STRUCTURAL ELUCIDATION OF TRICHOSPORIN-B-Ia, IIIa, IIId AND V FROM *TRICHODERMA POLYSPORUM*

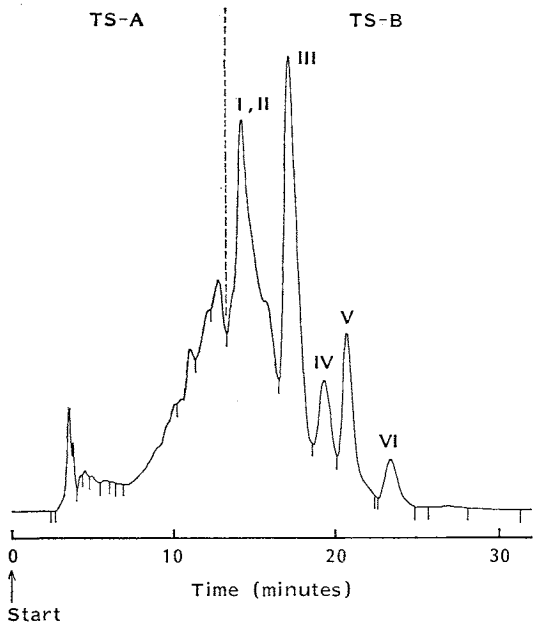
Sir:

Antibiotic peptaibols (=peptaibophols) represented by alamethicin[1] have received attention in terms of the activity against various microorganisms[2] as well as the formation of voltage-gated ion channels in membranes[3]. They contain an aminoalcohol and a high proportion of α-aminoisobutyric acid (Aib). We previously isolated trichopolyns I and II[4] of the same class from a culture broth of *Trichoderma polysporum* (Link ex Pers.) Rifai (Strain TMI 60146), which have a strong antagonistic inhibition against the growth of *Lentinus edodes*. Further examination on peptide components produced by *T. polysporum* has led to the isolation of new peptaibols, trichosporins (TS), having an uncoupling activity† similar to that of hypelcins[5] in mitochondria of rat livers. In this paper, we wish to report the structural elucidation of these peptides.

The culture filtrate of *T. polysporum* was subjected to an Amberlite XAD-2 column chromatography. Elution with MeOH yielded a brownish syrup, which was partitioned with EtOAc and water. The water soluble fraction showed many peaks on the HPLC chromatogram as illustrated in Fig. 1, the substances of these peaks being designated as TS-A, TS-B-I, II, III, IV, V and VI, respectively. In the present work, TS-B-I, III and V (**4**) were separated. TS-B-I and III were further fractionated by repeated preparative HPLC (Nakarai Cosmosil 5Ph column) giving TS-B-Ia (**1**), IIIa (**2**) and IIId (**3**) as pure compounds, respectively. The physical properties of the isolated peptides are summarized in Table 1.

The main component, TS-B-V (**4**), showed the following spectral data indicating the presence of amide bonds; IR (KBr) cm^{-1} 3300 (NH), 1660 (CO) and 1530 (NH); ^1H NMR δ 6.6~8.6 (NH); ^{13}C NMR (100 MHz, CD_3OH) δ 172~180 (CO). The amino acid analysis (Table 1) of the complete acid hydrolysate showed it to consist of the following seven kinds of amino acids (number of residues): Aib (8 or 9), Ala (3), Glu (3), Gly (1), Ile (1), Leu (1), Pro (1) and Val (1)†. The number of Aib residues in (**4**) was eventually determined to be eight by analysing its distortionless enhancement by polarization transfer (DEPT) NMR spectrum (8 quarternary carbon signals due to 8 Aib α-carbons at δ 57~58). Furthermore, the existence of three glutamine residues in (**4**) were deduced from the facts that (**4**) was not methylated with diazomethane and that the ^1H NMR spectrum showed six broad singlets (6H) at δ 6.65, 6.77, 6.80, 7.36, 7.43 and 7.45 due to three carboxamides. Since (**4**) was negative to both ninhydrin reaction and

Fig. 1. HPLC chromatogram of trichosporins.



Conditions: Mobile phase, CH_3OH - H_2O (85:15); flow rate, 0.5 ml/minute; a.u.f.s., 0.16; UV detection, 220 nm; column, Nakarai Cosmosil $5C_{18}$ (4.6×150 mm).

† The details will be reported elsewhere.

† The absolute configurations of phenylalaninol (Pheol) and the constituent amino acids of these peptides were determined as follows; the complete acid hydrolysates were led to 3,5-dinitrobenzoyl derivatives, followed by the HPLC analyses[9]. HPLC conditions: Mobile phase, *n*-hexane - dichloroethane - ethanol (50:5:1); flow rate, 1 ml/minute; UV detection, 254 nm; column, Sumitomo Chemical Sumipax OA-4100 consisting of urea derivatives of (*R*)-1-(α-naphthyl)ethylamine with (*S*)-valine (4 mm i.d. × 250 mm). The Pheol and all the optically active amino acids had L-configuration.

Table 1. Physical properties and amino acid compositions of trichosporin-B-Ia (1), IIIa (2), IIId (3) and V (4).

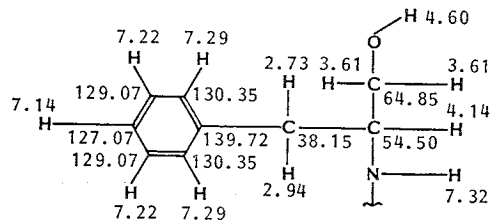
	1	2	3	4
MP (°C)	224~226	256~258	261~264	268~271
$[\alpha]_D^{25}$	−24.8°	−20.4°	−22.4°	−16.3°
	(*c* 0.42, MeOH)	(*c* 0.52, MeOH)	(*c* 0.34, MeOH)	(*c* 0.67, MeOH)
UV λ_{max}^{MeOH} nm (ε)	226 (4,300), 259 (340), 264 (280), 268 (240)	222 (5,600), 258 (220), 262 (190), 268 (150)	224 (5,400), 259 (340), 264 (270), 268 (240)	224 (4,200), 256 (220), 262 (170), 267 (150)
IR (KBr) cm^{-1}	3300, 1660, 1530	3300, 1660, 1530	3300, 1660, 1530	3300, 1660, 1530
^1H NMR (400 MHz, CD_3OH) δ				
CH_3CO	2.05	2.04	2.04	2.04
NH	6.5~8.6	6.6~8.6	6.6~8.6	6.6~8.6
Aromatic	7.1~7.3	7.1~7.3	7.1~7.3	7.1~7.3
MW (nominal)	1,965	1,949	1,935	1,949
Molecular formula	$C_{91}H_{151}N_{23}O_{25}$	$C_{91}H_{151}N_{23}O_{24}$	$C_{90}H_{149}N_{23}O_{24}$	$C_{91}H_{151}N_{23}O_{24}$
Amino acid compositions* (average rounded values)	Aib (8-9), Ala (2), Glu (3), Gly (1), Leu (2), Pro (1), Ser (1), Val (1)	Aib (8-9), Ala (3), Glu (3), Gly (1), Leu (2), Pro (1), Val (1)	Aib (8-9), Ala (3), Glu (3), Gly (1), Leu (1), Pro (1), Val (2)	Aib (8-9), Ala (3), Glu (3), Gly (1), Ile (1), Leu (1), Pro (1), Val (1)

* Acid hydrolysates were obtained under the following conditions: 6 N HCl, 100°C, 20 hours for (1) and 6 N HCl, 110°C, 24 hours for the rest.

above methylation, it was suggested that both terminal N and C of (4) are protected. The ^1H and ^{13}C NMR spectra and ^1H-^1H correlated spectroscopy (COSY) spectra of (4) showed the presence of an acetyl group (CH_3CO at δ 2.04 and CH_3CO at δ 23.30 and 173.53) and phenylalaninol† (Pheol, see Fig. 2). Thus, this compound was deduced to be a peptaibol where terminal *N* is protected by an acetyl group and *C*-terminal residue is linked with phenylalaninol like alamethicin. The amino acid sequence was determined through inspection of positive ion fast atom bombardment (FAB) mass fragmentation and ^{13}C-^1H correlated spectroscopy *via* long range coupling (COLOC)[6] NMR spectra. The FAB-MS (Fig. 3) showed the formation of two acylium ion series. One series (A), corresponding with the *N*-terminal oligopeptide ($C_{54}H_{93}N_{14}O_{15}$), begins at *m/z* 1,177 and loses Aib, Leu (or Ile), Gly, Aib, Ile (or Leu), Aib, Gln, 2Aib and 3Ala successively to afford the terminal *N*-acetylated Aib (*m/z* 128). The other one (B), corresponding with the *C*-terminal oligopeptide ($C_{37}H_{59}N_9O_9$), begins at *m/z* 774 to give the fragment ion peak formulated Pro-Val (*m/z* 197) by successive losses of Pheol+H^+, 2Gln, and 2Aib. The formation of these two complementary oligopeptide fragments is most likely

† See footnote on p. 814.

Fig. 2. ^{13}C and ^1H chemical shifts (δ) of phenylalalninol.



due to the preferential cleavage[1] of an Aib-Pro peptide bond. Additionally, the spectrum showed three ion peaks at *m/z* 1,950, 1,972 and 1,988 in the molecular ion region, which are considered as $(M+H)^+$, $(M+Na)^+$ and $(M+K)^+$, respectively. Thus, the complete sequence of (4) can be obtained by connecting these two oligopeptides, leading to the molecular formula ($C_{91}H_{151}N_{23}O_{24}$). The remaining problem, location of Ile and Leu, was solved in the following way. The ^{13}C-^1H COLOC NMR spectrum showed a correlated peak between the carbonyl carbon (δ 172.85) of Gly^{11} and the α-proton (δ 4.45) of Leu. Thus, Leu is situated at postition 12 and Ile at 9. This finding was further complemented by the observation of a two-bond coupling between Leu N*H* (δ 8.07) and Gly^{11} *C*O (δ 172.85) in the long range selective proton decoupling (LSPD)[7] experiment. Therefore,

Fig. 3. Positive ion FAB-MS of (4).

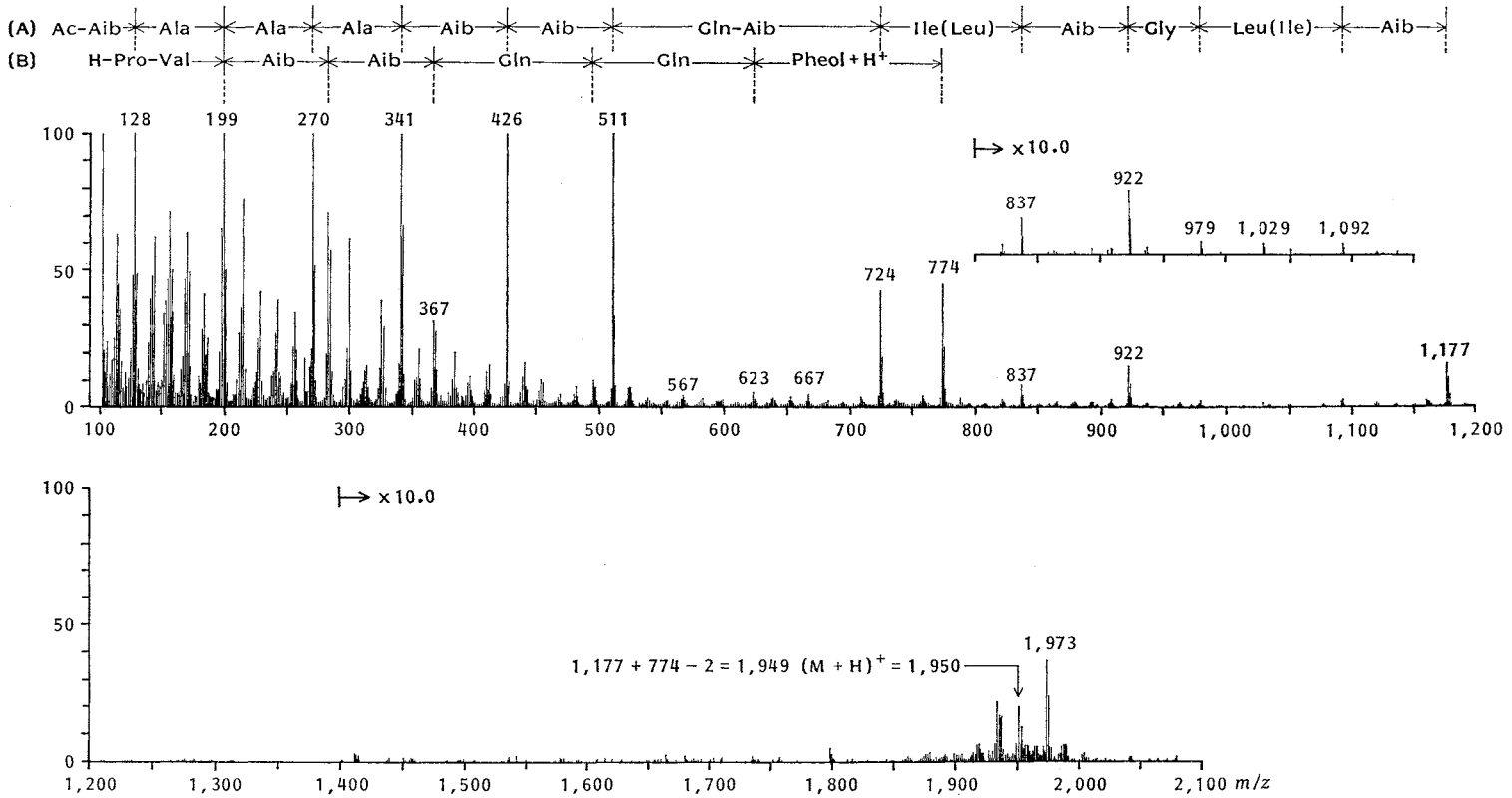


Table 2. Diagnostic ions observed in FAB-MS and the primary structures of trichosporin-B-Ia (1), IIIa (2), IIId (3) and V (4).

	Acylium ion, *m/z*																			
	Series A											Series B								
	Ac-1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Pheol
1	128	199	286	357	442	527	655	740	853	938	995		1,193,		197[a]	282	367			774
2	128	199	270	341	426	511	639	724	837	922	979	1,092	1,177,		197[a]	282	367		623[b]	774
3	128	199	270	341	426	511	639	724	823	908	965	1,078	1,163,		197[a]	282	367		623[b]	774
4	128	199	270	341	426	511	639	724	837	922	979	1,092	1,177,		197[a]	282	367	495	623[b]	774

1	Ac-Aib-Ala-**Ser**-Ala-Aib-Aib-Gln-Aib-**Leu**-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol
2	Ac-Aib-Ala-Ala-Ala-Aib-Aib-Gln-Aib-**Leu**-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol
3	Ac-Aib-Ala-Ala-Ala-Aib-Aib-Gln-Aib-**Val**-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol
4	Ac-Aib-Ala-Ala-Ala-Aib-Aib-Gln-Aib-Ile-Aib-Gly-Leu-Aib-Pro-Val-Aib-Aib-Gln-Gln-Pheol

[a] The fragment ion, *m/z* 197, could be formulated as H-Pro-Val.
[b] The fragment ion, *m/z* 623, results from the loss of Pheol+H^+.

the primary structure of (4) was determined as: Ac-Aib-L-Ala-L-Ala-L-Ala-Aib-Aib-L-Gln-Aib-L-Ile-Aib-Gly-L-Leu-Aib-L-Pro-L-Val-Aib-Aib-L-Gln-L-Gln-L-Pheol (Table 2).

The amino acid sequences of (1), (2) and (3) were determined through the amino acid analyses and comparison of the FAB-MS with those of (4). In the spectra, the fragment ions over *m/z* 199 of (1) were 16 mass units higher than those of (4). This is attributed to the replacement of Ala^3 in (4) by Ser in (1). Furthermore, the amino acid analysis indicated that Ile^9 in (4) is replaced by Leu in (1). On the other hand, the fragment ions over *m/z* 724 of (3) were 14 mass units lower than those of (4). Thus, Ile^9 in (4) is replaced by Val in (3). The fragment ion pattern of (2) was quite the same as that of (4). However, the amino acid analysis pointed out that Ile^9 in (4) is replaced by Leu in (2). Therefore, the primary structures of (1), (2) and (3)† were established as shown in Table 2. The isolation of the peptides (1), (2), (3) and (4) constitutes a first time discovery of suzukacillin[8] type peptaibol from fungus *T. polysporum*.

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† See footnote on p. 814.

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