Cyclic lipopeptides with fungicidal activity from the sea isolate of the bacterium *Bacillus subtilis*

G. K. Oleinikova,[a] T. A. Kuznetsova,[a]★ F. Huth,[b] H. Laatsch,[b] V. V. Isakov,[a] L. S. Shevchenko,[a] and G. B. Elyakov[a]

[a]*Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, 159 prosp. 100-Letiya Vladivostoka, 690022 Vladivostok, Russian Federation. Fax: +7 (423 2) 31 4050. E-mail: piboc@stl.ru*

[b]*Department of Organic Chemistry, University of Göttingen, 2 Tammannstr., D-37077 Göttingen, Germany. Fax: +494 (551) 39 3660*

A mixture of cyclic lipopeptides with fungicidal activity was extracted from the sea isolate of the bacterium *Bacillus subtilis* (KMM 457). HPLC separation gave two main individual peptides of this mixture (M = 1030 and 1044 Da). According to amino acid analysis and ^{1}H and ^{13}C NMR data, they belong to iturin antibiotics and are cyclic systems composed of the same seven α-amino acids (2 Asn, Glu, Tyr, Pro, Thr, and Ser) and one β-amino acid (3-aminotetradecanoic or 3-amino-13-methyltetradecanoic acid, respectively). The sequence of amino acids in these peptides was determined for the first time using tandem electrospray ionization mass spectrometry.

Key words: cyclic lipopeptides, facultative marine bacteria *Bacillus subtilis*, bacillomycins, electrospray ionization mass spectrometry.

In a search for biologically active metabolites in marine bacteria isolated from sea water, bottom sediments, and marine invertebrates, it was found[1] that the bacterium *Bacillus subtilis* (KMM 457) associated with the marine soft coral *Sarcophyton sp.* produces a mixture of peptides exhibiting fungicidal activity at a minimum inhibitive concentration <10 μg mL^{-1}. Terrestrial bacteria of the genus *Bacillus* are known to contain fungicidal peptidolipids classified as iturin antibiotics. They are characterized by a ring made up of seven α-amino acids (2 Asx, Glx, Tyr, Pro, Ser, and Thr) and one β-amino acid with a chain length ranging from C_{14} to C_{18}.[2—5] The main constituents of a mixture of peptides from *Bacillus subtilis* (KMM 457) are peptides **1** and **2** (M = 1030 and 1044 Da, respectively) with the same amino acid composition (L-Asx, D-Asx, L-Glx, D-Tyr, L-Pro, D-Ser, and L-Thr). The ^{1}H—^{1}H COSY NMR data for peptide **2** confirm the presence of the above amino acids, including two asparagine residues and a residue of glutamic acid (Fig. 1).

Analysis of the amino acid composition, molecular masses, and ^{1}H NMR spectra of the peptides show that molecules **1** and **2** differ from each other in the length and isomerism of β-amino acids (*n*-C_{14} for **1** and *i*-C_{15} for **2**). Indeed, ^{1}H NMR spectrum of peptide **1** contains a high-field doublet at δ 1.37 for the Me protons of threonine and a triplet at δ 0.86 for the Me protons of β-amino acid. The ^{1}H NMR spectrum of peptide **2** shows an analogous signal for the Me protons of threonine (δ 1.38) and another doublet for six protons of two Me groups in β-amino acid (δ 0.84).

The amino acid sequences in peptides **1** and **2** were determined from tandem electrospray ionization mass spectra (ESI-MS/MS) of these compounds and some

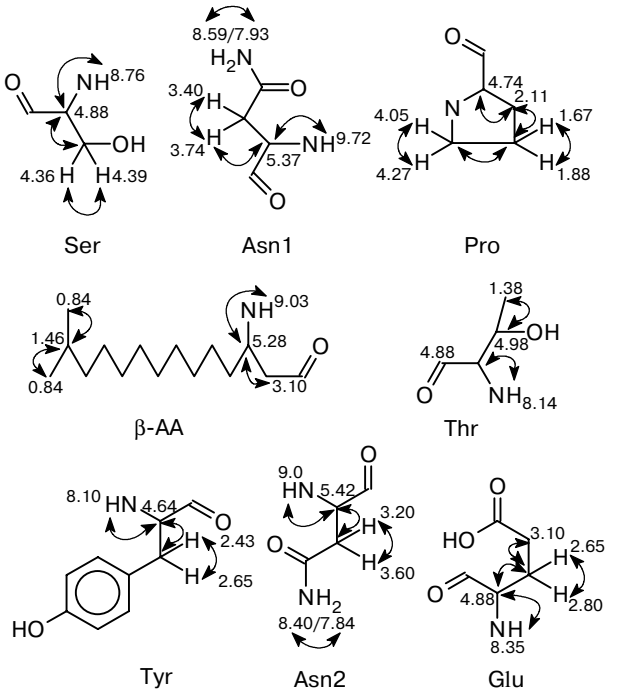
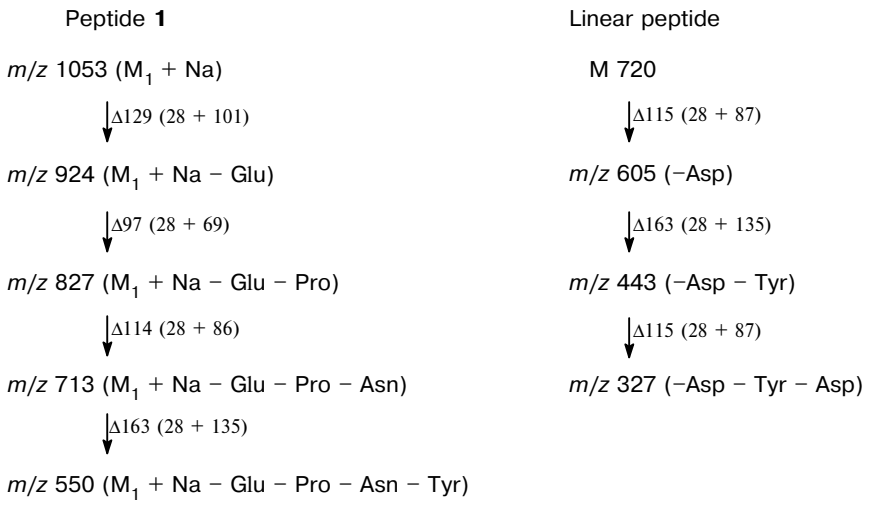


Fig. 1. Amino acid composition of peptide **2** (the numbers indicate the proton chemical shifts in the ^{1}H NMR spectrum; correlations in the ^{1}H—^{1}H COSY spectrum are marked with arrows; β-AA is the β-amino acid residue).

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 11, pp. 2131—2135, November, 2001.

1066-5285/01/5011-2231 $25.00

Scheme 1

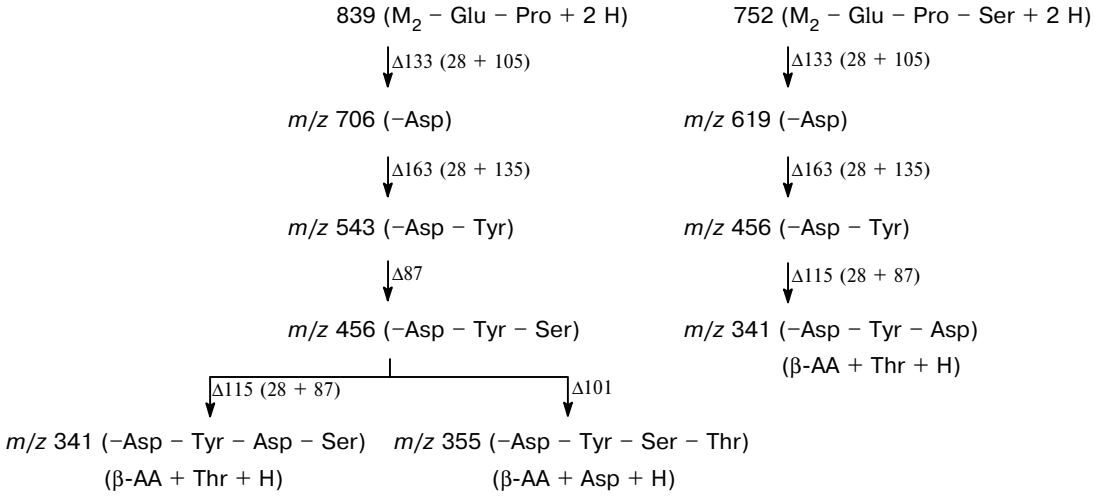


linear peptides produced by their partial acid hydrolysis. Analysis of fragmentation ions obtained from peptide **1** under ESI-MS conditions (Scheme 1, M_1 is the molecular mass of peptide **1**) gave the following sequence of four amino acids in the peptide: Glu—Pro—Asp—Tyr. Partial hydrolysis of compound **1** yielded a mixture of linear peptides. In addition, ESI-MS study of a peptide with M = 720 Da revealed a sequence of three amino acids: Asp—Tyr—Asp (see Scheme 1). Thus, the sequence of five amino acids in peptide **1** was unambiguously determined: Glu—Pro—Asn—Tyr—Asn.

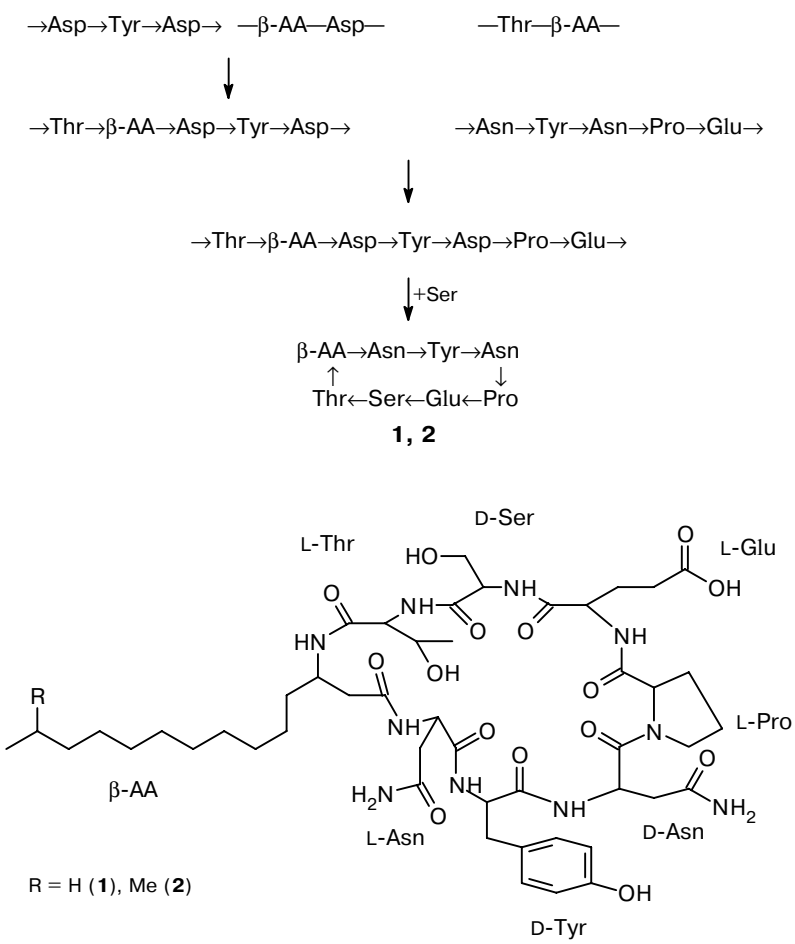
The β-amino acid residue (β-AA), threonine, and serine were located by analyzing fragmentation ions of linear peptides with M = 839 and 752 Da produced by partial acid hydrolysis of peptide **2** (Scheme 2; M_2 is the molecular mass of peptide **2**). The fragmentation ion with *m/z* 456 gives not only an ion with *m/z* 341 (β-AA + Thr + H), but also an ion with *m/z* 355 (β-AA + Asp + H), as a result of threonine detachment. An ion with *m/z* 752 from a linear peptide successively loses Asp, Tyr, and Asp to give fragments with *m/z* 619, 456, and 341, respectively.

Based on the results presented in Schemes 1 and 2, one can determine the sequence of amino acids in peptides **1** and **2** (Scheme 3). Under ESI-MS conditions, the fragmentation of cyclic lipopeptides **1** and **2** proceeds as follows. First, cleavage of the bond between a glutamic acid and serine produces a linear peptide; then amino acids are detached successively from its C-end with elimination of CO at each detachment act,[6,7] while the N-end loses only serine and threonine. The amino acid sequences found for peptides **1** and **2** indicate that the isolated peptides are identical with bacillomycins D1 and D2 produced by the terrestrial bacteria *Bacillus subtilis*.[8,9] Based on the literature data for these compounds,[9] we assume that a relative ar-

Scheme 2



Scheme 3



rangement of the D- and L-asparagine residues corresponds to the aforementioned sequence, while 3-aminoalkanoic acids have *R*-configuration characteristic of iturin acids obtained by hydrolysis of iturin A.[10]

Experimental

Cultivation of microorganisms and isolation of individual peptides were carried out according the known procedure.[1]

^{1}H and ^{13}C NMR spectra were recorded on Varian VXR 500 (499.9 and 125.7 MHz, respectively) and Bruker WM-300 (300.13 and 75.5 MHz, respectively) instruments in C_5D_5N with Me_4Si as the internal standard. Standard procedures were used in two-dimensional correlation spectrometry.

The stereochemistry of amino acids was determined using GLC by measuring the retention times in a chiral column of isopropyl esters of their *N*-trifluoroacetyl derivatives produced by the hydrolysis of peptides **1** and **2** (Table 1). The analysis was carried out on a Siemens Sicchromat 1 chromatograph with a flame ionization detector and a Permabond-L-Chirasil-Val capillary column (Machery, Nagel and Co., Dueren, Germany, 25 × 0.32 mm, He as a carrier gas).

ESI-MS/MS mass spectra were recorded on a Finnigan LCQ instrument (4.25 kV, MeOH, 3 μL min^{-1}).

Peptide 1. ^{1}H NMR (499.9 MHz), δ: 9.75 (br.s, H—D exchange, 1 H, NH Asn1); 9.06 (br.s, H—D exchange, 1 H, NH β-AA); 9.02 (br.s, H—D exchange, 1 H, NH Asn2); 8.78 (br.s, H—D exchange, 1 H, NH Ser); 8.62 (s, H—D exchange, 1 H, NH_{2a} Asn1); 8.43 (s, H—D exchange, 1 H, NH_{2a} Asn2); 8.38 (br.s, H—D exchange, 1 H, NH Glu); 8.17 (br.s,

Table 1. Retention time (t_r) for isopropyl esters of *N*-trifluoroacetyl derivatives of standard α-amino acids and for the products of complete acid hydrolysis of peptides **1** and **2**

Amino acid	t_r/min		
	Standard	**1**	**2**
D-Asn	27.12	27.14	27.08
L-Asn	27.26	27.25	27.23
D-Glu	31.13	—	—
L-Glu	31.28	31.23	31.26
D-Ser	14.03	14.05	14.09
L-Ser	15.26	—	—
D-Thr	9.10	—	—
L-Thr	9.53	9.56	9.53
D-Pro	22.27	—	—
L-Pro	22.35	22.41	22.39
D-Tyr	34.47	34.40	34.42
L-Tyr	35.10	—	—

H—D exchange, 1 H, NH Thr); 8.14 (br.d, H—D exchange, 1 H, NH Tyr, ^3J = 8.5 Hz); 7.96 (s, H—D exchange, 1 H, NH_{2b} Asn1); 7.87 (s, H—D exchange, 1 H, NH_{2b} Asn2); 7.51 (d, 2 H, C(3′)H, C(5′)H Tyr, ^3J = 9 Hz); 7.09 (d, 2 H, C(2′)H, C(6′)H Tyr, ^3J = 9 Hz); 5.40—5.30 (m, 3 H, C(2)H Asn2, C(2)H Asn1, C(3)H β-AA); 4.99 (m, 1 H, C(3)H Thr); 4.91 (m, 3 H, C(2)H Ser, C(2)H Glu, C(2)H Thr); 4.76 (t, 1 H, C(2)H Pro, ^3J = 6 Hz); 4.65 (br.m, 1 H, C(2)H Tyr); 4.41 (dd, 1 H, $C(3)H_{2a}$ Ser, ^2J = 12 Hz, ^3J = 4.5 Hz); 4.37 (dd, 1 H, $C(3)H_{2b}$ Ser, ^2J = 12 Hz, ^3J = 4.5 Hz); 4.30 (m, 1 H, $C(5)H_{2a}$ Pro); 4.07 (m, 1 H, $C(5)H_{2b}$ Pro); 3.76 (dd, 1 H, $C(3)H_{2a}$ Asn1, ^2J = 15 Hz, ^3J = 6 Hz); 3.62 (dd, 1 H, $C(3)H_{2a}$ Asn2, ^2J = 15 Hz, ^3J = 7.6 Hz); 3.41 (dd, 1 H, $C(3)H_{2b}$ Asn1, ^2J = 15 Hz, ^3J = 8 Hz); 3.22 (dd, 1 H, $C(3)H_{2b}$ Asn2, ^2J = 15 Hz, ^3J = 6 Hz); 3.14—2.96 (m, 4 H, $C(2)H_2$ β-AA, $C(4)H_2$ Glu); 2.83 (m, 1 H, $C(3)H_{2a}$ Glu); 2.66 (m, 2 H, $C(3)H_{2b}$ Glu, $C(3)H_{2a}$ Tyr); 2.45 (m, 1 H, $C(3)H_{2b}$ Tyr); 2.13 (m, 2 H, $C(3)H_2$ Pro); 1.89 (m, 1 H, $C(4)H_{2a}$ Pro); 1.68 (m, 1 H, $C(4)H_{2b}$ Pro); 1.59 (m, 1 H, $C(4)H_{2a}$ β-AA); 1.48 (m, 1 H, $C(4)H_{2b}$ β-AA); 1.37 (d, 3 H, $C(4)H_3$ Thr, ^3J = 7.2 Hz); 1.35—1.08 (m, 18 H, $C(5)H_2$—$C(13)H_2$ β-AA); 0.86 (t, 3 H, $C(14)H_3$ β-AA, ^3J = 6 Hz). ^{13}C NMR (125.7 MHz, C_5D_5N), δ: 173.68 (2 C_{quat}); 173.23 (C_{quat}); 172.88 (C_{quat}); 172.78 (2 C_{quat}); 172.56 (C_{quat}); 172.43 (C_{quat}); 171.14 (2 C_{quat}); 157.45 (C_{quat}); 131.28 (CH); 128.63 (C_{quat}); 116.10 (CH); 66.23 (CH); 63.50 (CH_2); 62.18 (CH); 59.34 (CH); 57.94 (CH); 55.82 (CH); 55.35 (CH); 52.81 (CH); 50.24 (CH); 48.52 (CH_2); 47.45 (CH); 41.88 (CH_2); 37.84 (CH_2); 37.15 (CH_2); 36.47 (CH_2); 35.66 (CH_2); 32.07 (CH_2); 31.75 (CH_2); 29.93 (CH_2); 29.89 (CH_2); 29.85 (2 CH_2); 29.83 (CH_2); 29.74 (CH_2); 29.57 (CH_2); 26.07 (CH_2); 24.97 (CH_2); 22.89 (CH_2); 20.81 (CH_3); 14.25 (CH_3). MS ((+)-ESI-MS), *m/z* (I_{rel} (%)): 1069 $[M + K]^+$ (4), 1053 $[M + Na]^+$ (100), 1031 $[M + H]^+$ (43). MS ((−)-ESI-MS), *m/z* (I_{rel} (%)): 1029 $[M - H]^-$ (100). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 1053 $[M + Na]^+$ (5), 1035 $[M + Na - H_2O]^+$ (41), 1025 $[M + Na - CO]^+$ (100), 1009 $[M + Na - CO_2]^+$ (46), 924 $[M + Na - Glu]^+$ (21), 827 $[M - Glu - Pro]^+$ (7). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 924 $[M']^+$ (45), 906 $[M' - H_2O]^+$ (18), 880 $[M' - CO_2]^+$ (100), 827 $[M' - Pro]^+$ (32), 713 $[M' - Pro - Asn]^+$ (45), 685 $[M' - Pro - Asn - CO]^+$ (43), 550 $[M' - Pro - Asn - Tyr]^+$ (7). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 605 (100), 587 $[M' - H_2O]^+$ (12), 504 $[M' - Thr]^+$ (10), 474 (16), 460 (77), 442 $[M' - Tyr]^+$ (4), 441 (10), 421 (13), 399 (14), 345 (64), 327 $[M' - Tyr - Asp]^+$ (100), 285 (19).

Peptide 2. ^{1}H NMR (499.9 MHz), δ: 9.72 (br.s, 1 H, NH Asn1); 9.03 (br.s, 1 H, NH β-AA); 9.00 (br.s, 1 H, NH Asn2); 8.76 (br.s, 1 H, NH Ser); 8.59 (s, 1 H, NH_{2a} Asn1); 8.40 (s, 1 H, NH_{2a} Asn2); 8.35 (br.s, 1 H, NH Glu); 8.14 (d, 1 H, NH Thr, ^3J = 8 Hz); 8.10 (d, 1 H, NH Tyr, ^3J = 8 Hz); 7.93 (s, 1 H, NH_{2b} Asn1); 7.84 (s, 1 H, NH_{2b} Asn2); 7.49 (d, 2 H, C(3′)H, C(5′)H Tyr, ^3J = 8 Hz); 7.07 (d, 2 H, C(2′)H, C(6′)H Tyr, ^3J = 8 Hz); 5.42 (m, 1 H, C(2)H Asn2); 5.37 (m, 1 H, C(2)H Asn1); 5.28 (m, 1 H, C(3)H β-AA); 4.98 (m, 1 H, C(3)H Thr); 4.88 (m, 3 H, C(2)H Ser, C(2)H Glu, C(2)H Thr); 4.74 (dd, 1 H, C(2)H Pro, ^3J = 3.2 Hz, ^3J = 6.5 Hz); 4.64 (m, 1 H, C(2)H Tyr); 4.39 (dd, 1 H, $C(3)H_{2a}$ Ser, ^2J = 12 Hz, ^3J = 4.5 Hz); 4.36 (dd, 1 H, $C(3)H_{2b}$ Ser, ^2J = 12 Hz, ^3J = 4.5 Hz); 4.27 (m, 1 H, $C(5)H_{2b}$ Pro); 4.05 (m, 1 H, $C(5)H_{2b}$ Pro); 3.74 (dd, 1 H, $C(3)H_{2a}$ Asn1, ^2J = 15 Hz, ^3J = 5 Hz); 3.60 (dd, 1 H, $C(3)H_{2a}$ Asn2, ^2J = 15 Hz, ^3J = 9 Hz); 3.40 (dd, 1 H, $C(3)H_{2b}$ Asn1, ^2J = 15 Hz, ^3J = 9 Hz); 3.20 (dd, 1 H, $C(3)H_{2b}$ Asn2, ^2J = 15 Hz, ^3J = 6 Hz); 3.12—2.92 (m, 4 H, $C(2)H_2$ β-AA, $C(4)H_2$ Glu); 2.80 (m, 1 H, $C(3)H_{2a}$ Glu); 2.65 (m, 2 H, $C(3)H_{2b}$ Glu, $C(3)H_{2a}$ Tyr); 2.43 (m, 1 H, $C(3)H_{2b}$ Tyr); 2.11 (m, 2 H, $C(3)H_2$ Pro); 1.88 (m, 1 H, $C(4)H_{2a}$ Pro); 1.67 (m, 1 H, $C(4)H_{2b}$ Pro); 1.58 (m, 1 H, $C(4)H_{2a}$ β-AA); 1.46 (m, 2 H, $C(4)H_{2b}$, $C(13)H_{2a}$ β-AA); 1.38 (d, 3 H, $C(4)H_3$ Thr, ^3J = 7 Hz); 1.29—1.07 (m, 16 H, C(5)—$C(12)H_2$ β-AA); 0.84 (d, 6 H, $C(14)H_3$, $C(15)H_3$ β-AA, ^3J = 7.5 Hz). ^{13}C NMR (75.5 MHz), δ: 173.72 (C_{quat}); 173.24 (C_{quat}); 172.86 (2 C_{quat}); 172.70 (2 C_{quat}); 172.48 (C_{quat}); 172.38 (C_{quat}); 171.71 (C_{quat}); 171.13 (C_{quat}); 157.46 (C_{quat}); 131.30 (CH); 128.62 (C_{quat}); 116.12 (CH); 66.16 (CH); 63.55 (CH_2); 62.21 (CH); 59.31 (CH); 58.01 (CH); 55.82 (CH); 55.39 (CH); 52.84 (CH); 50.30 (CH); 48.58 (CH_2); 47.42 (CH); 41.94 (CH_2); 39.20 (2 CH_2); 37.84 (CH_2); 37.11 (CH_2); 36.48 (CH_2); 35.71 (CH_2); 34.54 (CH_2); 32.07 (CH_2); 31.76 (CH_2); 30.17 (CH_2); 29.91 (CH_2); 29.88 (2 CH_2); 29.57 (CH_2); 28.14 (CH); 27.66 (CH_2); 26.05 (CH_2); 24.96 (CH_2); 22.89 (CH_2); 22.74 (2 CH_3); 20.83 (CH_3). ^{1}H—^{1}H COSY NMR (COSY 45, 300.1 MHz): NH Asn1 ↔ H(2) Asn1, NH β-AA ↔ H(3) β-AA, NH Asn2 ↔ H(2) Asn2, NH Ser ↔ H(2) Ser, NH_2 Asn2 ↔ NH_2 Asn2, NH_2 Glu ↔ NH_2 Glu, NH Glu ↔ H(2) Glu, NH Thr ↔ H(2) Thr, NH Tyr ↔ H(2) Tyr, H(2) Asn2 ↔ H(3) Asn2, H(2) Asn1 ↔ H(3) Asn1, H(2) β-AA ↔ H(3) β-AA, H(3) Thr ↔ H(4) Thr, H(2) Ser ↔ H(3) Ser, H(2) Glu ↔ H(3) Glu, H(2) Pro ↔ H(3) Pro, H(2) Tyr ↔ H(3) Tyr, $H(5)_a$ Pro ↔ $H(5)_b$ Pro, H(4) Pro, $H(3)_a$ Asn1 ↔ $H(3)_b$ Asn1, $H(3)_a$ Asn2 ↔ $H(3)_b$ Asn2, H(4) Glu ↔ H(3) Glu, $H(3)_a$ Glu ↔ $H(3)_b$ Glu, $H(3)_a$ Tyr ↔ $H(3)_b$ Tyr, H(3) Pro ↔ H(4) Pro, $H(4)_a$ Pro ↔ $H(4)_b$ Pro, H(13) β-AA ↔ H(14), H(15) β-AA.

Complete acid hydrolysis of peptides 1 and 2. Peptide **1** or **2** (1 mg) was dissolved in 6 *M* aqueous HCl (1 mL) in a glass tube. The tube was evacuated, sealed, and heated at 105 °C for 24 h. The reaction mixture was cooled, diluted with bidistilled water, and concentrated *in vacuo*. The α-amino acid composition was determined on an amino acid analyzer.[1]

Synthesis of isopropyl esters of *N*-trifluoroacetyl derivatives of complete acid hydrolyzates of peptides 1 and 2. Standard α-amino acids and peptide hydrolyzate (2 mg) were esterified with Pr^iOH (5 mL) by refluxing them for 2 h and saturating with gaseous HCl. The reaction mixture was concentrated *in vacuo*, dissolved in CH_2Cl_2 (4 mL), and stirred with 1 mL of $(CF_3CO)_2O$ at ~20 °C for 3 h. The resulting mixtures were concentrated *in vacuo*, dissolved in CH_2Cl_2 (2 mL), and analyzed by GLC.

Partial acid hydrolysis of peptides 1 and 2. Peptide **1** or **2** (1 mg) was dissolved in 2 *M* aqueous HCl (1 mL) and heated at 90 °C for 3 h in an atmosphere of nitrogen. The reaction mixture was diluted with bidistilled water, concentrated *in vacuo*, and analyzed by ESI-MS/MS.

Mixture of products of the partial hydrolysis of peptide 2. MS ((+)-ESI-MS), *m/z* (I_{rel} (%)): 1099 (27), 1067 (100), 1045 (98), 950 (23), 839 (50), 821 (57), 787 (32), 752 (92), 734 (68), 724 (34), 672 (46), 637 (52), 619 (33), 561 (34), 543 (16), 474 (47), 446 (20), 428 (15), 359 (28), 341 (22). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 839 (4), 821 $[M' - H_2O]^+$ (62), 706 $[M' - H_2O - Asp]^+$ (47), 651 (26), 543 $[M' - H_2O - Asp - Tyr]^+$ (100), 525 $[M' - 2\ H_2O - Asp - Tyr]^+$ (18), 410 $[M' - 2\ H_2O - 2\ Asp - Tyr]^+$ (3). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 752 (18), 734 $[M' - H_2O]^+$ (69), 619 $[M' - H_2O - Asp]^+$ (100), 456 $[M' - H_2O - Asp - Tyr]^+$ (98), 341 $[M' - H_2O - 2\ Asp - Tyr]^+$ (26). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 637 (12), 619 $[M' - H_2O]^+$ (9), 456 $[M' - H_2O - Tyr]^+$ (100), 341 $[M' - H_2O - Tyr - Asp]^+$ (37). MS ((+)-ESI-MS/MS), *m/z* (I_{rel} (%)): 619 (7), 601 $[M' - H_2O]^+$ (100), 591 (21), 456 $[M' - Tyr]^+$ (11), 438 $[M' - Tyr - H_2O]^+$ (8), 355

$[M' - Tyr - Thr]^+$ (13), 341 $[M' - H_2O - Tyr - Asp]^+$ (6). MS ((+)-ESI-MS/MS), m/z (I_{rel} (%)): 543 (35), 525 $[M' - H_2O]^+$ (100), 507 $[M' - 2\,H_2O]^+$ (9), 456 $[M' - Ser]^+$ (3), 355 $[M' - Ser - Thr]^+$ (25), 341 $[M' - Ser - Asp]^+$ (9). MS ((+)-ESI-MS/MS), m/z (I_{rel} (%)): 456 (52), 438 $[M' - H_2O]^+$ (66), 341 $[M' - Asp]^+$ (100), 338 (52), 299 (20), 258 (17).

This work was financially supported by the Russian Foundation for Basic Research (Project Nos. 00-04-48034 and 00-15-97397) and BMBF (Germany) (Grant No. 0310735).

References

1. G. L. Oleinikova, L. S. Shevchenko, T. A. Kuznetsova, and V. V. Mikhailov, *Antibiotiki i khimioterapiya* [*Antibiotics and Chemotherapy*], 1995, **40**, 19 (in Russian).
2. F. Peypoux, M. Guinand, G. Michel, L. Delcambe, B. Das, and E. Lederer, *Biochemistry*, 1978, **17**, 3992.
3. R. C. Gueldner, C. C. Reilly, P. L. Pussey, C. E. Costello, R. F. Arrendale, R. H. Cox, D. S. Himmelsbach, F. G. Cramley, and H. G. Cutler, *J. Agric. Food Chem.*, 1987, **36**, 366.
4. A. Isogai, S. Takayama, S. Murakoshi, and A. Suzuki, *Tetrahedron Lett.*, 1982, **23**, 3065.
5. R. Maget-Dana and F. Peypoux, *Toxicology*, 1994, **87**, 151.
6. P. B. Terent´ev, *Mass-spektrometriya v organicheskoi khimii* [*Mass Spectrometry in Organic Chemistry*], Vysshaya shkola, Moscow, 1979, 150 (in Russian).
7. K. Ishikawa, Y. Niwa, K. Hatakeda, and T. Goton, *Org. Mass Spectrom.*, 1988, **23**, 290.
8. F. Peypoux, M. T. Pommier, B. C. Das, F. Besson, L. Delcambe, and G. Michel, *J. Antibiot.*, 1984, **37**, 1600.
9. F. Peypoux, F. Besson, G. Michel, and L. Delcambe, *Eur. J. Biochem.*, 1981, **118**, 323.
10. U. Nagai, F. Besson, and F. Peypoux, *Tetrahedron Lett.*, 1979, **25**, 2359.

Received January 17, 2001;
in revised form May 17, 2001