METHYL ESTER OF BACILLOMYCIN D FROM THE MARINE ISOLATE OF *Bacillus subtilis* KMM 457

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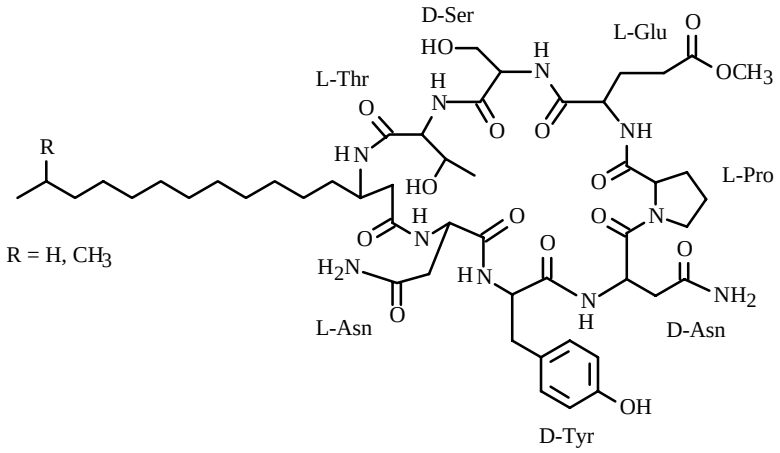
Isolation of the marine isolate of *Bacillus subtilis* KMM 457, which produced bacillomycin D and two peptides with *n*-C-14 and *iso*-C-15 β-amino acids, was previously reported. It possesses anticandidosic activity and belongs to the iturin group of antibiotics [1, 2]. In continuation of an investigation of the components of the butanol extract of the culture liquid of this strain, we isolated another compound that inhibited growth of the yeast-like fungus *Candida albicans* but was inactive relative to gram-positive and gram-negative bacteria. Repeated adsorption chromatography over silica gel of the butanol extract of the bacteria culture liquid with gradient elution by systems from $CHCl_3$:C_2H_5OH (1:1) to $CHCl_3$:C_2H_5OH:H_2O (100:100:8-17) produced a chromatographically pure fraction **1**.



Culture liquid (50 L) produced **1** as a white powder (15 mg) that had mp 257-260°C after reprecipitation from ethanol. It dissolved in polar solvents (alcohol, pyridine) but not in nonpolar solvents (hexane, $CHCl_3$, benzene). The IR spectrum of **1** (KBr) contained absorption bands for a peptide bond (1678, 1662, 1640, 1542, and 1510 cm^{-1}) and ester (1250 cm^{-1}). The UV spectrum (ethanol) had an absorption at 280-285 nm, characteristic of tyrosine. Based on the amino-acid analysis (6 N HCl, 100°C, 24 h), six α-amino acids Asp, Tyr, Glu, Pro, Thr, and Ser were present in the ratio 2:1:1:1:1:1. The $CHCl_3$ extract of the hydrolysate also contained an aliphatic β-amino acid that gave a positive reaction with ninhydrin. The amino-acid composition was identical to that of bacillomycin D. Matrix-activated laser desorption/ionization mass spectrometry (MALDI MS) of the fraction **1** showed two peaks of molecular weights 1044 and 1058 Da. Therefore, the isolated fraction was the sum of two homologous peptides with the same amino-acid composition but different β-amino acids. The ratio of intensities of the molecular-ion peaks showed that the peptide with a C_{15}-β-amino acid ($[M]^+$ 1058) dominated.

The PMR spectrum (300 MHz, C_5D_5N, δ, ppm, J/Hz, 0 = TMS) of **1** contained signals for amide and carbamide protons: 9.8 (1H, d, J = 8.3, NH Asn1), 9.1 (1H, br.s, NH β-AA, 1H NH Asn2), 8.6 (1H, br.s, NH_2 Asn1), 8.4 (1H, br.s, NH_2 Asn2), 8.25 (1H, br.s, NH Glu), 8.1-7.9 (m, 1H, NH_2 Asn1, 1H NH_2 Asn2, 1H NH Tyr, 1H NH Thr, 1H NH Ser), 7.15 and 7.5 (d, tyrosine aromatic protons), seven α- and one β-methine proton at 5.35-3.7 (1H C-3 β-AA, 1H C-2 Asn1, 1H C-2 Asn2, 1H

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C-2 Tyr, 1H C-2 Glu, 1H C-2 Pro, 1H C-2 Thr, 1H C-2 Ser), *iso*-propyl of a β-amino acid and threonine methyl at 0.85 (d, J = 6.6) and 1.35 (d, J = 10.9), respectively, and a weak multiplet at 0.86 for the methyl of the *n*-β-amino acid of the peptide.

The ^{13}C NMR spectrum of **1** (75 MHz, C_5D_5N, δ, ppm, 0 = TMS) confirmed the presence of C atoms for peptide carbamide and carboxylic (signals at 169-175), tyrosine (signals at 115.9, 128.3, 131.0, and 157.4), α- (45-67) and β-positions (30-45) to carbamide, methoxy (51.2), threonine methyl (20.67), and methyls of *iso*- (22.54) and *n*- (14.05) β-amino acids. Comparison of the NMR spectra for **1** and bacillomycin D showed that they have an identical set of signals with the exception of the signals at 3.5 (PMR spectrum) and 51.2 ppm (^{13}C NMR spectrum). Based on the literature, these belong to H and C of a methoxy group and are lacking in spectra of bacillomycin D. The PMR spectrum of **1** was consistent with the presence of two amides in asparagines and lacking an amide of glutamic acid. 2D NMR spectroscopy (HMBC) showed coupling of methoxy protons with the carbonyl of an acid. Therefore, the glutamic acid occurs in **1** as the methyl ester.

Thus, we found that the fraction **1** is the methyl ester of bacillomycin D and is a new representative of iturin antibiotics [3, 4] (Fig. 1). They are synthesized by different strains of *B. subtilis* as a combination of homologs at the β-amino acid and have a definite set, number, sequence, and configuration of amino acids in the peptide ring [5, 6].

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