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Tylopeptins A and B, New Antibiotic Peptides from *Tylopilus neofelleus*

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Two new peptides, tylopeptins A and B, were isolated from the methanol extract of the fruiting body of the mushroom, *Tylopilus neofelleus*. These peptides were identified as peptaibols possessing an acetylated *N*-terminal residue, fourteen amino acids, and leucinol as the *C*-terminal amino alcohol. Sequential determination and complete ^{1}H and ^{13}C resonance assignments were based on positive ion FAB mass spectroscopy and two dimensional NMR techniques. These peptides were subsequently shown to be active against some Gram-positive bacteria, but inactive against pathogenic fungi and Gram-negative bacteria.

Linear hydrophobic peptides from fungi called peptaibols are well known to have antibiotic activity against phytopathogenic fungi and Gram-positive bacteria. Their antibiotic activity is mediated *via* the formation of amphipathic helices that interact with the phospholipid bilayers perturbing their permeability by forming voltage-gated ion channels.[1~4]

Peptaibols are characterized by the high content of α,α-dialkylated amino acids such as α-aminoisobutyric acid (Aib, U) and isovaline (Iva, J), and contain an acetylated *N*-terminus and *C*-terminal amino alcohol. Based on their chain lengths, they are classified into the long sequence peptaibols with 18~20 residues such as alamethicins[5], trichosporins[6], trichorzins[7], chrysospermins[8], and longibrachins[9], the short sequence peptaibols with 11~16 residues as exemplified by emerimicins[10], zervamicins[11], antiamoebins[12], harzianins[13], and bergofungin[14], and the lipopeptaibols with 7 or 11 residues and an *N*-terminal lipid chain such as trichogin[15], and trichodecenins[16].

In the course of the searching for novel antibiotic peptides from various mushrooms, we have isolated the short sequence peptaibols, tylopeptins A and B, from *Tylopilus neofelleus* (Fig. 1). This report describes the isolation, structure determination, and biological activities of tylopeptins A and B.

Fig. 1. Amino acid sequence of tylopeptins A and B from *Tylopilus neofelleus*

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tylopeptin A :	Ac	L-Trp	L-Val	L-Aib	D-**Iva**	L-Ala	L-Gln	L-Ala	L-Aib	L-Ser	L-Aib	L-Ala	L-Leu	L-Aib	L-Gln	L-Leuol
Tylopeptin B :	Ac	L-Trp	L-Val	L-Aib	L-**Aib**	L-Ala	L-Gln	L-Ala	L-Aib	L-Ser	L-Aib	L-Ala	L-Leu	L-Aib	L-Gln	L-Leuol

Bold-faced letters indicate those amino acids which differ in the sequences: Aib, U: α-aminoisobutyric acid, Iva, J: isovaline, Leuol, LO: Leucinol.

Table 1. Physico-chemical properties of tylopeptins A and B.

	Tylopeptin A	Tylopeptin B
Appearance	White powder	White powder
MP	215~218 °C	214~217 °C
$[\alpha]_D^{25}$	-36 °(*c* 0.005, MeOH)	-25 °(*c* 0.01, MeOH)
Molecular formula	$C_{73}H_{120}N_{18}O_{19}$	$C_{72}H_{118}N_{18}O_{19}$
Molecular weight		
FAB-MS(*m/z*)	1554 $(M+H)^+$, 1576 $(M+Na)^+$	1540 $(M+H)^+$ 1562 $(M+Na)^+$
UV λ_{max} nm, MeOH	290 (sh), 280, 227	289 (sh), 280, 226
IR ν_{max} (KBr) cm^{-1}	3432, 3315, 2924 1656, 1542, 1458	3420, 3310, 2930 1658, 1536, 1386
TLC (Rf values)[a]	0.44	0.44
HPLC[b]	27.2 min	25.0 min
Solubility		
Soluble	MeOH, DMSO	MeOH, DMSO
Insoluble	H_2O	H_2O

[a] Silica gel plate (Silica gel 60 F_{254}, Merck), methanol - chloroform (3 : 7).
[b] MetaSil 5 μ ODS, 4.6 mm i.d.×250 mm, solvent: 80% MeOH, flow rate: 0.7 ml/minute, detector: UV 220 nm.

Results and Discussion

Isolation and Purification

The dried fruiting bodies of *Tylopilus neofelleus* (70 g, dry weight) was extracted with methanol (1 liter×2). The methanolic extract (18 g of dark brown sticky substance) was partitioned sequentially between water and hexane (500 ml×3), chloroform (500 ml×3), and ethyl acetate (500 ml×3). The chloroform extract (3.5 g) was chromatographed on a silica gel column eluted with $CHCl_3$/MeOH (20 : 1 increasing to 3 : 1). The residue eluted with $CHCl_3$/MeOH (5 : 1) was subsequently purified by semipreparative HPLC (MAXSIL 5 μ C_{18}, Phenomenex, 10 mm i.d.×250 mm, MeOH/H_2O (80 : 20); UV detection at 210 nm, flow rate 3 ml/minute) to yield tylopeptin A (6 mg, Rt 24.1 minutes) and B (4 mg, Rt 22.5 minutes).

Physico-chemical Properties and Amino Acid Composition of Tylopeptins A and B

Physico-chemical properties of tylopeptins A and B are summarized in Table 1. The IR absorptions at 3315 (NH), 1656 and 1542 cm^{-1} are characteristic of peptides. The UV absorption at 280 nm indicated the presence of aromatic ring(s). Their relatively nonpolar characteristics and negative response to the ninhydrin test were indicative of a cyclic or a *N*-terminal substituted linear peptide.

The amino acid compositions of tylopeptins A and B were determined by HPLC analysis on a Supelcosil LC-18 column of the complete acid hydrolysates (6 N HCl, 110 °C, 24 hours), after derivatization of the amino acids as phenylthiocarbamyl amino acids. Tylopeptin A was shown to contain Aib (4), Ala (3), Glx (2), Iva (1), Leu (1), Leuol (1), Ser (1), Trp (1), and Val (1). Tylopeptin B differs from tylopeptin A in that it contains Aib instead of Iva (1) found in tylopeptin A. The two Glx residues obtained in the total hydrolysates were assigned to Gln from the later observation of the *syn* and *anti* ε-protons of the two carboxamide groups in the ^1H NMR spectra.

The absolute stereochemistry of all the amino acids of tylopeptins A and B was determined to be L by GC analysis of the hydrolysates of tylopeptins A and B, except for that of isovaline which was determined to be D.

Table 2. ^{1}H and ^{13}C NMR chemical shifts of tylopeptin A in DMSO-d_6.

	δ_C	δ_H
Ac		
C-1	170.5	
C-2	22.6	1.87
Trp^{1}		
N-H		8.3 d, 6.5
C=O	172.8	
α	54.6	4.45, m
β	27.0	3.16 dd,4.2,15
		3.01 dd,6.3,15
1-NH		10.8
2	123.3	7.2 s
3	110.0	
4	118.0	7.51 d, 7.5
5	120.5	7.05 t, 7.5
6	118.2	6.97 t, 7.5
7	111.3	7.33 d, 7.5
8	136.2	
9	127.2	
Val^{2}		
N-H		7.8 brd
C=O	172.5	
α	59.4	3.87 m
β	29.2	2.17 m
γ	18.5	0.86 d, 6.4
δ	19.1	0.90 d, 6.7
Aib^{3}		
N-H		8.21 s
C=O	175.0	
α	55.8	
β	23.3	1.32 s
γ	23.9	1.39 s
Iva^{4}		
N-H		7.90s
C=O	176.5	
α	58.4	
β	25.5	2.18 m
		1.66 m
γ	7.2	0.73 t, 7.4
δ	22.5	1.32 s

	δ_C	δ_H
Ala^{5}		
N-H		7.82 d, 5.0
C=O	175.2	
α	51.0	3.97 m
β	16.4	1.45 d, 7.3
Gln^{6}		
N-H		7.97 d, 6.1
C=O	173.3	
α	54.9	4.0 m
β	27.2	1.97 m
γ	31.4	2.15 m
		2.27 m
C=O	173.2	
NH$_2$		6.67 br, 7.11 br
Ala^{7}		
N-H		
C=O	173.9	7.57 d, 5.7
α	51.3	4.04 m
β	16.0	1.33 d, 7.1
Aib^{8}		
N-H		7.91 s
C=O	175.6	
α	55.5	
β	24.1	1.35 s
γ	26.0	1.44 s
Ser^{9}		
N-H		7.64 d, 3.9
C=O	171.7	
α	60.2	3.88 m
β	60.4	3.77 m
O-H		4.8 d, 5.8
Aib^{10}		
N-H		7.84 d, 5.7
C=O	175.4	
α	55.6	
β	26.1	1.45 s
γ	25.9	1.38 s

	δ_C	δ_H
Ala^{11}		
N-H		7.62 d, 5.1
C=O	174.4	
α	51.1	3.98 m
β	16.4	1.40 d, 7.0
Leu^{12}		
N-H		7.73 d, 5.88
C=O	173.2	
α	53.3	3.94 m
β	39.2	1.53 m
γ	26.1	1.77 m
δ	22.7	0.86 d, 6.7
ε	23.4	0.84 d, 6.7
Aib^{13}		
N-H		7.55 s
C=O	173.8	
α	56.1	
β	23.1	1.38 s
γ	23.8	1.37 s
Gln^{14}		
N-H		7.19 d, 6.0
C=O	170.9	
α	53.3	3.97 m
β	27.0	1.83 m
γ	31.5	2.10 m
		2.22 m
C=O	173.3	
NH$_2$		7.14 br, 6.7 br
Leuol^{15}		
N-H		7.03 m
α	48.7	3.78 m
β^{1}	63.9	3.21 m
		3.32 m
β	48.7	1.35 m
γ	39.5	1.65 m
δ	22.7	0.80 d, 6.4
δ^{1}	21.6	0.82 d, 6.5
O-H		4.49 t, 5.9

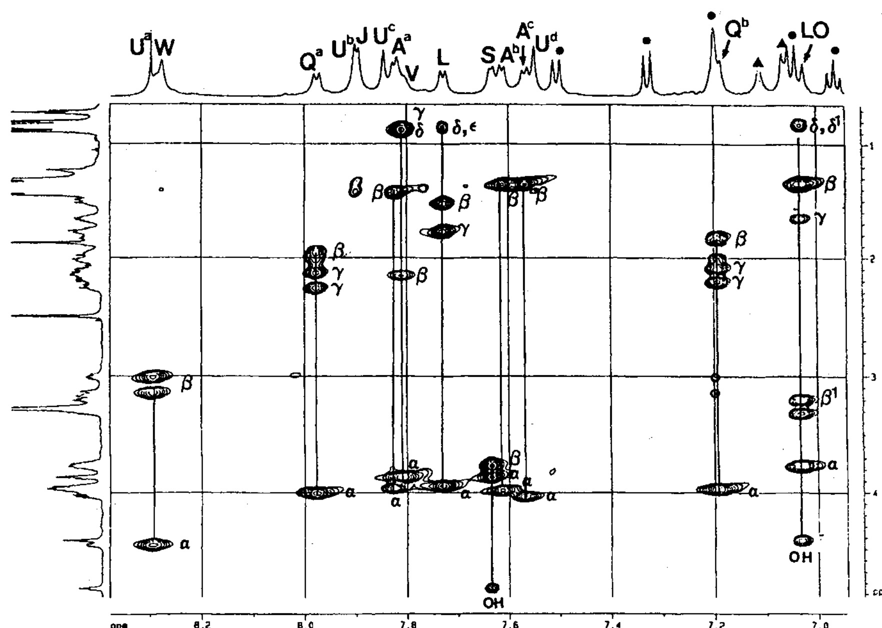
Sequence Determination and Complete NMR Assignment of Tylopeptin A

Tylopeptin A showed molecular ion peaks at *m/z* 1554 $(M+H)^+$ and 1576 $(M+Na)^+$ in the FABMS spectrum and the molecular formula was determined as $C_{73}H_{120}N_{18}O_{19}$ by HRFABMS [Found *m/z* 1553.9021, Calcd. 1553.9055 for $(M+H)^+$]. The proton NMR spectrum of tylopeptin A showed resonances characteristic of a peptide in DMSO-d_6 solvent. Amide and C^{α} proton signals were observed at δ 7~8 and δ 3.5~4.5, respectively, and upfield side-chain proton signals were also observed. A sharp singlet at δ 1.87 indicated that the *N*-terminal residue was acetylated.

The proton chemical shifts for each amino acid moiety were assigned by COSY and HOHAHA NMR experiments. Ten doublet amide NH signals were assignable, while five singlet amide signals that did not show correlation peaks in the HOHAHA spectrum indicated the disubstitution at α position (Fig. 2). Amide proton signals for fourteen amino

Fig. 2. HOHAHA spectrum of tylopeptin A (600 MHz, DMSO-d_6).

Amide protons correlated with C^{α} proton and aliphatic proton resonances.
U=Aib, J=Iva, LO=leuol, ▲=CO–NH_2 for glutamine, ●=aromatic proton signals of tryptophan.



acid residues and an amino alcohol were as follows: δ 8.3 (NH-Trp), 8.21 (NH-Aib[a]), 7.97 (NH-Gln[a]), 7.91 (NH-Aib[b]), 7.90 (NH-Iva), 7.84 (NH-Aib[c]), 7.82 (NH-Ala[a]), 7.80 (NH-Val), 7.73 (NH-Leu), 7.64 (NH-Ser), 7.62 (NH-Ala[b]), 7.57 (NH-Ala[c]), 7.55 (NH-Aib[d]), 7.19 (NH-Gln[b]), 7.03 (NH-Leuol) (Fig. 2).

The amino acid sequence of tylopeptin A was determined by 2D NMR analysis. The ^{13}C NMR spectrum of tylopeptin A revealed the amide carbonyl resonances of fourteen amino acid residues and an amino alcohol (Fig. 3). Of these, nine amide carbonyl resonances were determined from correlation peaks to each C^{α} proton signal in the HMBC spectrum (Fig. 4): δ 175.2 (CO-Ala^5), 174.4 (CO-Ala^{11}), 173.9 (CO-Ala^7), 173.7 (CO-Gln^6), 173.2 (CO-Leu^{12}), 172.8 (CO-Trp^1), 172.5 (CO-Val^2), 171.7 (CO-Ser^9), 170.9 (CO-Gln^{14}). The HMBC spectrum showed correlations of NH(Aib^3)/CO(Val^2), NH(Iva^4)/CO(Aib^3), NH(Ala^5)/CO(Iva^4), NH(Gln^6)/CO(Ala^5), NH(Ala^7)/CO(Gln^6), NH(Aib^8)/CO(Ala^7), NH(Leu^{12})/CO(Ala^{11}), NH(Aib^{13})/CO(Leu^{12}), and NH(Leuol^{15})/CO(Gln^{14}) indicating the presence of segments of Val^2-Aib^3-Iva^4-Ala^5-Gln^6-Ala^7-Aib^8, Aib-Ser, and Ala^{11}-Leu^{12}-Aib^{13}-Gln^{14}-Leuol^{15}. However, the correlation peaks showing the sequence of NH(Val^2)/CO(Trp^1), NH(Ser^9)/CO(Aib^8) and NH(Ala^{11})/CO(Aib^{10}) were not observed in the HMBC spectrum (Fig. 4).

Conclusive evidence for the amino acid sequence of tylopeptin A was derived from the ROESY spectrum, which showed the correlation peaks of methyl(Ac)/NH(Trp^1), NH(Aib^8)/NH(Ser^9), NH(Ser^9)/NH(Aib^{10}), NH(Gln^{14})/NH(Leuol^{15}) (Fig. 5). This sequence was further confirmed by the FABMS fragments as follows: m/z 1308 (Ac-Trp-Val-Aib-Iva-Ala-Gln-Ala-Aib-Ser-Aib-Ala-Leu-Aib)$^+$, 1223 (1308-Aib)$^+$, 1110 (1223-Leu)$^+$, 1039 (1110-Ala)$^+$, 954 (1039-Aib)$^+$, 867 (954-Ser)$^+$, 782 (867-Aib)$^+$, 583 (Ac-Trp-Val-Aib-Iva-Ala)$^+$, 512 (583-Ala)$^+$, 413 (512-Aib)$^+$, 328 (Ac-Trp-Val)$^+$, 229 (Ac-Trp)$^+$. Thus, the structure of tylopeptin A was concluded to be Ac-Trp^1-Val^2-Aib^3-Iva^4-Ala^5-Gln^6-Ala^7-Aib^8-Ser^9-Aib^{10}-Ala^{11}-Leu^{12}-Aib^{13}-Gln^{14}-Leuol^{15}.

Sequence Determination of Tylopeptin B

Tylopeptin B showed molecular ion peaks at m/z 1540 $(M+H)^+$ and 1562 $(M+Na)^+$ in the FABMS spectrum. The molecular formula of tylopeptin B was determined to be $C_{72}H_{118}N_{18}O_{19}$. The amino acid sequence of tylopeptin B was derived from the FABMS fragments based on the

Fig. 3. ^{1}H-decoupled ^{13}C NMR spectrum of tylopeptin A and B (100 MHz, DMSO-d_6).
▲ = CO–NH_2 for two glutamines.

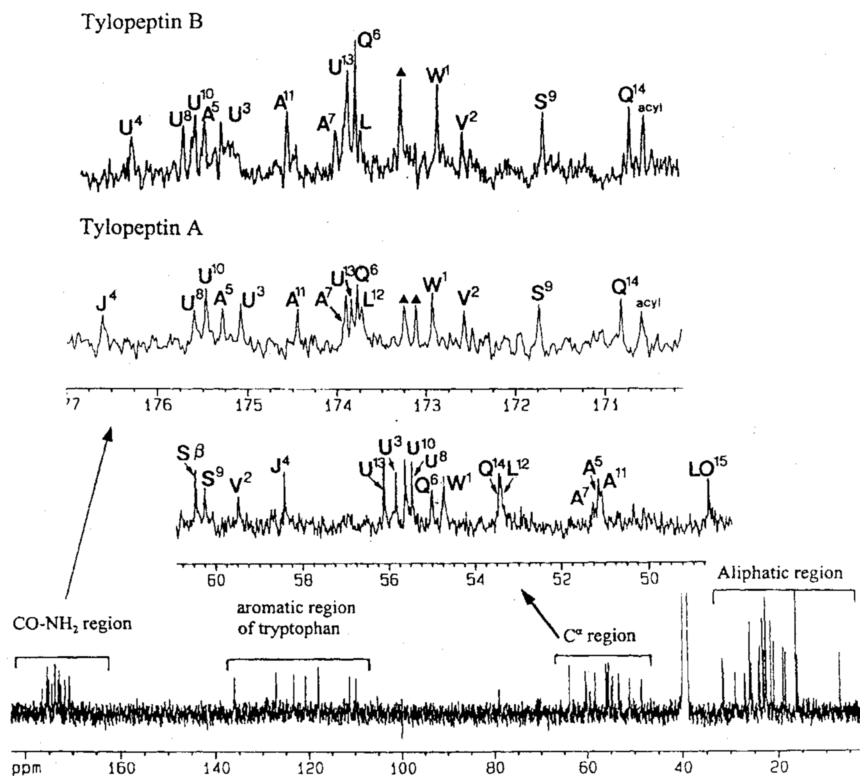


Fig. 4. HMBC correlations for determining the sequence of amino acids for tylopeptin A in DMSO-d_6.

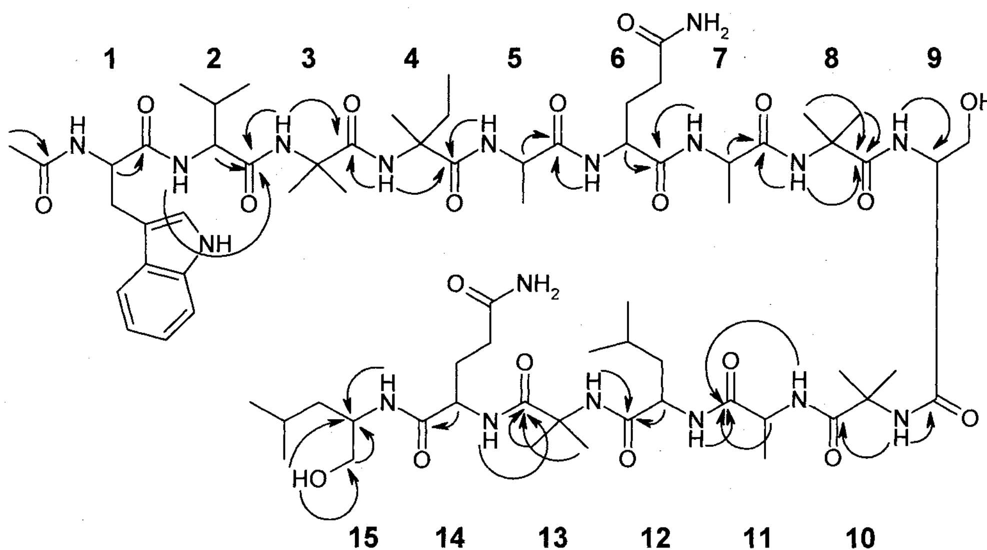
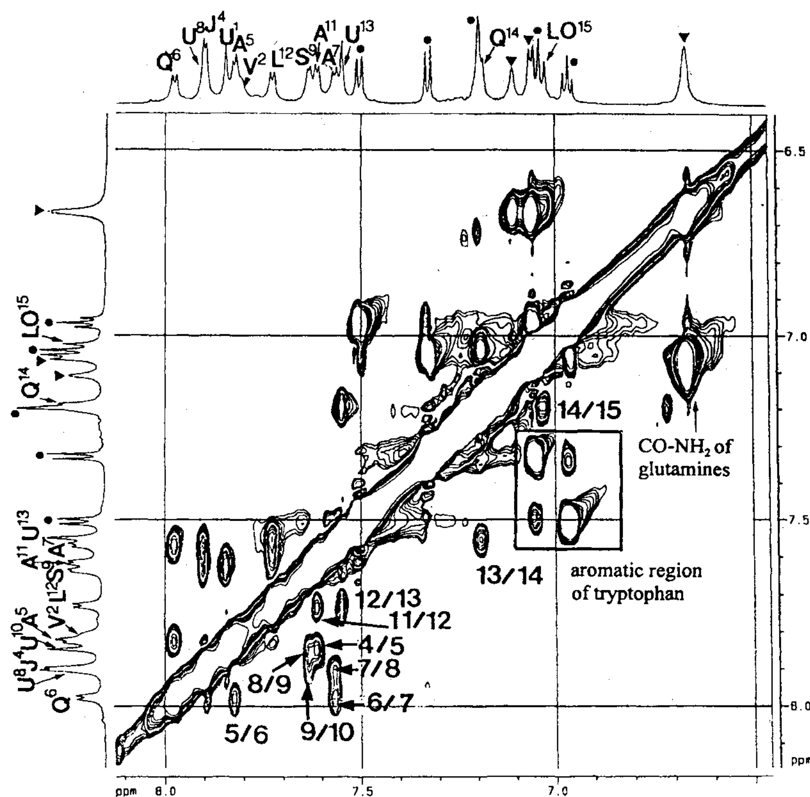


Fig. 5. Amide proton region of the ROESY spectrum of tylopeptin A (600 MHz, DMSO-d_6).
▲=CO-NH_2 for glutamine, ●=aromatic proton signals of tryptophan.



amino acid sequence of tylopeptin A. The sequence was described by successive loss of Leuol+Gln, Aib, Leu, Ala+Aib, Ser, Aib, Ala+Gln, Ala, Aib, Aib, Val (*m*/*z* 1294, 1209, 1096, 940, 853, 768, 569, 498, 413, 328, 229) in FABMS spectrum (Fig. 5). Comparison of the mass spectral data suggested that tylopeptins A and B were homologous peptides possessing the identical *N*-acyl terminus and *C*-terminal alcohol with their amino acid composition differing only by the presence of Aib or Iva at position 4.

^{1}H and ^{13}C NMR assignments (DMSO-d_6) of the tylopeptin B are summarized in the Materials and Methods section. The complete sequence of tylopeptin B was elucidated by HOHAHA, ROESY and HMBC spectra as well as FABMS. The structure of tylopeptin B was concluded to be Ac-Trp^1-Val^2-Aib^3-Aib^4-Ala^5-Gln^6-Ala^7-Aib^8-Ser^9-Aib^{10}-Ala^{11}-Leu^{12}-Aib^{13}-Gln^{14}-Leuol^{15}.

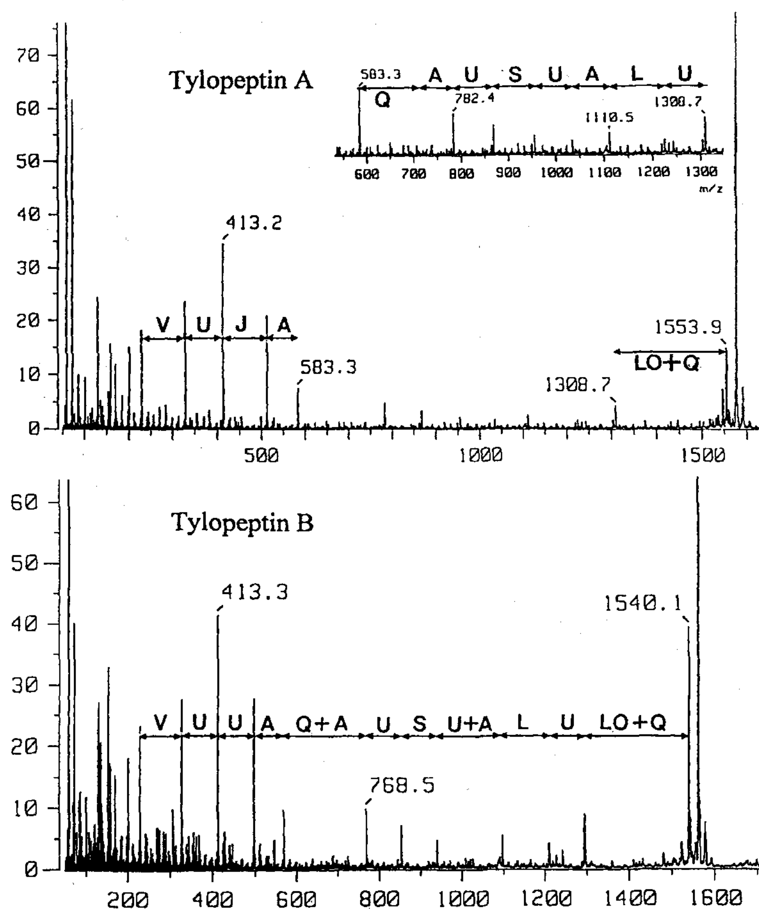
Biological Activity

The antimicrobial activity of tylopeptin A and B were examined against phytopathogenic fungi and Gram-positive and -negative bacteria. As shown in Table 3, tylopeptin A and B were active against Gram-positive bacteria, *Bacillus subtilis*, *Staphylococcus aureus* R-209, *Staphylococcus aureus* IFO-12732, and *Corynebacterium lilium*, and were inactive against several yeasts, fungi, and Gram-negative bacteria.

Conclusion

In the present study, we isolated and identified two new peptaibols, tylopeptins A and B, from fresh fruiting bodies of the mushroom *Tylopilus neofelleus*. To our knowledge, this is the first report isolating peptaibols from fruiting bodies of mushrooms. Peptaibols are usually found as the metabolites of fungi of the genus *Trichoderma*, while they

Fig. 6. Positive ion FAB mass spectra of tylopeptins A and B.



are also found in other fungi such as *Emericellopsis*[14] and *Apiocrea*[8] strains.

Tylopeptins contain several Aib units, and the *C*-terminus is formed by an amino alcohol leucinol. Of the peptaibols, tylopeptin A and B are rare in that they contain the tryptophan moiety at the *N*-terminus. Tylopeptin A differs from tylopeptin B by only one substitution at position 4 in the sequence, Iva→Aib. The Iva→Aib substitution is commonly observed in peptaibols.

Materials and Methods

Materials

Tylopilus neofelleous was collected in an area of Mt. Moak at Junrabukdo, Korea, and identified by Dr. CHO. (CHO-5738).[17] Whole latex was kept dry at the laboratory of Cell Function Regulator Research Unit in KRIBB.

General Methods

UV spectra were measured on a Kontron Uvicon 930 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on Bruker DRX300 and DMX600 NMR spectrometers, respectively. Two dimensional NMR spectra were recorded on a Bruker AM600 NMR spectrometer. All two-dimensional homonuclear spectra were recorded in pure-phase absorption mode. ROESY spectra were recorded with the mixing time of 400 ms. HMBC experiments were performed using 80 ms duration optimized for 6.25 (6.3) Hz.

FAB mass spectra were recorded on a JMS-HX 110A/HX110A (Jeol, JAPAN) FAB mass spectrometer. The solution was mixed with 3-nitrobenzyl alcohol as the matrix on the FAB probe tip.[18] Optical rotation and IR spectra were obtained on a Schmidt+Haensch Polartronic polarimeter and on Perkin Elmer 16000 FTIR spectrometers, respectively.

Table 3. Antimicrobial activity of tylopeptins A and B.

Test organism[a]	Diameter of inhibition zone (mm)[b]	
	tylopeptin A	tylopeptin B
Bacillus subtilis IAM 1609	14	13
Staphylococcus aureus R-209	13	15
Staphylococcus aureus IFO-12732	16	17
Corynebacterium lilium (wild type)	18	15
Streptococcus sp. (wild type)	-[c]	-
Salmonella typhimurium KCTC 1926	-	-
Escherichia coli AB 1157	-	-
Pasteurella haemolytica (wild type)	-	-
Pasteurella multocida (wild type)	-	-
Saccharomyces cerevisiae KCTC7039	-	-
Candida albicans IAM 4905	-	-
Aspergillus niger ATCC 9642	-	-
Magnaporthe gricea IFO 5994	-	-
Colletotrichum lagenarium IFO 7513	-	-
Fusarium solani (wild type)	-	-
Alternaria mali IFO 8594	-	-
Mucor ramannianus IAM 6218	-	-

[a] Bacteria was tested on LB agar, fungi on PDA agar, and yeast on SAB agar medium.
[b] Determined by the agar diffusion test with 50 µg of tylopeptin A and B on a 8 mm paper disc.
[c] No activity.

Amino Acids Analysis

The absolute configurations of the amino acids were determined by GC after hydrolysis of tylopeptins. Tylopeptins A and B (500 µg) were treated with 6 N HCl at 110°C for 24 hours in a sealed tube. The excess HCl was removed by N_2 gas. Derivatization of the amino acids and amino alcohol was conducted as previously described[19]. Retention times of the *N*-trifluoroacetyl isopropyl ester derivatives were compared with the standards. The GC analyses were performed with a Hewlett Packard series II 5890 gas chromatograph on a chirasil-L-Val (*N*-propionyl-L-valine-*tert*-butylamide polysiloxane) quartz capillary column (Chrompack, 25 m length, 0.2 mm i.d.). GC analytical conditions were the same as described previously[19].

Tylopeptin B

Tylopeptin B was obtained as a white powder. Positive ion FABMS: *m/z* 1562 $(M+Na)^+$, 1540 $(M+H)^+$,1294, 1209, 1096, 940, 853, 768, 569, 498, 413, 328, 229. ^1H NMR (600 MHz, DMSO-d_6, 40°C): amino acid, Ac-Trp[1] δ 1.87 (3H, s), 3.0 (1H, dd, J=6.3, 15.0), 3.16 (1H, dd, J=4.2, 15.0), 4.44 (1H, m), 6.96 (1H, t, J=7.5), 7.05 (1H, t, J=7.5), 7.20 (1H, s), 7.32 (1H, d, J=7.5), 7.50 (1H, d, J=7.5), 8.3 (1H, d, 6.5), 10.8 (1H, s); Val[2] δ 0.85 (3H, d, J=6.3), 0.89 (3H, d, J=6.6), 2.16 (1H, m), 3.87 (1H, m), 7.8 (1H, br d); Aib[3] δ 1.32 (3H, s), 1.39 (3H, s), 8.29 (1H, s); Aib[4] δ 1.33 (3H, s), 1.38 (3H, s), 8.0 (1H, s), Ala[5] δ 1.45 (3H, d, J=7.2), 3.96 (1H, m), 7.85 (1H, d, J=5.0); Gln[6] δ 1.98 (1H, m), 2.15 (1H, m), 2.25 (1H, m), 4.0 (1H, m), 6.67 (1H, br), 7.11 (1H, br), 8.0 (1H, d, J=6.0); Ala[7] δ 1.33 (3H, d, J=7.1), 4.04 (1H, m), 7.57 (1H, d, J=5.7); Aib[8] δ 1.35 (3H, s), 1.44 (3H, s), 7.96 (1H, s); Ser[9] δ 3.86 (2H, m), 3.87 (2H, m), 7.64 (1H, d, J=3.9); Aib[10] δ 1.38 (3H, s), 1.45 (3H, s), 7.88 (1H, s); Ala[11] δ 1.40 (3H, d, J=7.26), 3.98 (1H, m), 7.61 (1H, d, J=5.1); Leu[12] δ 0.84 (3H, d, J=6.66), 0.86 (3H, d, J=6.56), 1.53 (2H, m), 1.76 (1H, m), 3.93 (1H, m), 7.73 (1H, d, J=5.9); Aib[13] δ 1.36 (3H, m), 1.38 (3H, m), 7.53 (1H, s); Gln[14] δ 1.83 (2H, m), 2.1 (1H, m), 2.2 (1H, m), 3.96 (1H, m), 6.68 (1H, br s), 7.14 (1H, br s), 7.19 (1H, m); Leuol[15] δ 0.80 (3H, d, J=6.42), 0.82 (3H, d, J=6.45), 1.35 (2H, m), 1.65 (1H, m), 3.21 (1H, m), 3.32 (1H, m), 3.78 (1H, m), 7.03 (1H, m). ^{13}C NMR (600 MHz, DMSO-d_6, 40°C): amino acid, Ac-Trp[1] δ 22.6, 26.9, 54.9, 109.8, 111.3, 118.0, 118.2, 120.8, 123.3, 127.9, 136.1, 170.7, 172.9; Val[2] δ 18.8, 19.0, 28.9, 59.5,

172.6; Aib^{3} δ 23.4, 23.9, 55.7, 175.2; Aib^{4} δ 25.2, 26.4, 55.6, 176.2; Ala^{5} δ 16.4, 51.2, 175.4; Gln^{6} δ 27.0, 31.6, 55.1, 173.3, 173.7; Ala^{7} δ 16.0, 51.4, 174.0; Aib^{8} δ 24.1, 26.0, 55.5, 175.6; Ser^{9} δ 60.3, 60.5, 171.7; Aib^{10} δ 25.9, 26.0, 55.4, 175.5; Ala^{11} δ 16.4, 51.1, 174.5; Leu^{12} δ 22.7, 23.4, 26.1, 39.2, 53.5 173.7; Aib^{13} δ 23.0, 23.8, 56.1, 173.9; Gln^{14} δ 27.0, 31.4, 53.3, 170.8, 173.3; $Leuol^{15}$ δ 21.0, 21.6, 22.7, 39.5, 48.6, 63.9.

Biological Activity

Antimicrobial activity of tylopeptins A and B were determined against phytopathogenic fungi and Gram-positive and negative bacteria by the agar diffusion method using paper discs (8 mm diameter). Fifty microgram of compounds was applied onto each paper disc. Inhibition zones were measured after incubation at 27°C for 24 hours for yeasts, at 37°C for 24 hours for bacteria and at 27°C for 48 hours for fungi.

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