Two New Tryptophan-Derived Alkaloids from the Okinawan Marine Sponge Aplysina Sp.

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TWO NEW TRYPTOPHAN-DERIVED ALKALOIDS FROM THE OKINAWAN MARINE SPONGE *APLYSINA* SP.

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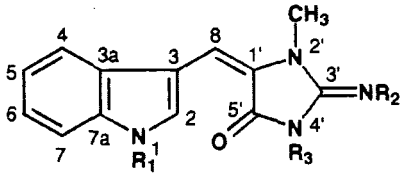
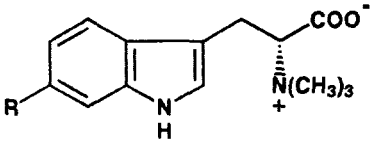
ABSTRACT.—Two new indole alkaloids, isoplysin A [**2**] and D-6-bromohypaphorine [**3**], have been isolated from the Okinawan marine sponge *Aplysina* sp. and their structures were elucidated by spectral and chemical means.

Aplysinopsin [**1**] is a tryptophan-derived marine natural product possessing cytotoxic activity, isolated from sponges of several families as well as scleractinian corals (1). It has also been reported that **1** is a substance that induces symbiosis in Anthozoa (2). During our studies on bioactive substances from Okinawan marine organisms (3), we recently isolated a new nucleoside derivative, aplysidine, from the Okinawan marine sponge *Aplysina* sp. (4). The extract of this sponge contained aplysinopsin [**1**] as a major alkaloid and further examination of the constituents of this sponge resulted in the isolation of two new tryptophan derivatives, isoplysin A [**2**] and D-6-bromohypaphorine [**3**]. In this paper we describe the isolation and structure elucidation of **2** and **3**.

The sponge *Aplysina* sp. (family Aplysinidae) was collected off the Kerama Islands, Okinawa, and kept frozen until used. The MeOH extract of the sponge was partitioned between EtOAc and H_2O, and the aqueous portion was subsequently extracted with *n*-BuOH. The *n*-BuOH-soluble material was subjected to Si gel cc [$CHCl_3$-*n*-BuOH-AcOH-H_2O (1.5:6:1:1) followed by *n*-BuOH-AcOH-H_2O (6:1:1)] and further purified on ODS (MeOH) and Sephadex LH-20 (MeOH) columns to give isoplysin A (**2**, 0.01% yield wet wt) and D-6-bromohypaphorine (**3**, 0.001%), respectively.

The hrfabms of **2** established the molecular formula, $C_{14}H_{14}N_4O$ (*m/z* 255.1239, $[M+H]^+$, Δ −2.6 mmu). The ir spectrum indicated the presence of amino (3410 cm^{-1}) and amide carbonyl (1670 cm^{-1}) groups. The ^{1}H-nmr spectrum of **2** showed proton signals due to two *N*-methyl groups at δ 3.22 (3H, s) and 3.54 (3H, s), an olefin at δ 7.28 (1H, s), and a monosubstituted indole at δ 7.22 (1H, td, *J*=6.8 and 1.5 Hz), 7.23 (1H, td, *J*=6.8 and 1.5 Hz), 7.51 (1H, dd, *J*=6.8 and 1.5 Hz), 8.05 (1H, dd, *J*=6.8 and 1.5 Hz), 8.97 (1H, d, *J*=2.9 Hz), and 12.09 (1H, br s, D_2O-exchangeable). The latter was supported by the uv absorption maxima observed at 220, 278, and 405 nm. The eims spectrum of **2** showed intense fragment ion peaks at *m/z* 155 and 169, assignable to fragments **a** and **b**, respectively (5). These spectral data resembled those of aplysinopsin [**1**]. On irradiation of H-8 at δ 7.28 a strong





1 R_1=H, R_2=H, R_3=CH_3
2 R_1=H, R_2=CH_3, R_3=H
4 R_1=CH_3, R_2=H, R_3=CH_3

3 R=Br
5 R=H

nOe (14%) was observed for 2′-*N*-methyl at δ 3.54, which suggested the 8*E* configuration. From these observations it was implied that **2** was an isomer of aplysinopsin [**1**] having a methyl group at the 3′-N position instead of the 4′-N position. This structure was confirmed by the following chemical evidence. Treatment of compound **2** with KOH in DMSO at room temperature for 10 min converted it into a rearranged product through ring-opening and closure; the spectral data of this product were completely identical with those of aplysinopsin [**1**]. Heating of **2** in a 1N NaOH solution at 80° for 2 h gave indole-3-carbaldehyde. Furthermore, methylation both of **2** and **1** by treatment with CH_3I and KOH in DMSO afforded compound **4** in quantitative yield.

The fabms of **3** showed pseudomolecular ion peaks $(M+H)^+$ at *m/z* 325 and 327 in a ratio of ca. 1:1, implying that **3** contains one bromine atom. The molecular formula of **3** was established as $C_{14}H_{17}BrN_2O_2$ by hrfabms (*m/z* 325.0571, $(M+H)^+$ for $C_{14}H_{18}{}^{79}BrN_2O_2$, Δ +1.9 mmu). The ^{1}H-nmr spectrum of **3** showed signals due to a disubstituted indole at δ 7.17 (1H, dd, J=8.7 and 2.2 Hz), 7.25 (1H, d, J=2.5 Hz), 7.53 (1H, d, J=8.2 Hz), 7.56 (1H, d, J=1.7 Hz), and 11.20 (1H, br s, D_2O-exchangeable). In the ^{1}H-nmr spectrum of the 5-bromoindole derivative [**6**], H-4 resonated at a lower field (by ca. 0.5 ppm) than H-7, while in the 6-bromoindole derivatives (7–10) H-4 and H-7 have almost the same chemical shifts. For **3**, H-4 and H-7 appeared at δ 7.53 and 7.56, respectively, indicating that **3** is a 6-bromoindole derivative. A methyl signal resonating at δ_H 3.30 and δ_C 51.7 in **3** was assigned as a trimethylammonium group. The debromo derivative [**5**] of **3** was prepared by hydrogenolysis of **3** with Raney-Ni/H_2 in EtOH. The ^{1}H-nmr, fabms, and $[\alpha]_D$ data of **5** were consistent with those of D-hypaphorine obtained by methylation of D-tryptophan with CH_3I and $KHCO_3$ in MeOH (11).[1] Compound **3** was thus determined to be D-6-bromohypaphorine. This is the first isolation of D-6-bromohypaphorine from a natural source, although L-6-bromohypaphorine has been isolated from the sponge *Pachymatisma johnstoni* (12).

Isoplysin A [**2**] was found to be weakly cytotoxic against murine lymphoma L-1210 (IC_{50} 11.5 μg/ml) and human epidermoid carcinoma KB (31% inhibition at 20 μg/ml) cells, while D-6-bromohypaphorine [**3**] showed no significant effects. Aplysinopsin [**1**] and methylaplysinopsin [**4**] had almost comparative cytotoxicity (IC_{50} values against L-1210 and KB cells, respectively: **1**, 2.3 and 6.4 μg/ml; **4**, 3.5 and 6.7 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL METHODS.—Mps were determined on a Yanaco MP-J3 melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco DIP-370 digital polarimeter. Uv and ir spectra were taken on a Shimadzu Uv-220 spectrometer and a Jasco ir Report-100 spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. The resonances of residual DMSO at δ_H 2.50 and δ_C 39.5 were used as internal references for ^{1}H- and ^{13}C-nmr spectra, respectively. Fabms and eims were obtained on a JEOL HX-110 and a JEOL DX-303 spectrometer, respectively.

SPONGE MATERIAL.—The sponge *Aplysina* sp. (order Verongida, family Aplysinidae; Nardo, 1834) was (1 kg, wet wt) collected off the Kerama Islands, Okinawa, Japan, and kept frozen until used. The specimen was a collagenous, medium brown sponge with large dark fibers <1 mm wide. The choanosome was iscavernous and sunk around the fibers. There are large reticulate meshes, 5 mm wide. The fiber has concentric layers of bark and a central pith, with some detritus at the nodes. The voucher specimen (SS-231) was deposited at Sir George Fisher Center, James Cook University, Townsville, Australia.

EXTRACTION AND ISOLATION.—The MeOH extract of the sponge (45 g) was partitioned be-

[1]The synthesis of D-hypaphorine has not so far been reported, while L-hypaphorine was previously prepared synthetically (13).

tween EtOAc (500 ml×3) and H_2O (500 ml). The aqueous layer was subsequently extracted with *n*-BuOH (500 ml×3). The *n*-BuOH-soluble material (1.2 g) was subjected to Si gel cc (2.3×39 cm) with $CHCl_3$-*n*-BuOH-AcOH-H_2O (1.5:6:1:1) followed by *n*-BuOH-AcOH-H_2O (6:1:1). The fraction eluted from 110 ml to 430 ml with $CHCl_3$-*n*-BuOH-AcOH-H_2O (1.5:6:1:1) was purified by ODS cc (2.4×9 cm) with MeOH to give **2** (106 mg, 0.01% wet wt), and the fraction eluted from 50 ml to 150 ml with *n*-BuOH-AcOH-H_2O (6:1:1) was purified on a Sephadex LH-20 column (2×100 cm) with MeOH to give **3** (13.4 mg, 0.001%).

Isoplysin A [**2**].—Yellow needles: mp 310° (dec); uv (MeOH) λ max 220 (ε 14000), 278 (5400), and 405 nm (13000); ir (KBr) ν max 3410, 3050, 1670, 1390, 1220, and 1100 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; HMBC correlations (C/H) C-2/H-8, C-3/H-2, C3a/H-2, C-3a/H-5, C3a/H-7, C-3a/H-8, C-4/H-6, C-5/H-4, C-6/H-7, C-7/H-5, C-7a/H-2, C-7a/H-4, C-7a/H-6, C-1′/H-8, and C-5′/H-8; eims *m/z* $[M]^+$ 254, $[M-Me]^+$ 239, 169, and 155; fabms (positive ion, glycerol matrix) *m/z* $(M+H)^+$ 255; hrfabms found *m/z* 255.1239, calcd for $C_{14}H_{15}N_4O$ $[M+H]^+$ 255.1246.

CONVERSION OF ISOPLYSIN A [**2**] INTO APLYSINOPSIN [**1**].—To a suspension of **2** (1.5 mg) in DMSO (0.7 ml) was added KOH (2.6 mg) and the mixture was stirred at room temperature for 10 min. After removal of KOH by filtration and evaporation of the filtrate, the residue was purified by Si gel cc with $CHCl_3$-MeOH (85:15) to give **1** (0.9 mg, 60%).

CONVERSION OF ISOPLYSIN A [**2**] INTO INDOLE-3-CARBALDEHYDE.—A suspension of **2** (1.0 mg) in 1 N aqueous NaOH (0.2 ml) was heated at 80° for 2 h. After evaporation of the solvent, the residue was purified by Si gel cc with $CHCl_3$-MeOH (95:5) to give indole-3-carbaldehyde (0.3 mg, 53%): colorless solid; ^1H nmr ($CDCl_3$) δ 10.08 (1H, s), 8.71 (1H, br s), 8.33 (1H, m), 7.85 (1H, d, *J*=2.9 Hz), 7.45 (1H, m), and 7.34 (2H, m); eims *m/z* $[M]^+$ 145.

PREPARATION OF METHYLAPLYSINOPSIN [**4**].—To a suspension of **2** (1.5 mg) in DMSO (0.1 ml) was added KOH (5.3 mg) and MeI (5 μl) and the mixture was stirred at room temperature for 10 min. After removal of KOH by filtration and evaporation of the filtrate, the residue was purified by Si gel cc with $CHCl_3$-MeOH (95:5) to give **4** (1.4 mg, 93%): yellow solid; mp 178° (dec); ^1H nmr (270 MHz, DMSO-d_6) δ 8.66 (1H, s), 7.90 (1H, d, *J*=7.8 Hz), 7.48 (1H, d, *J*=7.8 Hz), 7.23 (1H, t, *J*=7.8 Hz), 7.15 (1H, t, *J*=7.8 Hz), 6.42 (1H, s), 3.85 (3H, s), 3.25 (3H, s), and 3.07 (3H, s); eims *m/z* $[M]^+$ 268. By the same procedure, compound **4** was obtained from **1**.

D-6-BROMOHYPAPHORINE [**3**].—Yellow needles: mp 275° (dec); $[\alpha]^{19}D$ −27° (*c*=0.80, MeOH-CF_3COOH, 8:1); uv (H_2O) λ max 293 (ε 8000), 285 (9300), and 227 nm (56000); ir (KBr) ν max 3400 and 1620 cm^{-1}; ^1H nmr (270 MHz, DMSO-d_6+CF_3COOH) δ 11.20 (1H, s, NH), 7.56 (1H, d, *J*=1.7 Hz, H-7), 7.53 (1H, d, *J*=8.2 Hz, H-4), 7.25 (1H, d, *J*=2.5 Hz, H-2), 7.17 (1H, dd, *J*=8.7 and 2.2 Hz, H-5), 4.39 (1H, dd, *J*=11.5 and 3.3 Hz, H-9), 3.53 (1H, dd, *J*=13.7 and 3.3 Hz, H-8), 3.30 (1H, dd, *J*=13.7 and 11.5 Hz, H-8′), and 3.30 (9H, s, $N(CH_3)_3$); ^{13}C nmr

TABLE 1. ^1H- and ^{13}C-Nmr Data of Compounds **1** and **2** in DMSO-d_6.

Position	^1H		^{13}C	
	1	**2**	**1**	**2**
1	11.50 (br s)	12.09 (br s)		
2	8.69 (d, *J*=2.4 Hz)	8.97 (d, *J*=2.9 Hz)	127.2 (d)	130.9 (d)
3			108.8 (s)	108.4 (s)
3a			127.7 (s)	127.7 (s)
4	7.87 (br d, *J*=7.5 Hz)	8.05 (dd, *J*=6.8 and 1.5 Hz)	118.1 (d)	118.4 (d)
5	7.09 (t, *J*=7.5 Hz)	7.22 (td, *J*=6.8 and 1.5 Hz)	119.4 (d)	122.7 (d)
6	7.15 (t, *J*=7.5 Hz)	7.23 (td, *J*=6.8 and 1.5 Hz)	121.7 (d)	120.6 (d)
7	7.41 (br d, *J*=7.5 Hz)	7.51 (dd, *J*=6.8 and 1.5 Hz)	111.7 (d)	112.3 (d)
7a			135.5 (s)	135.8 (s)
8	6.43 (s)	7.28 (s)	102.6 (d)	115.0 (d)
1′			126.4 (s)	122.2 (s)
2′-NMe	3.25 (s)	3.54 (s)	24.4 (q)	28.8 (q)
3′			150.5 (s)	151.8 (s)
3′-NMe		3.22 (s)	26.2 (q)	—
4′-NH or NMe	3.05 (s)	9.52 (br s)	26.9 (q)	
5′			162.2 (s)	160.3 (s)

(DMSO-d_6) δ 168.3 s, 137.0 s, 125.7 d, 125.6 s, 121.5 d, 120.2 d, 114.2 d, 114.1 s, 106.5 s, 74.1 d, 51.7 q, and 22.2 t; fabms (positive ion, glycerol matrix) *m/z* [(M+H)$^+$; 1:1] 325 and 327; hrfabms found *m/z* (M+H)$^+$ 325.0571, calcd for $C_{14}H_{18}{}^{79}BrN_2O_2$ (M+H)$^+$ 325.0552.

PREPARATION OF COMPOUND **5**.—A solution of **3** (1.7 mg) in MeOH (0.5 ml) containing Raney-Nickel (3 mg) was stirred at room temperature under H_2 for 1 day. After removal of the catalyst by filtration and evaporation of the filtrate, the residue was purified by Si gel cc with $CHCl_3$-MeOH-H_2O (6:6:1) to give compound **5** (0.4 mg, 31%): colorless oil; $[\alpha]^{20}D$ −87° (*c*=0.05, MeOH); uv (MeOH) λ max 290 (ε 4900), 282 (5700), 221 nm (33700); ^{1}H nmr (270 MHz, DMSO-d_6) δ 10.85 (1H, br s, NH), 7.61 (1H, d, *J*=7.7 Hz, H-7), 7.33 (1H, d, *J*=7.7 Hz, H-4), 7.16 (1H, d, *J*=2.2 Hz, H-2), 7.07 (1H, t, *J*=6.9 Hz, H-6), 6.98 (1H, t, *J*=7.2 Hz, H-5), 3.67 (1H, dd, *J*=9.1 and 4.2 Hz, H-9), 3.21 (2H, s, H-8), and 3.18 (9H, s, $N(CH_3)_3$); fabms (positive ion, glycerol matrix) *m/z* (M+H)$^+$ 247.

SYNTHESIS OF D-HYPAPHORINE.—To a suspension of D-tryptophan (0.2 g) and $KHCO_3$ (1 g) in MeOH (20 ml) was added CH_3I (1 ml) and the mixture was stirred at room temperature for 1 day. After removal of $KHCO_3$ by filtration and evaporation of the filtrate, the residue was purified by Si gel cc with $CHCl_3$-MeOH-H_2O (6:6:1) to give D-hypaphorine (208 mg, 86%): colorless solid; mp 248° (dec); $[\alpha]^{20}D$ −86° (*c*=1.0, MeOH); uv (MeOH) λ max 290 (ε 8800), 282 (10300), and 220 nm (63800); ir (KBr) ν max 3450 and 1620 cm^{-1}; ^{1}H nmr (270 MHz, DMSO-d_6) δ 10.86 (1H, br s, NH), 7.61 (1H, d, *J*=7.7 Hz, H-7), 7.33 (1H, d, *J*=8.2 Hz, H-4), 7.16 (1H, d, *J*=2.2 Hz, H-2), 7.07 (1H, td, *J*=6.6 Hz, H-6), 6.98 (1H, td, *J*=6.6 Hz, H-5), 3.67 (1H, dd, *J*=9.5 and 4.4 Hz, H-9), 3.22 (2H, s, H-8), and 3.18 (9H, s, NMe_3); fabms (positive ion, glycerol matrix) *m/z* (M+H)$^+$ 247.

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