatine to afford **2**, all spectral data of which were found to be identical with those of natural **2** including optical rotaions {synthetic **2**, $[\alpha]_D^{22}$ -5.1° (*c* 1.6, MeOH)}. Thus the absolute configuration at C-3 of aplysillamide B (**2**) was concluded to be *S*.

Table 1. Antimicrobial Activities of Aplysillamides A (1) and B (2)

Compound	MIC values (μg/mL)									
	C.alb	*C.neo*	*P.var*	*A.nig*	*T.men*	*S.aur*	*S.lut*	*B.sub*	*E.col*	*Myco*
1	133	66	33	133	33	16	16	66	133	66
2	133	33	16	66	33	16	16	66	133	33

Fungi: *Candida albicans*, *Cryptococcus neoformans*, *Paecilomyces variotii*, *Aspergillus niger*, and *Trichophyton mentagrophytes*.
Bacteria: *Staphyrococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Escherichia coli*, and *Mycobacterium* sp. 607.

Aplysillamides A (**1**) and B (**2**) are new antimicrobial guanidine alkaloids with a C_{11} acyl chain from the sponge *Psammaplysilla purea*. To our knowledge, the isolation of alkaloids with an agmatine unit from marine sponges is vary rare,[7] although many guanidine alkaloids incorporating a homoagmatine unit have been reported.[8-10] Compounds **1** and **2** exhibited modest antimicrobial activity against some fungi and bacteria as shown in Table 1. The comparison of antifungal activity of **1** and **2** indicated that reduction of the double bond at C-2 resulted in a slight increase in the activity. Aplysillamide A (**1**) was cytotoxic against murine lymphoma L1210 and human epidermoid carcinoma KB cells (IC_{50} 5.5 and 5.8 μg/mL, respectively), while compound **2** showed no cytotoxicity (IC_{50} > 10 μg/mL).

EXPERIMENTAL

Collection, Extraction, and Isolation. The dark brown sponge (1.5 kg, wet weight), *Psammaplysilla purea* Carter, was collected off Ishigaki Island, Okinawa, and kept frozen until used. The sponge was extracted with MeOH (1 L x 2). After evaporation of the solvent, the residue (55.6 g) was partitioned between EtOAc (500 mL x 3) and H_2O (500 mL). The EtOAc soluble material (3.30 g) was subjected to a silica gel column with $CHCl_3$/*n*-BuOH/AcOH/H_2O (1.5:6:1:1) and a C_{18} column (Develosil LOP ODS 24S) with $CH_3CN/H_2O/CF_3CO_2H$ (35:65:0.1) and then MeOH. The fraction (36.4 mg) eluting with MeOH was subjected to C_{18} HPLC {YMC Pack AM323 ODS, 10 x 250 mm; eluent, $CH_3CN/H_2O/CF_3CO_2H$ (42:58:0.1); flow rate, 2.5 mL/min; UV detection at 254 nm} to afford aplysillamides A (**1**, 3.2 mg, 0.002 %, wet wt, t_R 24.0 min) and B (**2**, 2.8 mg, 0.002 %, t_R 22.8 min).

Aplysillamide A (1). Colorless oil; UV (MeOH) λ_{max} 225 nm (ε 10000); IR (KBr) ν_{max} 3400, 2840, 1680, 1610, 1200, and 1130 cm^{-1}; ^1H NMR (DMSO-*d6*) δ 7.75 (1H, t, *J* = 5.5 Hz, NH-1), 7.57 (1H, br.s, NH-4'), 7.5 ~ 6.7 (3H, br., NH-5' and NH_2-5'), 5.60 (1H, s, H-2), 3.07 (2H, m, H_2-1'), 3.06 (2H, m, H_2-4'), 2.58 (2H, m, H_2-4), 1.75 (3H, s, H_3-11), 1.5 ~ 1.4 (6H, m, H_2-5, H_2-2', and H_2-3'), 1.3 ~ 1.15 (8H, m, H_2-6, H_2-7, H_2-8, and H_2-9), and 0.85 (3H, t, *J* = 6.6 Hz, H_3-10); ^{13}C NMR (DMSO-*d6*) δ 165.7 (s, C-1), 156.7 (s, C-5'), 152.1 (s, C-3), 119.2 (d, C-2), 40.4 (t, C-1'), 37.5 (t, C-4'), 31.8 (t, C-4), 31.2 (t), 29.0 (t), 28.5 (t), 27.5 (t), 26.4 (t), 26.0 (t), 24.2 (q, C-11), 22.0 (t, C-9), and 13.9 (q, C-10); EIMS *m/z* 296 $(M)^+$, 281, 267, 253, 239, 225, 212, 199, 157, 130, 114, 100, 86, and 73; FABMS *m/z* 297 $(M+H)^+$; HRFABMS (glycerol) *m/z* 297.2670 [$(M+H)^+$, calcd for $C_{16}H_{33}N_4O$, 297.2654].

Aplysillamide B (2). Colorless oil; $[\alpha]_D^{21}$ -2.4° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3400, 2840, 1680, 1610, 1200, and 1130 cm^{-1}; ^1H NMR (DMSO-*d6*) δ 7.79 (1H, t, *J* = 5.5 Hz, NH-1), 7.59 (1H, br.s, NH-4'), 7.5 ~ 6.7 (3H, br, NH-5' and NH_2-5'), 3.09 (2H, m, H_2-1'), 3.04 (2H, m, H_2-4'), 2.04 (1H, m, H-2), 1.85 (1H, m, H-2), 1.82 (1H, m, H-3), 1.41 ~ 1.10 (13H, m), 0.85 (3H, d, *J* = 7.3 Hz, H_3-11), and 0.82 (3H, t, *J* = 6.6 Hz, H_3-10); ^{13}C NMR (DMSO-*d6*) δ 170.8 (s, C-1), 156.7 (s, C-5'), 43.2 (t, C-2), 40.5 (t, C-1'), 36.2 (t, C-4'), 31.2 (t), 30.0 (d, C-3), 29.2 (t), 29.04 (t), 28.99 (t), 28.5 (t), 26.4 (t), 26.0 (t), 22.0 (t, C-9), 19.4 (q, C-11), and 13.9 (q, C-10) FABMS *m/z* 299 $(M+H)^+$; HRFABMS (glycerol) *m/z* 299.2799 [$(M+H)^+$, calcd for $C_{16}H_{35}N_4O$, 299.2811].

(3*E*,2*S*)-1-Benzyloxymethoxy-2-methyl-3-nonene (4). To a solution of oxalyl chloride (0.66 mL, 7.6 mmol) in CH_2Cl_2 (13 mL) at -78 °C was slowly added DMSO (0.8 mL, 11.4 mmol) in CH_2Cl_2 (1 mL), and successively (2*S*)-3-benzyloxymethoxy-2-methylpropan-1-ol (3 800 mg, 3.8 mmol), which was prepared from methyl (2*R*)-3-hydroxy-2-methylpropionate (commercially available), in CH_2Cl_2 (5.2 mL). After stirring at -78 °C for 30 min, Et_3N (2.5 mL, 18.5 mmol) was added to the reaction mixture, and stirring was continued at -50 °C for 1 h. After addition of saturated aqueous NH_4Cl and extraction with EtOAc, the organic phase was washed with H_2O and brine and dried over $MgSO_4$. Evaporation of the solvent afforded crude aldehyde, which was subjected to the following reaction without separation. To a solution of *n*-hexyltriphenylphosphonium bromide (2.2 g, 5.15 mmol) in THF (20 mL) at 0 °C was added a hexane solution of 1.6 M *n*-butyllithium (3.6 mL, 4.8 mmol). After stirring at 0 °C for 30 min, the crude aldehyde (700 mg) in THF (2 mL) was added dropwise to the reaction mixture at 0 °C, and stirring was continued for 12 h at room temperature. After addition of saturated aqueous NH_4Cl (30 mL), the reaction mixture was extracted with ether, washed with H_2O and then brine, and dried over $MgSO_4$. After evaporation, the residue was subjected to a silica gel column (hexane/EtOAc, 100:1) to give compound **4** (739 mg, 71 %): colorless oil; $[\alpha]_D^{21}$ +28° (*c* 0.38, $CHCl_3$); IR (neat) ν_{max} 2970, 2940, 2880, 1460, 1380, 1120, and 1050 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.36 ~ 7.26 (5H, m), 5.42 (1H, dt, *J* = 11.5 and 7.2 Hz), 5.19 (1H, dd, 9.1 and 11.5 Hz), 4.76 (2H, s), 4.60 (2H, s), 3.42 (2H, d, *J* = 6.3 Hz), 2.77 (1H, m), 2.06 (2H, m), 1.43 ~ 1.22 (6H, m), 1.00 (3H, d, *J* = 7.0 Hz), and 0.89 (3H, t, *J* = 6.7 Hz); FABMS *m/z* 277 $(M+H)^+$; HRFABMS (3-nitrobenzyl alcohol) *m/z* 277.2180 (M^++H, calcd for $C_{18}H_{29}O_2$, 277.2168).

(2*S*)-2-Methylnonanol (5). To a solution of **4** (710 mg, 2.57 mmol) in EtOH (9 mL) at room temperature was added 50 % Raney-Ni W2 in EtOH (4 mL), and the mixture was stirred for 66 h under H_2 atmosphere. After filtration with celite, the solvent was evaporated to give a residue, which was purified by a

silica gel column (hexane/EtOAc, 3:1) to afford compound **5** (293.2 mg, 72 %): colorless oil; $[\alpha]_D^{21}$ -13° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3300, 2960, 2930, 2850, 1460, 1380, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.51 (1H, dd, *J* = 5.9 and 8.2 Hz), 3.41 (1H, dd, *J* = 6.4 and 8.2 Hz), 1.59 (1H, m), 1.43 ~ 1.22 (12H, m), 0.91 (3H, d, *J* = 5.7 Hz), and 0.88 (3H, t, *J* = 6.9 Hz); Anal. calcd for $C_{10}H_{22}O$: C 75.94, H 14.02; found C 75.09, H 14.02.

(3*S*)-3-Methyldecanenitrile (6). To a solution of **5** (288 mg, 1.8 mmol) in CH_2Cl_2, Et_3N (1.6 mL, 11.3 mmol), and 4-(dimethylamino)pyridine (33 mg, 0.27 mmol) at room temperature was added *p*-toluenesulfonyl chloride (688 mg, 3.6 mmol), and the mixture was stirred for 2 h. After addition of MeOH (0.5 mL), stirring was continued for 30 min at room temperature. The reaction mixture was partitioned between EtOAc and H_2O, and the organic layer was washed with brine and dried over $MgSO_4$. After evaporation, the residue was subjected to a silica gel column (hexane/EtOAc, 10:1) to give the tosylate (470 mg, 83 %). To a solution of the tosylate (462 mg, 1.49 mmol) in DMSO (10 mL) was added NaCN (219 mg, 4.46 mmol), and stirring was continued for 1 h at 70 °C. The reaction mixture was partitioned between EtOAc and H_2O, and the EtOAc layer was washed with brine, dried over $MgSO_4$, and evaporated. The crude product was subjected to a silica gel column (EtOAc/H_2O, 100:1) to give compound **6** (240 mg, 97%): colorless oil; $[\alpha]_D^{21}$ +3.3° (*c* 1.3, $CHCl_3$); IR (neat) ν_{max} 2950, 2920, 2850, 2240, 1460, 1420, and 1380 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.31 (1H, dd, *J* = 5.7 and 16.7 Hz), 2.23 (1H, dd, *J* = 9.3 and 16.7 Hz), 1.84 (1H, m), 1.42 ~ 1.25 (12H, m), 1.08 (3H, d, *J* = 6.7 Hz), and 0.89 (3H, t, *J* = 6.7 Hz); EIMS *m/z* 167 (M^+); HREIMS *m/z* 167.1646 (M^+, calcd for $C_{11}H_{21}N$, 167.1674).

(3*S*)-3-Methyldecanoic acid (7). A solution of **6** (224 mg, 1.34 mmol) in EtOH (23 mL) containing NaOH (2.9 g, 72.5 mmol) was stirred at room temperature for 30 min. To the mixture was added 30 % aqueous H_2O_2 (23 mL), and the mixture was heated at refluxing temperature for 22 h. After addition of 1 M Na_2SO_3 (23 mL) and then 2 N HCl (80 mL), the reaction mixture was extracted with CH_2Cl_2, washed with brine, dried over $MgSO_4$, and evaporated to give compound **7** (234 mg, 94 %): colorless oil; $[\alpha]_D^{21}$ -5.8° (*c* 0.58, $CHCl_3$); IR (neat) ν_{max} 3600 ~ 2400, 2970, 2940, 2860, 1710, 1470, 1420, 1300, 2130, and 950 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.35 (1H dd, *J* = 5.9 and 14.9 Hz), 2.14 (1H, dd, *J* = 8.1 and 14.9 Hz), 1.96 (1H, m), 1.32 ~ 1.23 (12H, m), 0.96 (3H, d, *J* = 6.6 Hz), and 0.88 (3H, t, *J* = 6.9 Hz); FABMS *m/z* 186 (M^+); HRFABMS (3-nitrobenzyl alcohol) *m/z* 186.1633 (M^+, calcd for $C_{11}H_{22}O_2$, 186.1620).

Synthesis of Aplysillamide B (2). To a mixture of **7** (229 mg, 1.23 mmol) and *N*-hydroxysuccinimide (177 mg, 1.53 mmol) in dioxane (4.5mL) at 0 °C was added dicyclohexylcarbodiimide (289 mg, 1.40 mmol) in dioxane (2.2 mL), and stirring was continued at 4 °C in the dark for 20 h. After evaporation, the residue was dissolved in hexane/EtOAc. Insoluble material was filtered off, and then the filtrate was evaporated to give a crude *N*-hydroxysuccinimide ester (374 mg), which was subjected to the following reaction without purification. To a solution of agmatine sulfate (296 mg, 1.30 mmol) in H_2O (28 mL), which was adjusted to pH 8 with $NaHCO_3$, was added the crude *N*-hydroxysuccinimide ester (374 mg) in THF (28 mL). The mixture was left in the dark at room temperature for 44 h. The reaction mixture was partitioned between EtOAc and 1 N HCl, and the aqueous layer was neutralized by addition of 1N $NaHCO_3$, extracted with EtOAc, and evaporated. The crude product was purified by a Sep Pak C_{18} column (MeOH/H_2O/CF_3CO_2H, 80:20:0.2) to afford **2** (53 mg, 13 %).

ACKNOWLEDGMENTS

We thank Prof. T. Sasaki, Kanazawa University, for the cytotoxicity test. This work was partly supported by a Grant-in-Aid from Ciba-Geigy Foundation (Japan) for the Promotion of Science and by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture of Japan.

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(*Received in Japan* 12 *January* 1995; *accepted* 6 *February* 1995)