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KONBAMIDIN, A NEW INDOLE ALKALOID FROM THE OKINAWAN MARINE SPONGE *IRCINIA* SP.

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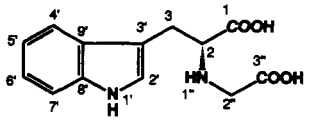
ABSTRACT.—A new indole alkaloid, konbamidin [**1**], has been isolated from the Okinawan marine sponge *Ircinia* sp. and the structure determined by spectral data and its synthesis.

Many indole alkaloids have been isolated from marine sources and shown to exhibit interesting biological activities (1). In our search for bioactive metabolites from Okinawan marine organisms (2), we examined extracts of a sponge, *Ircinia* sp. (Dictyoceratida, Thorectidae), and obtained a new indole alkaloid, named konbamidin [**1**]. In this paper we describe the isolation, structure elucidation, and synthesis of **1**.

The sponge *Ircinia* sp. was collected off Konbu, Okinawa Island, Japan, and kept frozen until used. The MeOH extract of the sponge was partitioned between EtOAc and H_2O. The *n*-BuOH-soluble portion of the aqueous phase was subjected to Si gel cc ($CHCl_3$-*n*-BuOH-AcOH-H_2O, 1.5:6:1:1) followed by gel filtration on Sephadex LH-20 (MeOH) to give konbamidin [**1**] (0.002%, wet wt).



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The eims of konbamidin [**1**] showed a molecular ion peak at *m/z* 262 and the molecular formula was established as $C_{13}H_{14}N_2O_4$ by hreims data [*m/z* 262.0925 (M^+) for $C_{13}H_{14}N_2O_4$, Δ −2.9 mmu]. Uv absorptions at 222, 281, and 290 nm indicated the presence of an indole ring. The ^{1}H- and ^{13}C-nmr data (Table 1) of **1** revealed signals corresponding to a tryptophan residue and a

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Konbamidin [**1**].

Position	^{1}H[a]	*J* (Hz)	^{13}C[b]	H coupled with C[c]
1			171.5 s	H-2, H-3
2	4.46 dd	6.8, 6.0	62.2 d	H-3, H-2″
3	3.58 dd	15.5, 6.0	25.7 t	H-2
	3.55 dd	15.5, 6.8		
1′				
2′	7.30 s		124.5 d	H-3
3′			107.3 s	H-2, H-3, H-2′, H-4′
4′	7.65 d	8.0	118.1 d	H-6′
5′	7.11 t	8.0	119.0 d	H-7′
6′	7.19 t	8.0	121.7 d	H-4′
7′	7.42 d	8.0	111.5 d	H-5′
8′			135.9 s	H-2′, H-4′, H-6′
9′			126.3 s	H-2′, H-4′, H-5′, H-7′
1″				
2″	3.96 d	16.9	48.6 t	H-2
	3.90 d	16.9		
3″			171.5 s	H-2″

[a]In CD_3OD.
[b]In D_2O.
[c]HMBC correlations.

methylene group (δ_H 3.90, d, J=16.9 Hz and δ_H 3.96, d, J=16.9 Hz; δ_C 48.6, t). An HMBC (3) correlation between the methylene protons and a carboxyl carbon (δ_C 171.5) indicated the presence of a carboxymethyl group. The ^{13}C-nmr chemical shifts of the carboxymethyl group were very similar to those of a glycine moiety (4). The connection from the carboxymethyl group to C-2 through N-1″ was verified by an HMBC correlation between H_2-2″ and C-2. Thus, the structure of konbamidin was elucidated as **1**. The absolute stereochemistry at C-2 of konbamidin was determined by comparison with spectral data of (*R*)-**1** and (*S*)-**1** derived from D- and L-tryptophan by reaction with glyoxylic acid and $NaBH_3CN$ in MeOH, respectively (Scheme 1). All spectral data (^{1}H- and ^{13}C-nmr, ir, uv, and eims) of konbamidin were identical with those of both of (*R*)- and (*S*)-**1**, while the sign and value of the $[\alpha]_D$ (+15.0°) of konbamidin corresponded to that (+6.8°) of (*R*)-**1** but not that (−12.6°) of (*S*)-**1**. The *R*-configuration at C-2 of **1** was further confirmed by chiral hplc analysis of konbamidin and the synthetic (*R*)-**1** and (*S*)-**1**.

Konbamidin [**1**] is the third example of an indole alkaloid from a sponge of the genus *Ircinia* (5). Biogenetically, konbamidin [**1**] may be derived from one mol each of glycine and 3-indoleacrylic acid, since 3-indoleacrylic acid was isolated together with **1** from this sponge. Compound **1** exhibited cytotoxicity against HeLa cells in vitro with an IC_{50} value of 5.4 μg/ml, while (*S*)-**1** was not significantly cytotoxic.

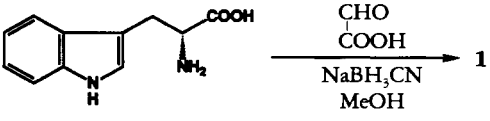
EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were determined on a Jasco DIP-370 polarimeter. ^{1}H- and ^{13}C-nmr spectra were recorded on a JEOL EX-400 and a Bruker ARX-500 spectrometer, respectively. The 3.35 ppm resonance of residual CD_2HOD in CD_3OD and the 29.8 ppm resonance of Me_2CO in D_2O were used as internal references. Eims and fabms spectra were obtained on a JEOL DX-303 spectrometer operating at 70 eV and on a JEOL HX-110 spectrometer.

ANIMAL MATERIAL.—The sponge *Ircinia* sp. was collected by scuba off Konbu, Okinawa Island, Japan, and kept frozen until used. This specimen was a medium brown, amorphous sponge when preserved but its morphology could not be determined from the sample available. It contained much macroscopic accreted material, and had a slightly darker surface skin, probably conulose, without much skin on the specimen. The sponge was compressible but tough, and its interior had ropes of filaments in a thick swirling pattern, with sandgrains and foreign material throughout the mesohyl. The sponge had internal fiber development; the fibers were fasciculated and cored with sandgrains in the central region of the sponge, and all ran at right angles to the surface. Some secondary fibers occurred and did not appear to be cored with foreign material. The sponge's filaments were 5–7 μm wide, and it did not have a thick sand cortex. A voucher specimen (SS-717) was deposited at Sir George Fisher Center, James Cook University, Townsville, Queensland, Australia.

EXTRACTION AND ISOLATION.—The sponge (0.75 kg, wet wt) was extracted with MeOH (1.0 liter×2). The MeOH extract (44 g) was partitioned between EtOAc (500 ml×3) and H_2O (500 ml), and the aqueous layer was subsequently extracted with *n*-BuOH (500 ml×3). The *n*-BuOH-soluble portion was evaporated under reduced pressure to give a residue (2.3 g), which was chromatographed on a Si gel column (2.5×34 cm) eluted with $CHCl_3$-*n*-BuOH-AcOH-H_2O (1.5:6:1:1). The fraction eluting from 830 to 1160 ml was separated by a Sephadex LH-20 column (1.0×90 cm) with MeOH to give konbamidin [**1**, 3.0 mg].

Konbamidin [**1**].—A colorless powder; $[\alpha]^{21}_D$ +15.0° (c=0.27, MeOH); ir (KBr) ν max 3400 (OH), 1700 (sh, C=O), and 1640 cm^{-1}; uv (MeOH) λ max (log ε) 222 (4.26), 281 (3.52), and 290 (3.43) nm; ^{1}H and ^{13}C nmr (Table 1); eims *m/z* 262 $[M]^+$; hreims *m/z* 262.0925 $[M+H]^+$, calcd for $C_{13}H_{14}N_2O_4$, 262.0954.



SCHEME 1

Synthesis of (R)-*konbamidin* [**1**] *from* D-*tryptophan*.—To a solution of D-tryptophan (105 mg, 0.51 mM) in MeOH (10 ml) was added glyoxylic acid (120 mg, 1.6 mM) and $NaBH_3CN$ (31 mg). After the mixture was stirred at 0° for 10 min, glyoxylic acid (9 mg, 0.12 mM) was added and stirred at 0° for 10 min followed by evaporation under reduced pressure to give a residue. The residue was partitioned between *n*-BuOH (30 ml×2) and H_2O (30 ml) and the *n*-BuOH layer was evaporated to give a residue, which was purified by a Sephadex LH-20 column (1.0×90 cm, eluent MeOH) to afford *(R)*-**1** (31.0 mg, 23%) and D-Trp (72 mg). *(R)*-**1**: A colorless powder; $[\alpha]^{19}D$ +6.8° (*c*=1.1, MeOH); ir (KBr) ν max 3400 (OH), 1710 (C=O), and 1620 cm^{-1}; uv (MeOH) λ max (log ϵ) 222 (4.68), 280 (3.92), and 290 (3.84) nm; ^1H nmr (CD_3OD) δ_H 3.55 (1H, dd, *J*=15.5 and 6.8 Hz, H-3), 3.59 (1H, dd, *J*=15.5 and 5.7 Hz, H-3), 3.90 (1H, d, *J*=16.7 Hz, H-2″), 3.96 (1H, d, *J*=16.7 Hz, H-2″), 4.47 (1H, dd, *J*=6.8 and 5.7 Hz, H-2), 7.11 (1H, t, *J*=8.0 Hz, H-5′), 7.20 (1H, t, *J*=8.0 Hz, H-6′), 7.30 (1H, s, H-2′), 7.41 (1H, d, *J*=8.0 Hz, H-7′), and 7.65 (1H, d, *J*=8.0 Hz, H-4′); ^{13}C nmr (D_2O) δ_C 25.2 (t, C-3), 48.6 (t, C-2″), 60.8 (d, C-2), 106.1 (s, C-3′), 111.6 (d, C-7′), 117.9 (d, C-4′), 119.1 (d, C-5′), 121.8 (d, C-6′), 124.7 (d, C-2′), 126.1 (s, C-9′), 135.9 (s, C-8′), 169.3 (s, C-3″), and 171.6 (s, C-1); eims *m/z* 262 $[M]^+$; fabms (positive, glycerol) *m/z* 263 $[M+H]^+$; hrfabms found *m/z* 263.1049 $[M+H]^+$, calcd for $C_{13}H_{15}N_2O_4$, 263.1032.

According to essentially the same procedure as described above, L-Trp (101 mg) afforded *(S)*-**1** (69.6 mg, 54%); $[\alpha]^{21}D$ −12.6° (*c*=1.9, MeOH) and L-Trp (41 mg).

CHIRAL HPLC ANALYSIS.—The absolute stereochemistry of konbamidin [**1**] was determined by chiral hplc analysis [Sumichiral OA-5000, Sumitomo Chemical Industry, 4×150 mm, flow rate: 1.5 ml/min; eluent MeOH-H_2O (85:15) containing 2.0 mmol/liter of $CuSO_4$; detection: uv at 254 nm]. Retention times of konbamidin [**1**], *(S)*-**1**, and *(R)*-**1** were 33.2, 14.8, and 33.2 min, respectively.

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