Journal of Peptide Science
J. Peptide Sci. 2006; **12**: 321–327
Published online 21 October 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.728

JOURNAL OF PEPTIDE SCIENCE

Sequence diversity of the peptaibol antibiotic suzukacillin-A from the mold *Trichoderma viride*

CORINA KRAUSE,[a] JOCHEN KIRSCHBAUM,[a] GÜNTHER JUNG[b] and HANS BRÜCKNER[a]*

[a] Interdisciplinary Research Center, Department of Food Sciences, University of Giessen, Giessen, Germany
[b] Institute of Organic Chemistry, University of Tübingen, Tübingen, Germany

Received 3 August 2005; Accepted 10 August 2005

Abstract: From the culture broth of the mold *Trichoderma viride*, strain 63 C-I, the polypeptide antibiotic suzukacillin (SZ) was isolated. A peptide mixture named SZ-A was obtained by crystallization from crude SZ. Individual peptides from SZ-A were isolated by semipreparative HPLC and sequences were determined by HPLC-ESI-MS. The data confirm a general sequence of SZ-A published previously and in addition establish the individual sequences of 15 acetylated eicosa peptides with *C*-terminal alcohols. The major peptide SZ-A4 (21% of all peptides) shows the sequence:

Ac-Aib-Ala-Aib-Ala-Aib-Ala^{6}-Gln-Aib-Lx^{9}-Aib-Gly-Aib^{12}-Aib-Pro-Vx^{15}-Aib-Vx^{17}-Gln-Gln-Fol. Amino acid exchanges of the peptaibol are located in position 6 (Ala/Aib), 9 (Vx/Lx), 12 (Aib/Lx), 17 (Aib/Vx) and possibly at position15 (Val/Iva) (uncommon abbreviations: Aib (α-aminoisobutyric acid); Iva (D-isovaline); Lx (L-leucine or L-isoleucine); Vx (L-valine or D-isovaline); Fol (L-phenylalaninol)). Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: peptaibols; peptide antibiotics; α-aminoisobutyric acid; isovaline; suzukacillin-A; electrospray ionization mass spectrometry; sequencing

INTRODUCTION

From the culture broth of the mold *Trichoderma viride*, strain 63C-I, a polypeptide antibiotic named suzukacillin (SZ) was isolated in crystalline form. Investigation of SZ by TLC showed that this material comprises two major groups of peptides named SZ-A ($R_f = 0.18$) and SZ-B ($R_f = 0.70$) (Figure 1). Analysis of the amino acid composition showed among other amino acids, the presence of nonproteinogenic α-aminoisobutyric acid (Aib or U) [1,2]. In the following, L-phenylalaninol (L-Fol) and D-isovaline (Iva or J) in SZ-A were detected by ^{13}C-NMR and enantioselective gas chromatography [3,4]. Sequencing of trifluoroacetyl peptide methyl esters from partial hydrolysates of SZ-A using gas chromatography mass spectrometry (GC-MS) provided a preliminary sequence of SZ-A, including several amino acid exchange positions [3]. Sequences were refined on the basis of an approach used for sequencing the related 18-residue peptaibol trichotoxin [4,5]. Selective trifluoroacetolysis of SZ-A furnished peptides that could be resolved using silica gel and Sephadex G-10 chromatography. These fragments were converted into a mixture of di-, tri- and tetra-peptides by partial hydrolysis with 12 M HCl. The peptide mixtures were analyzed by GC-MS as their trifluoroacetyl peptide methyl esters or, after reduction of fragments, as *N*-ethylpolyaminoalcohol *O*-trimethylsilyl ethers. Overlapping sequences, together with field desorption and fast atom bombardment mass spectrometry of fragments, quantitative and enantioselective amino acid analysis and ^{13}C-NMR spectroscopy provided a general sequence of the eicosa peptide. In particular, the presence of *N*-terminal acetylated Aib (U) and *C*-terminal amino alcohol L-Fol were established and three amino acid exchange positions were localized (Figure 6) [6].

The sequences of SZ indicated a close sequential and conformational similarity with alamethicin, another *N*-acetylated eicosapeptide alcohol of the peptaibol family [3,6,7]. Methods such as ultraviolet and infrared spectroscopy, circular dichroism in various solvents and, in particular, ^{13}C nuclear magnetic resonance spectroscopy have been used for a comparative study of SZ and alamethicin [3]. Owing to amphiphilic, detergent-like properties, SZ causes hemolysis of erythrocytes [8] and exhibits weak antibiotic properties [1].

SZ modifies cation permeability of model membranes whereby oligomeric aggregates form transmembrane channels, whose conductance is dependent on the membrane potential [9–11]. Biological activities were correlated with moderate membrane permeabilization [12]. X-ray diffraction and NMR studies revealed high-resolution structures for several peptaibols and showed that these peptides adopt α-helical structures with a proline bend. Analysis of conductance levels provided support for the 'barrel stave' model of channel formation by bundles of parallel transmembrane helices [10,13–17] with varying pore sizes depending on the number of monomers forming a single pore.

One of the most characteristic features of peptaibols is their pronounced microheterogenicity, as a result

*Correspondence to: H. Brückner, University of Giessen, Interdisciplinary Research Center, Department of Food Sciences, Heinrich-Buff-Ring 26, 35392 Giessen, Germany;
e-mail: hans.brueckner@ernaehrung.uni-giessen.de

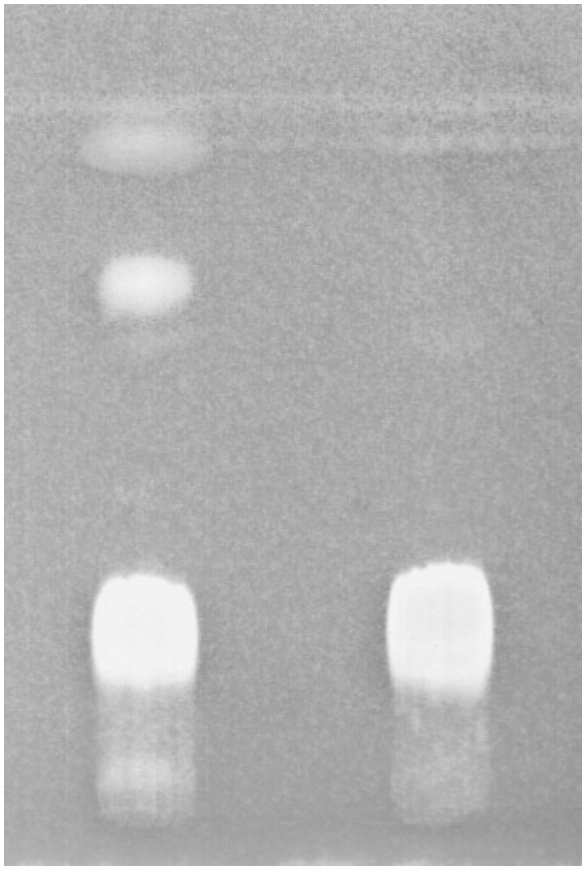

Figure 1 TLC of crude suzukacillin (SZ) and suzukacillin-A (SZ-A) isolated from *Trichoderma viride* 63C-1 and purified by crystallization (TLC: silica gel, chloroform/methanol 7/3, v/v).

of the nonribosomal biosynthesis of these fungal metabolites [18–21]. Single and multiple exchanges of amino acids accumulate in various defined positions and result in a complex pattern of closely related sequence analogs [22,23]. This has recently also been demonstrated for alamethicins [7,24], antiamoebins [25], stilboflavins [26] and trichovirins [27].

Since in previous work a general sequence and several exchange positions but no individual peptide sequences were assigned [6] we reinvestigated SZ-A using advanced techniques, in particular semipreparative HPLC together with on-line HPLC electrospray ionization mass spectrometry (HPLC-ESI-MS) and enantioselective GC-MS using chiral capillary columns.

EXPERIMENTAL

Materials and Methods

Chemicals. Acetonitrile and methanol of gradient grade quality were purchased from Merck (Darmstadt, Germany); chloroform was from Carl Roth (Karlsruhe, Germany); trifluoroacetic acid and L-phenylalaninol were from Fluka (Deisenhofen, Germany); α-methylalanine (Aib, U) was from Sigma; and DL-isovaline (Iva, J) was synthesized in our laboratory via the Strecker procedure. For TLC, precoated silica gel plates 60 F_{254} (no. 5714, Merck) were used.

Origin of Suzukacillin-A

Crude SZ was kindly provided by Dr T. Ooka, Japan [2]. Crystals of SZ-A were obtained from a solution of SZ in chloroform/acetone by addition of an excess of acetone. This material, uniform on TLC but still microheterogeneous (Figures 1 and 2), was used for sequencing.

Instrumental

For HPLC, an HP 1100 series instrument comprising Model G1322A degasser, G1312A binary pump, G1313A autosampler, G1316A column thermostat, G1314A UV/VIS detector, and HP ChemStation software for LC (Rev. A.04.02) was used (all from Agilent, Waldbronn, Germany or Palo Alto, CA, USA). The SZs were detected at a wavelength of 205 nm.

For HPLC, analytical column no. I (150 mm × 4.6 mm i.d.) filled with Kromasil KR100, with 3.5-µm particle size (EKA Chemicals, Bohus, Sweden), was used. For semipreparative HPLC, column no. II (150 mm × 10 mm i.d.) packed with the same batch of Kromasil was employed. Eluents and gradient elution conditions are compiled in Table 1.

For confirmation of the presence of SZ-A constituents and determination of their stereochemistry, a standard mixture was prepared composed of Aib, Gly and the L-enantiomers of Ala, Glu, Leu, Ile, Val and Fol and compared to a total hydrolysate of SZ-A. Enantioselective analyses of the standard mixture and SZ-A by GC on Chirasil-L-Val were performed similarly to procedures already described [27,28]. Components were converted into trifluoroacetyl amino acid 1-propyl esters or bistrifluoroacetyl derivatives of the amino alcohol, which were analyzed by GC-MS on a Chirasil-L-Val capillary column [28]. Isovaline was analyzed as *N*-acetyl-isovaline-(1)-propyl

Table 1 Gradient program and eluents at column temperatures of 35 °C for (a) analytical column no. I at flow rate 1 ml/min and (b) semipreparative column no. II at flow rate 3.5 ml/min. Eluent A, MeCN/MeOH/water 39/39/22, v/v/v; eluent B, MeCN/MeOH 1 : 1, v/v. Note that for analytical gradient elution 0.1% TFA was added to eluents A and B

(*a*)			(*b*)		
	Eluent			Eluent	
Time (min)	A (%)	B (%)	Time (min)	A (%)	B (%)
0	100	0	0	100	0
25	100	0	25	100	0
30	70	30	30	90	10
35	50	50	35	70	30
45	0	100	45	0	100
50	0	100	50	0	100
51	100	0	51	100	0
56	100	0	56	100	0

ester since the acetyl derivatives of DL-Iva are much better resolved on Chirasil-L-Val in comparison to the corresponding *N*-perfluoroacyl esters [4] (chromatogram not shown).

Mass Spectrometry

For HPLC-ESI-MS, an LCQ™ MS (Thermo Electron Corp., San Jose, CA, USA) was used. Nitrogen served as sheath and auxiliary gas and helium (purity >99.9990%, Messer-Griesheim, Krefeld, Germany) as collision gas. SZs were analyzed either by on-line HPLC-MS, using HPLC column no. I, or via direct infusion into the MS. Sequence analysis was carried out by positive and negative ionization. The m/z values were recorded in centroid mode and have an accuracy of 0.5 m/z (Table 2).

Conditions for direct infusion and positive (negative) ionization mode were: spray voltage 4.00 kV (4.00 kV), heated capillary temperature 230 °C, capillary voltage +3.0 V (−3.0 V), tube lens offset +30.0 V (−30.0 V), sheath gas 50 units, auxiliary gas 5 units, and maximum ion time 1000 ms. For on-line HPLC-MS, the temperature of the heated capillary was set to 250 °C, sheath gas to 65 units, and auxiliary gas

Table 2 Diagnostic ions (m/z) of suzukacillin-A

	Suzukacillin-A														
Diagnostic ions	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10a**	**10b**	**11a**	**11b**	**12**	**13**
$(M + H)^+$	1909	1923	1923	1937	1937	1923	1951	1937	1951	1951	1965	1951	1965	1979	1965
$(M + H - H_2O)^+$	1891	1905	1905	1919	1919	1905	1933	1919	1933	1933	1947	1933	1947	1961	1947
$(M + Na)^+$	1931	1945	1945	1959	1959	1945	1973	1959	1973	1973	1987	1973	1987	n.d.	1987
b_2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	213
b_3	284	284	284	284	284	284	284	284	284	284	284	284	284	284	284
b_4	355	355	355	355	355	355	355	355	355	355	355	355	355	355	355
b_5	440	440	440	440	440	440	440	440	440	440	440	440	440	440	440
b_6	511	511	511	511	525	511	525	511	511	511	511	511	511	525	511
b_7	639	639	n.d.	639	n.d.	639	n.d.	639	639	639	639	639	639	n.d.	639
b_8	724	724	724	724	738	724	738	724	724	724	724	724	724	738	724
b_9	823	837	823	837	851	837	851	837	837	823	837	837	837	851	837
b_{10}	908	922	908	922	936	922	936	922	922	908	922	922	922	936	922
b_{11}	965	979	965	979	993	979	993	979	979	965	979	979	979	993	979
b_{12}	1050	1064	1050	1064	1078	1064	1078	1063	1092	1078	1092	1092	1092	1106	1092
b_{13}	1135	1149	1135	1149	1163	1149	1163	1149	1177	1163	1177	1177	1177	1191	1177
b_{16}	1417	n.d.	1417	n.d.	1445	n.d.	1445	1431	n.d.	n.d.	1459	n.d.	n.d.	n.d.	n.d.
b_{17}	1502	1516	1516	1530	1530	1516	1544	1530	1544	1544	1558	1544	1558	1572	1558
b_{18}	n.d.	1644	n.d.	1658	1658	1644	1672	1658	1672	n.d.	1686	n.d.	1686	1700	1686
b_{19}	1758	1772	1772	1786	1786	1772	1800	1786	1800	1800	1814	1800	1814	1828	1814
y_{7p}	774	774	788	788	774	774	788	788	774	788	788	774	788	788	788
$(y_{7P} - H_2O)$	756	756	770	770	756	756	770	770	756	770	770	756	770	770	770
y_{7P} − AA (20)	623	623	637	637	623	623	637	637	623	637	637	623	637	637	637
y_{7P} − AA (20–19)	495	495	509	509	495	495	509	509	495	509	509	495	509	509	509
y_{7P} − AA (20–18)	367	367	381	381	367	367	381	381	367	381	381	367	381	381	381
y_{7P} − AA (20–17)	282	282	282	282	282	282	282	282	282	282	282	282	282	282	282
y_{7P} − AA (20–16)	197	197	197	197	197	197	197	197	197	197	197	197	197	197	197
M^-	1908	1922	1922	1936	1936	1922	1950	1936	1950	1950	1964	1950	1964	1978	1964
y_{3N}	407	407	407	407	407	407	407	407	407	407	407	407	407	407	407
y_{4N}	492	492	506	506	492	492	506	506	492	506	506	492	506	506	506
y_{5N}	577	577	591	591	577	577	591	591	577	591	591	577	591	591	591
y_{6N}	676	676	690	690	676	676	690	690	676	690	690	676	690	690	690
y_{7N}	773	773	787	787	773	773	787	787	773	787	787	773	787	787	787
y_{12N}	1184	1198	1198	1212	1212	1198	1226	1212	1226	1226	1240	1226	1240	1240	1240
y_{13N}	1269	1283	1283	1297	1297	1283	1311	1297	1311	1311	1325	1311	1325	1325	1325
y_{14N}	1397	1411	1411	1425	1425	1411	1439	1425	1439	1439	1453	1439	1453	1453	1453
y_{15N}	1468	1482	1482	1496	1496	1482	1510	1496	1510	1510	1524	1510	1524	1538	1524
y_{16N}	1553	1567	1567	1581	1581	1567	1595	1581	1595	1595	1609	1595	1609	1623	1609
y_{17N}	1624	1638	1638	1652	1652	1638	1666	1652	1666	1666	1680	1666	1680	1694	1680
y_{18N}	1709	1723	1723	1782	1782	1723	1751	1782	1751	1751	1765	1751	1765	1779	1765
y_{19N}	1780	1794	1794	1808	1808	1794	1822	1808	1822	1822	1836	1822	1836	1850	1836
y_{20N}	1865	1879	1879	1893	1893	1879	1907	1893	1907	1907	1921	1907	1921	1935	1921

n.d. = not detected.

to 20 units. For automated mass calibration, a mixture of caffeine (m/z 195.1), Met-Arg-Phe-Ala (m/z 524.3) and the perfluorinated Ultramark 1621 (m/z 1022.0, 1122.0, 1222.0, 1322.0, 1422.0, 1522.0, 1622.0, 1722.0, 1822.0, 1921.9) was used. The negative-ion mode was performed via infusion of a solution ($c = 0.1\%$, w/v) of SZ-A in MeOH containing 2% aqueous ammonia. For sequence analysis of SZ peptides, $(M + H)^+$ and $(M)^-$ molecular ions were chosen as precursor ions for MS^n.

The notation used for fragment assignments in the positive-ion mode, referring to *a*, *b* and *c* acylium ions, is in accordance with those used by us previously [7,24,27]. The negative-ion mode produced the *y*-series of fragment ions without protonation [7], and is denoted y_N in Table 2.

RESULTS AND DISCUSSION

Among various octadecylsilyl phases tested, the Kromasil KR 100 phase together with the gradient elution conditions described in Table 1 provided highest resolution for SZ-A peptides. The fingerprint HPLC of analytical column no. I is shown in Figure 2, which demonstrates the microheterogeneity of SZ-A, despite its crystallinity and uniformity on TLC. These characteristic features had also been recognized previously at lower resolution [6].

For sequence studies, SZ-A peptides were separated by employing semipreparative HPLC on column no. II filled with the same batch of the Kromasil stationary phase. Using the gradient program listed in Table 1, 13 peptide fractions were collected and combined. The numbers of the peaks assigned in the chromatogram shown in Figure 2 correspond to the fractions resulting from semipreparative chromatography.

Enantioselective amino acid analysis by GC-SIM-MS of the derivatized total hydrolysates of the peptides of SZ-A corroborated the presence of Aib, Gly, D-Iva, L-Val, L-Leu, L-Ile, L-Ala, L-Glu (from Gln), L-Pro and L-Fol. Analysis revealed that each of the fractions contained D-Iva together with isomeric L-Val as well as L-Leu and isomeric L-Ile. Since these isomers cannot be distinguished by HPLC-ESI-MS of the peptaibols Val and Iva are denoted as Vx, and Leu and Ile as Lx in the SZ-A sequences compiled in Figure 6.

Using analytical HPLC-ESI-MS and direct infusion ESI-MS of isolated fractions applying collision induced dissociation (CID) energy in positive and negative ionization mode, the sequences of the SZ-A peptides were determined. The diagnostic ions determined are listed in Table 2.

Positive-ion MS-MS of $(M + H)^+$ precursor ions generated the *b*- and *y*-series of fragment ions. The resulting *y*-series are internal fragments and are marked y_P (P for positive-ion mode). MS-MS of M^- in negative-ion mode also formed the *y*-series and were designated y_N (N refers to negative mode). Some of the diagnostic ions, such as $(M + H)^+$, $(M + Na)^+$, b_{13} and y_{7P} resulted from on-line HPLC-ESI-MS. Typically, characteristic fragment ions of the b_{13} and y_{7P} series were generated by cleavage of the extremely labile Aib^{13}–Pro^{14} bond [7]. The majority of the diagnostic ions were identified via HPLC-ESI-MS^n ($n = 2$–4) in positive-ion mode from specific precursor ions $(M + H)^+$ and fragment ions b_{13} and y_{7P}, as well as b_{10} and b_8. The b_3 ions of the peptides SZ-A2, SZ-A4, SZ-A10a and SZ-A13 were only recorded using direct infusion at positive ionization mode.

From these fragments, sets of diagnostic fragment ions of the b_3–b_{12} series and internal fragment ions of y_{7P} were generated and provided sequence information on the amino acid positions 3–13 and 16–20. Figure 3 shows the diagnostic fragment ions of SZ-A4 after HPLC-ESI-MS in the positive-ion mode. HPLC-ESI-MS-MS of b_{13} of SZ-A4 provided the mass spectrum shown in Figure 4, and the mass spectrum demonstrating formation of diagnostic ions from HPLC-ESI-MS-MS of y_{7P} is presented in Figure 5.

The generation of b_7 ions was not observed in each case because of the very stable Gln–Aib bonds.

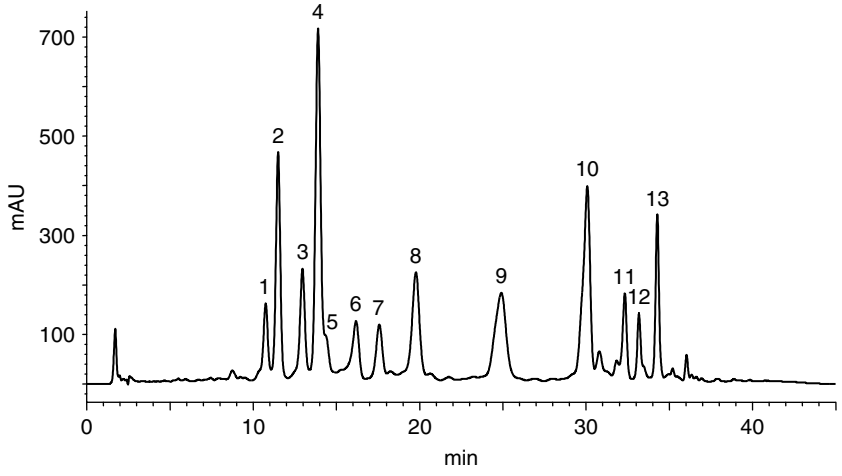

Figure 2 Analytical HPLC of crystalline suzukacillin-A. Numbers correspond to peptide sequences presented in Figure 6.

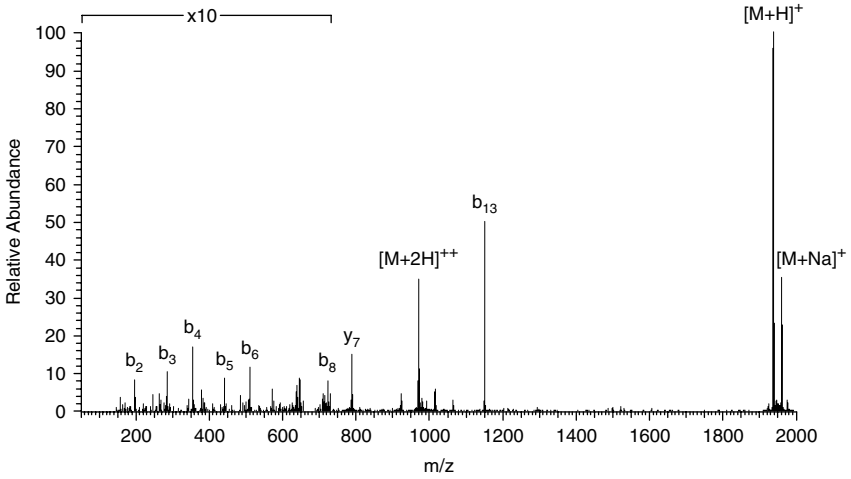

Figure 3 Characteristic mass spectrum (positive-ion mode) of suzukacillin-A4 resulting from HPLC-ESI-MS analysis.

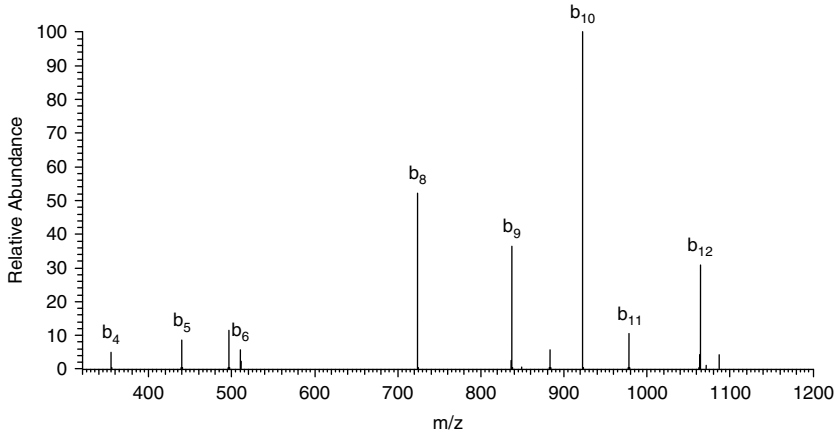

Figure 4 Mass spectrum (positive-ion mode) with fragment ions of b_{13} of suzukacillin-A4 resulting from HPLC-ESI-MS-MS.

The amino acids in these positions were confirmed by intense signals (y_{13N} and y_{12N}) using the negative ionization mode and direct infusion of SZ-A dissolved in a mixture of methanol/water (v/v, 1/1) containing 1% ammonia. Since only the mass difference of 128 amu was detected, the exclusive presence of Gln in SZ-A was confirmed [6] and no sequences containing Glu were found as in alamethicins F30 [7].

The b_1 fragment ions, which should be generated from the *N*-acetyl-Aib occurring in all peptides, were not detected under the conditions of positive ESI-MS. Thus, the *N*-terminal amino acids were determined from fragments resulting from *y*-series of negative ions generated from MS-MS of $(M)^-$ by applying direct infusion in the negative ionization mode. The mass differences between y_{20N} and M^- (43 amu) and y_{19N} to M^- (128 amu) represent the *N*-terminal fragment Ac-Aib. Diagnostic product ions y_{19N} to y_{12N} were also analyzed via negative ionization MS-MS from M^- and MS^3 of fragment ion y_{17N} (Table 2). The *C*-terminal positions of Fol in all SZ-A peptides were deduced from the mass difference of 151 amu between y_{7P} (m/z 774 or 788) and the internal fragments y_{7P}-AA(20) (m/z 623 or 637).

The smallest fragment detectable in HPLC-ESI-MS-MS and direct infusion ESI-MS-MS at positive mode of y_{7P} showed m/z 197, representing Pro^{14}-Vx^{15}. Because the y_{7P} ion results from cleavage of the labile Aib–Pro bond, Pro has to be the first amino acid in the y_{7P} sequence. This was also deduced from the series of negative ions y_{7N}, y_{6N} and y_{5N}, providing mass differences of 97 amu (Pro) and 99 amu (Vx).

From the data, 15 individual sequences of peptides of SZ-A were deduced and compared to the general sequence of SZ-A that had been reported previously (Figure 6). The analysis confirms exchange positions 6 (A/U) and 17 (Vx/U) of the general sequence SZ-A. No Aib was detected in position 9 (Lx/Vx) but an additional exchange was established in position 12 (U/Lx). Furthermore, relative quantities of SZ-A peptides could be calculated from the HPLC elution profile.

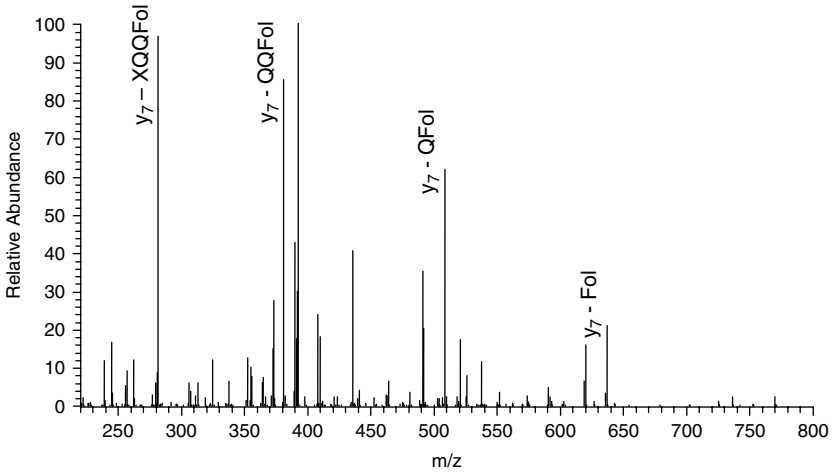

Figure 5 Mass spectrum (positive-ion mode) with fragment ions of y_{7P} of suzukacillin-A4 resulting from HPLC-ESI-MS-MS.

no.		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	%
1	Ac	U	A	U	A	U	A	Q	U	Vx	U	G	U	U	P	Vx	U	U	Q	Q	Fol	4.2
2	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	U	U	P	Vx	U	U	Q	Q	Fol	11.8
3	Ac	U	A	U	A	U	A	Q	U	Vx	U	G	U	U	P	Vx	U	Vx	Q	Q	Fol	6.1
4	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	U	U	P	Vx	U	Vx	Q	Q	Fol	20.9
5	Ac	U	A	U	A	U	U	Q	U	Lx	U	G	U	U	P	Vx	U	U	Q	Q	Fol	1.7
6	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	U	U	P	Vx	U	U	Q	Q	Fol	3.5
7	Ac	U	A	U	A	U	U	Q	U	Lx	U	G	U	U	P	Vx	U	Vx	Q	Q	Fol	3.1
8	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	U	U	P	Vx	U	Vx	Q	Q	Fol	8.3
9	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	Lx	U	P	Vx	U	U	Q	Q	Fol	10.7
10a	Ac	U	A	U	A	U	A	Q	U	Vx	U	G	Lx	U	P	Vx	U	Vx	Q	Q	Fol	15.7
10b	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	Lx	U	P	Vx	U	Vx	Q	Q	Fol	
11a	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	Lx	U	P	Vx	U	U	Q	Q	Fol	4.7
11b	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	Lx	U	P	Vx	U	Vx	Q	Q	Fol	
12	Ac	U	A	U	A	U	U	Q	U	Lx	U	G	Lx	U	P	Vx	U	Vx	Q	Q	Fol	2.9
13	Ac	U	A	U	A	U	A	Q	U	Lx	U	G	Lx	U	P	Vx	U	Vx	Q	Q	Fol	6.5
	Ac	U	A	U	A	U	A (U)	Q	U	U (L)	U	G	L	U	P	V	U	J (U)	Q	Q	Fol	

Figure 6 Sequences of suzukacillin-A (SZ-A) peptides nos. 1–13 and their relative quantities (%) in comparison with a general sequence of SZ-A [6]; abbreviations according to one-letter nomenclature; Ac = acetyl; U = α-aminoisobutyric acid; Lx = Leu or Ile; Vx = Val or Iva (isovaline, J); Fol = L-phenylalaninol. Chiral amino acids are of the L-configuration with the exception of D-Iva. For HPLC see Figure 2; for TLC see Figure 1.

The isomeric amino acids denoted as Vx and Lx are not distinguishable under our routine conditions of ESI-MS. Their differentiation was not part of this work. This is a basic problem of ESI-MS sequencing. It can be safely assumed that these amino acid exchanges do not influence bioactivity very much. Therefore, many sequences just indicating the exchange positions of these isomers have been included in the peptaibol database [22].

CONCLUSION

SZ-A is a microheterogeneous mixture of at least 15 metabolic fungal peptides belonging to the peptaibol family [22]. This mixture might also be considered as a natural peptaibol library [26,29,30]. The peptides are distinguished by their conserved and dynamic domains, the latter characterized by exchange of certain amino acids. The sequential comparison shows that neutral SZ-A peptides are closely related to alamethicin F-50 peptides, which possess no negatively charged *C*-terminal glutamic acid. The most conspicuous difference is the presence of Pro in position two of alamethicins. This may cause the minor, but distinct, differences found in the characteristics of potential-dependent pore formation [10] of these two membrane-modifying peptaibols.

REFERENCES

1. Ooka T, Shimojima Y, Akimoto T, Takeda I, Senoh S, Abe J. A new antibacterial peptide "Suzukacillin". *Agric. Biol. Chem.* 1966; **30**: 700–702.
2. Ooka T, Takeda I. Studies of peptide antibiotic suzukacillin. Part II. *Agric. Biol. Chem.* 1972; **36**: 112–119.
3. Jung G, König WA, Leibfritz D, Ooka T, Janko K, Boheim G. Structural and membrane modifying properties of suzukacillin, a peptide antibiotic related to alamethicin. Part A. Sequence and conformation. *Biochim. Biophys. Acta* 1976; **433**: 164–181.
4. Brückner H, Nicholson GJ, Jung G, Kruse M, König WA. Gas chromatographic determination of the configuration of isovaline in antiamoebin, samarosporin (emerimicin IV), stilbellin, suzukacillins and trichotoxins. *Chromatographia* 1980; **13**: 209–214.
5. Brückner H, König WA, Aydin M, Jung G. Trichotoxin A40. Purification by counter-current distribution and sequencing of isolated fragments. *Biochim. Biophys. Acta* 1985; **827**: 51–62.
6. Katz E, Aydin M, Lucht N, König W, Ooka T, Jung G. Sequence and conformation of suzukacillin A. *Liebigs Ann. Chem.* 1985; **1985**: 1041–1062.
7. Kirschbaum J, Krause C, Winzheimer RK, Brückner H. Sequences of alamethicins F30 and F50 reconsidered and reconciled. *J. Pept. Sci.* 2003; **9**: 799–809.
8. Irmscher G, Jung G. Die hämolytischen Eigenschaften der membran-modifizierenden Peptidantibiotika Alamethicin, Suzukacillin and Trichotoxin. *Eur. J. Biochem.* 1977; **80**: 165–174.
9. Mueller P, Rudin DO. Action potentials induced in biomolecular lipid membranes. *Nature* 1968; **217**: 713–719.
10. Boheim G, Janko K, Leibfritz D, Ooka T, König WA, Jung G. Structural and membrane modifying properties of suzukacillin, a peptide antibiotic related to alamethicin. Part B. Pore formation in black lipid films. *Biochim. Biophys. Acta* 1976; **433**: 182–199.
11. Schindler H. Autocatalytic transport of the peptide antibiotics suzukacillin and alamethicin across lipid membranes. *FEBS Lett.* 1979; **104**: 157–160.
12. Grigoriev PA, Schlegel B, Kronen M, Berg A, Härtl A, Gräfe U. Differences in membrane pore formation by peptaibols. *J. Pept. Sci.* 2003; **9**: 763–768.
13. Sansom MSP. Alamethicin and related peptaibols – model ion channels. *Eur. Biophys. J.* 1993; **22**: 105–124.
14. Chugh JK, Wallace BA. Peptaibols: models for ion channels. *Biochem. Soc. Trans.* 2001; **29**: 565–570.
15. Whitmore L, Wallace BA. Analysis of peptaibol sequence composition: implications for in vivo synthesis and channel formation. *Eur. Biophys. J.* 2004; **33**: 233–237.
16. Chugh JK, Brückner H, Wallace BA. Model for a helical bundle channel based on the high-resolution crystal structure of Trichotoxin A50E. *Biochemistry* 2002; **41**: 12934–12941.
17. Duclohier H, Wroblewski H. Voltage-dependent pore formation and antimicrobial activity by alamethicin and analogues. *J. Membr. Biol.* 2001; **184**: 1–12.
18. Rindfleisch H, Kleinkauf H. Biosynthesis of alamethicin. *FEBS Lett.* 1976; **62**: 276–280.
19. Kleinkauf H, von Döhren H. Nonribosomal biosynthesis of peptide antibiotics. *Eur. J. Biochem.* 1990; **192**: 1–15.
20. Reiber K, Neuhof T, Ozegowski JH, von Döhren H, Schwecke T. A nonribosomal peptide synthetase involved in the biosynthesis of ampullosporins in *Sepedonium ampullosporum*. *J. Pept. Sci.* 2003; **9**: 701–713.
21. Raap J, Erkelens K, Ogrel A, Skladnev DA, Brückner H. Fungal biosynthesis of non-ribosomal peptide antibiotics and α,α-dialkylated amino acid constituents. *J. Pept. Sci.* 2005; **11**: 331–338.
22. Whitmore L, Chugh JK, Snook CF, Wallace BA. The peptaibol database: a sequence and structure resource. *J. Pept. Sci.* 2003; **9**: 663–665.
23. Degenkolb T, Berg A, Gams W, Schlegel B, Gräfe U. The occurrence of peptaibols and structurally related peptaibiotics in fungi and their mass spectrometric identification via diagnostic fragments ions. *J. Pept. Sci.* 2003; **9**: 666–678.
24. Psurek A, Neusüß C, Degenkolb T, Brückner H, Balaguer E, Imhof D, Scriba GKE. Detection of new amino acid sequences of alamethicins F30 by nonaqueous capillary electrophoresis – mass spectrometry. *J. Pept. Sci.* 2005; in press; DOI:10.1002/psc.720
25. Jaworski A, Brückner H. New sequences and new fungal producers of peptaibol antibiotics antiamoebins. *J. Pept. Sci.* 2000; **6**: 149–167.
26. Jaworski A, Brückner H. Sequences of polypeptide antibiotics stilboflavins, natural peptaibol libraries of the mold *Stilbella flavipes*. *J. Pept. Sci.* 2001; **7**: 433–447.
27. Jaworski A, Kirschbaum J, Brückner H. Structures of trichovirin II, peptaibol antibiotics from the mold *Trichoderma viride* NRRL 5243. *J. Pept. Sci.* 1999; **5**: 341–351.
28. Pätzold R, Brückner H. Chiral separation of amino acids by gas chromatography. In *Quantitation of Amino Acids and Amines by Chromatography*, *Journal of Chromatography Library*. Vol **70**, Molnár-Perl I (ed.). Elsevier: Amsterdam, 2005; 98–118.
29. Jung G. Natural peptide libraries of microbial and mammalian origin. In *Combinatorial Peptide and Nonpeptide Libraries*, Jung G (ed.). Wiley-VCH: Weinheim, 1996; 1–18.
30. Lehr N, Antelo L, Meffert A, Anke H, Weber R, Sterner O. Antiamoebins, myrocin B and the basis of antifungal antibiosis in the coprophilous fungus *Stilbella erythrocephala*. *FEMS Microbiol. Ecol.* 2005; in press.