

The additional crystallization of the dipeptide (I) from n-butanol gave 1.64 g (36%) of crystals with mp 106-107°C and $[\alpha]_{546}^{20} +1.4^\circ$ (c 4.0; MeOH). PMR (500 MHz, CDCl₃): 1.34 (3H, d, $J_{\text{CH}_3\text{CH}} = 7$ Hz, CH_3CH), 1.45 (9H, s, Me_3C), 4.65 (1H, q, CHCH_3), 5.0 s, 5.14 d, 5.17 d (4H, 2COOCH_2), 4.94 (1H, d, NH-Ala), 6.89 (1H, d, NH-Glu), 7.35 (10H, m, 2Ph).

From the DBEs of D-Glu and L-Glu we obtained authentic samples of dipeptides (I) and (II), respectively: (I) with mp 106-108°C, $[\alpha]_{546}^{20} +1.6^\circ$ (c 4.0; MeOH), $+7.4^\circ$ (c 4.0; DMFA); the literature gave bp 99-101°, $[\alpha]_D^{25} +7^\circ$ (DMFA) [1], mp 106.5-107°, $[\alpha]_D^{20} -8.2^\circ$ (EtAc) [4], mp 98-100°, $[\alpha]_D^{20} -37.6^\circ$ (c 4.0; MeOH); the literature gave bp 59-61°, $[\alpha]_D^{25} -34.8^\circ$ (MeOH) [1].

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ANTIBIOTICS FROM STRAINS OF *Bacillus pumilus* ISOLATED FROM A MARINE SPONGE *Dendrilla* sp.

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Continuing a study of the secondary metabolites of microorganisms associated with marine invertebrates [1], we have investigated two strains (D-7 and D-12) from the sponge *Dendrilla* sp. collected in November, 1986, off the coast of Madagascar. We have shown that during their development the strains D-7 and D-12 synthesize substances with an antimicrobial activity. From their morphological characteristics and physiological tests, the microorganisms were identified as *Bacillus pumilus* [2].

Bacillus pumilus D-7 was grown on Yoshimitsu-Kimura medium in a thermostat at 30°C for 120 h. The culture liquid (20 liters) was chromatographed on a column of Polikhrom-1 with elution by 8, 20, and 40% ethyl alcohols successively. The fractions isolated were tested for antimicrobial activity by the agar diffusion method and by bioautography, using *Staphylococcus aureus* as the test culture.

The fraction eluted by 20% alcohol was separated in the CHCl_3 - CH_3OH (3:1, 2:1, and 1:1) systems on a column of silica gel that had been treated beforehand with a mixture of 0.2 M solutions of KH_2PO_4 and $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ (1:1, v/v). Evaporation of the CHCl_3 - CH_3OH (1:1) fraction yielded 200 mg of compound (I); mass spectrum: 425 (M + H); UV spectrum, λ_{max} (methanol): 246, 314 nm, while the ^1H and ^{13}C NMR spectra of (I) coincided with the corresponding spectra of antibiotic B isolated previously by Japanese workers from a soil strain of *B. pumilus*, A1-77 [3].

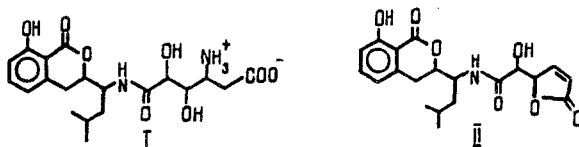
Strain D-12 was grown on Yoshimitsu-Kimura medium on a shaking machine at 25°C for 48 h. The culture liquid (20 liters) was chromatographed on a column of Polikhrom-1. The

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fraction eluted by 40% alcohol was purified on a column of TSK-Gel Toyopearl HW-40F in a $C_2H_5OH-H_2O$ gradient system [(33:67) \rightarrow (80:20)]. The fraction having the highest absorption at 315 nm was separated by high-performance liquid chromatography on a Bondapak C-18 column in the 84% acetonitrile-0.02 M $NaH_2PO_4 \cdot 2H_2O$ (50:50) system. As a result, 25 mg of a substance identical with compound (I) and antibiotic B according to its mass, UV, and 1H and ^{13}C NMR spectra, was obtained.

The $CHCl_3-CH_3OH$ (3:1) fraction (from strain D-7) was chromatographed on silica gel plates in the $CHCl_3-CH_3OH$ (20:1) system. This gave 5 mg of compound (II), with the mass spectrum 412 ($M + Na$) and the UV spectrum, λ_{max} (methanol): 246, 314 nm. The 1H NMR spectrum of (II) coincided with that of antibiotic F isolated previously from strain Al-77 [3].

Thus, the strains B. pumilus D-7 and D-12, isolated from the marine sponge Dendrilla sp., synthesize the same antibiotics as the soil strain B. pumilus Al-77.



It has been shown that compound (I) inhibits the development of Staphylococcus aureus ATCC 21027, Bacillus subtilis ATCC 6633, Micrococcus luteus VKM V-109, and Escherichia coli K-13 in concentrations of 500, 100, 6.25, and 1000 $\mu g/ml$, respectively.

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