Fungal Metabolites. Part 3.[1] Structural Elucidation of Antibiotic Peptides, Trichosporin-B-IIIb, -IIIc, -IVb, IVc, -IVd, -VIa and -VIb from *Trichoderma polysporum*. Application of Fast-Atom Bombardment Mass Spectrometry/Mass Spectrometry to Peptides containing a Unique Aib–Pro Peptide Bond

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Trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and -VIb are components of an antibiotic peptide mixture produced by *Trichoderma polysporum*. Each component was purified by reversed-phase high-performance liquid chromatography (HPLC). The amino acid sequences of these peptides, which have an unstable peptide bond, Aib–Pro, were elucidated by fast-atom bombardment mass spectrometry (FAB MS) and fast-atom bombardment mass spectrometry/mass spectrometry (FAB MS/MS) with the help of NMR spectroscopy. The molecular weights of these peptides were all *ca.* 2000 and the structures were very similar.

Trichosporin (TS)-Bs,[2] which were isolated from a culture broth of *Trichoderma polysporum*, are antibiotic icosapeptides and have an uncoupling activity[3] against rat liver mitochondria in the presence of inorganic phosphate. In addition, we have recently shown[4] that these peptides have the potential to release catecholamines from adrenal chromaffin cells. TS-Bs contain a high proportion of two unusual amino acids, α-aminoisobutyric acid (Aib) and isovaline (Iva), and belong to a class of peptaibols such as alamethicin[5] and suzukacillin[6] which have unique biological activities,[7–10] *e.g.*, voltage-dependent ion-channel formation. Peptaibols are structurally characterized as follows: their N-terminal amino acids are protected by an acetyl group and their C-terminal residues are linked with an amino alcohol.

Fast-atom bombardment mass spectrometry (FAB MS) has been the most suitable method for the determination of amino acid sequences of peptaibols, since Edman degradation and enzymatic methods cannot be applied to these compounds. In our previous paper, the sequences of TS-B-Ia, -IIIa, -IIId and -V were determined mainly by FAB MS with a double-focusing mass spectrometer. However, complete elucidation of sequences by only FAB MS is not very easy though peptaibols are oligopeptides whose molecular weights are 2000 daltons at most. This is caused by another structural feature, in that most peptaibols contain a very labile Aib–Pro peptide bond.[11,12] This peptide bond tends to cleave preferentially when samples are bombarded. Therefore, owing to the formation of two acylium ion series,[13,14] their FAB MS spectra become complicated in the low-mass region and do not show fragment ions which have an intact Aib–Pro segment in the high-mass region. In the case of two Aib–Pro peptide bonds[15] existing in the sequence, the spectrum becomes more complicated. Thus, chemical treatment, such as partial hydrolysis, or NMR measurements must be performed in order to confirm the peptide sequences.

Fast-atom bombardment mass spectrometry/mass spectrometry[16,17] (FAB MS/MS) is a useful technique for peptide sequencing. We have now isolated trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and -VIb by repeated preparative HPLC and have determined their amino acid sequences by FAB MS and FAB MS/MS. In this paper, we report on the structural elucidation of the trichosporin-Bs peptides, which is mostly dependent on the clear fragmentation by FAB MS/MS. To our knowledge, this is the first application of FAB MS/MS to peptaibols containing a characteristic peptide bond, Aib–Pro.

Results and Discussion

Separation of Trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and VIb.—As described in the previous paper, a peptide mixture containing trichosporin-Bs [Fig. 1(a)] was obtained from a culture broth of *T. polysporum* and purified by HPLC with a reversed-phase ODS column. TS-B-III, -IV, and -VI appeared to be almost pure based on HPLC analyses [Fig. 1(b)]. However, all of them showed heterogeneity in their NMR spectra and from the results of amino acid analysis. When further separation of the peaks was tried by HPLC, a different type of reversed-phase column (phenyl type) was found to be helpful in fractionating new trichosporin-Bs [Fig. 1(c), (d) and (e)]. Preparative HPLC with this column gave nine pure peptides. Among these purified peptides, the structures of TS-B-IIIa and -IIId have already been reported.[2]

Characterisation of Trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and VIb.—The proportions of normal amino acids in the peptides were established from amino acid analyses of the acid hydrolysates. The number of Aib and Iva residues was determined based on the signals of quaternary carbons observed in the ^{13}C NMR spectra (Aib: δ_C 57–58, Iva: δ_C 60–61). All the peptides were negative to ninhydin reagent and were not esterified with diazomethane. These facts indicate the absence of both free amino and free carboxy groups in the molecules. Furthermore, their ^{1}H and ^{13}C NMR spectra showed the presence of an acetyl group and a phenylalaninol[2] (Pheol) moiety in each peptide. Thus, it was found that the *N*- and the *C*-terminals of the peptides are protected by an acetyl group and a phenylalaninol, respectively, as occurs in alamethicin and the

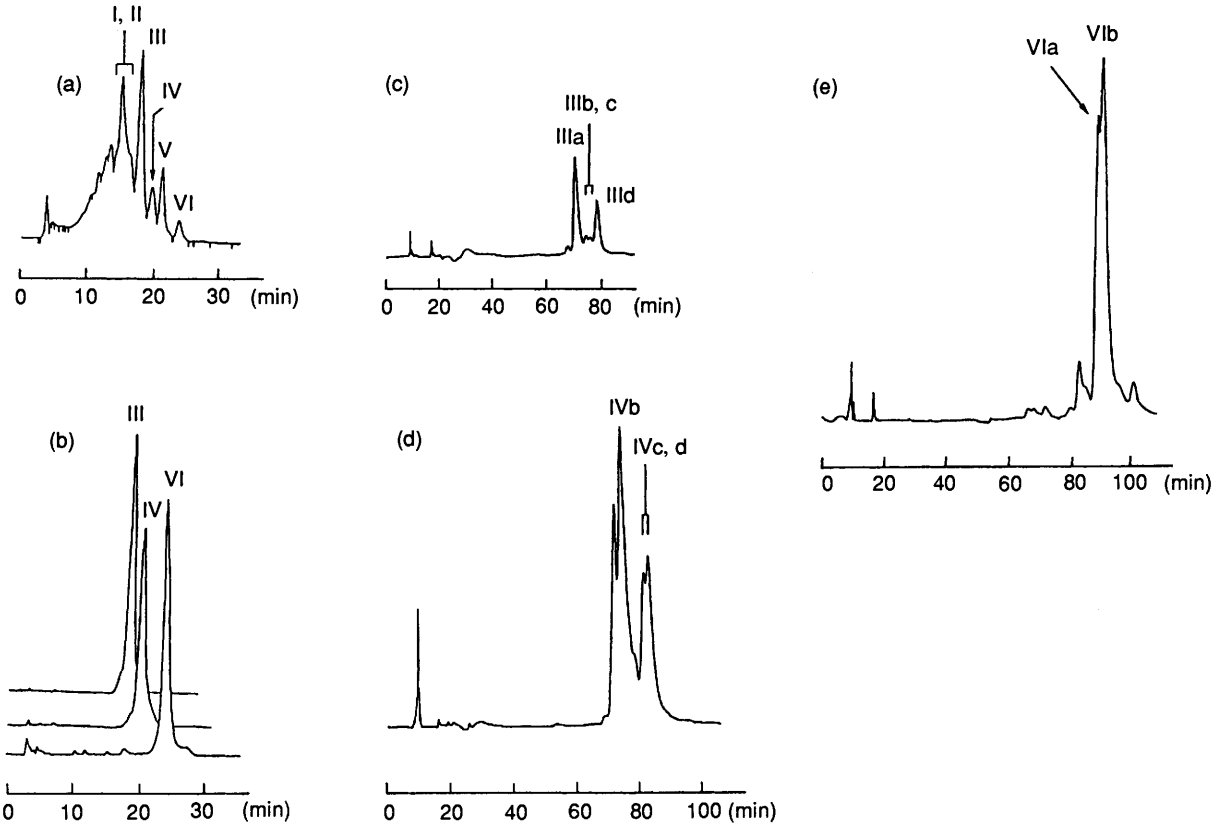

Fig. 1. HPLC profiles of trichosporin-Bs. (a) Crude trichosporin-Bs obtained from the culture broth of *Trichoderma polysporum*. (b) Purified trichosporin-B-III, -IV and -VI by preparative HPLC. (c), (d) and (e) Heterogeneity of trichosporin-B-III, -IV and -VI. Analytical conditions: eluant, methanol–water (85:15, v/v); flow rate, 0.5 cm^3 min^{-1} for (a) and (b), 0.6 cm^3 min^{-1} for (c), (d) and (e); detector, UV (220 nm); column, Nacalai Cosmosil ODS (4.6 mm i.d. × 150 mm) for (a) and (b), Nacalai Cosmosil Ph (8 mm i.d. × 250 mm) for (c), (d) and (e); column temperature, 40 °C.

known TS-Bs. At the same time, the presence of three glutamine residues was ascertained from the ^1H NMR spectra.

The absolute configuration of the optically active amino acids involving an amino alcohol was determined by HPLC with a chiral column. For the analyses, the acid hydrolysates were converted into the 3,5-dinitrobenzoates. The results revealed that Iva has the *R*-configuration and the others the *S*-configuration.

The circular dichroism (CD) spectra of these peptides showed negative Cotton effects at 207 and 221 nm. This means that each trichosporin-B takes a right-handed helical form,[5,6] as do other Aib-containing peptaibols such as alamethicin and suzukacillin. The amino acid proportions and the characteristics of these peptides are summarized in Table 1.

Structure of Trichosporin-B-VIb.—TS-B-VIb [$C_{92}H_{153}N_{23}O_{24}$, MW 1963 (nominal)] should be a linear peptide judging from the evidence described above. Fig. 2 shows the FAB MS spectrum of TS-B-VIb measured using a double-focusing mass spectrometer. The ion peaks at *m/z* 1986 in the molecular ion region can be assigned to the sodium adduct ion $[M + Na]^+$. Careful inspection of the spectrum reveals the presence of two acylium ion series, suggesting the preferential cleavage of one Aib–Pro bond. One series, beginning at *m/z* 1177, gives some sequence-specific ions, such as those at *m/z* 922, 511, 426, 341, 270, 199 and 128. Since the ion of *m/z* 128 can be assigned to Ac–Aib, this series results from the *N*-terminal oligopeptide. The other one, which defines the *C*-terminal oligopeptide, is expected to start at *m/z* 788. Therefore, the entire molecule can be obtained by combining these two acylium ion series between Aib and Pro.

However, unambiguous information on the fragmentation of the *C*-terminal oligopeptide could not be obtained from this spectrum. Moreover, the sequence from blocks 7–13 could not be determined clearly because of the absence of sequence-specific ions. In order to clarify these points we applied the FAB MS/MS technique. All FAB MS/MS experiments described in this paper were carried out by collisionally activated dissociation (CAD) using a triple-stage quadrupole mass spectrometer.[16]

Unfortunately, the CAD spectrum of the $(M + H)^+$ ion (1964) afforded no acceptable results. Thus, CAD spectra of some characteristic daughter ions in the normal FAB MS spectrum were recorded. Fig. 3 shows a part of the CAD spectrum of the peak at *m/z* 1177. The sequence-specific ions observed at *m/z* 837, 979 and 1092 can lead, for the sequence 9–13, to Ile (or Leu)–Aib–Gly–Leu (or Ile)–Aib. However, the sequence 7–8 could not be determined because sequence-specific daughter ions were not found between *m/z* 511 and 724 in the spectrum. Thus, the daughter ions of the peak at *m/z* 724 were examined (Fig. 4). The ions of *m/z* 639, which is identical with glutamine acylium ion, and *m/z* 511 can lead, for the sequence 7–8, to Gln–Aib. Therefore, the amino-acid sequence of the *N*-terminal oligopeptide of TS-B-VIb is as follows: Ac–Aib–Ala–Ala–Ala–Aib–Aib–Gln–Aib–Ile (Leu)–Aib–Gly–Leu (Ile)–Aib.

The next problem was to determine the *C*-terminal sequence. Fig. 5 shows the CAD spectrum of the *m/z* 788 ion derived from the *C*-terminal oligopeptide. This spectrum shows very clear fragmentation from the ion with *m/z* 788 compared with the normal FAB MS spectrum (Fig. 2). This acylium ion series loses Pheol, 2Gln, Iva (or Val) and Aib successively to give the *m/z* 197 ion which is identical with Pro + Val (or Iva). However, the location of Pro could not be determined. In order to clarify this location, a CAD spectrum of the *m/z* 197 ion was taken (Fig. 6)

Table 1. Characteristics and amino acid proportions for trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and -VIb.

		TS-B-IIIb	TS-B-IIIc	TS-B-IVb	TS-B-IVc	TS-B-IVd	TS-B-VIa	TS-B-VIb
Formula		$C_{90}H_{149}N_{23}O_{24}$	$C_{90}H_{149}N_{23}O_{24}$	$C_{92}H_{153}N_{23}O_{24}$	$C_{91}H_{151}N_{23}O_{24}$	$C_{91}H_{151}N_{23}O_{24}$	$C_{92}H_{153}N_{23}O_{24}$	$C_{92}H_{153}N_{23}O_{24}$
MW (nominal)		1935	1935	1963	1949	1949	1963	1963
M.p. (°C)[a]		232–235	249–252	191–194	246–250	244–247	239–242	250–254
Molecular ellipticity	207 nm	−3 377 000	−3 296 000	−3 180 000	−3 057 000	−3 726 000	−3 080 000	−3 205 000
[θ] (° cm^2 mol^{-1})	221 nm	−2 271 000	−2 256 000	−2 247 000	−2 176 000	−2 795 000	−2 260 000	−2 390 000
	Glu	3.11(3)	2.84(3)	2.87(3)	2.83(3)	2.99(3)	2.82(3)	2.94(3)
	Gly	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)	1.00(1)
	Ala	3.99(4)	3.76(4)	2.96(3)	2.23(2)	2.82(3)	2.23(2)	2.80(3)
	Aib[b]	7	7	7	9	7	9	7
Amino acid proportions	Val[c]	1.09(1)	1.06(1)	1.00(1)	1.89(2)	1.88(2)	1.08(1)	1.06(1)
(6 mol dm^{-3} HCl; 24 h)	Iva[b]			1		1		1
	Ile	0.82(1)	0.85(1)				0.84(1)	0.89(1)
	Leu	1.01(1)	1.06(1)	1.79(2)	0.92(1)	0.94(1)	1.07(1)	1.05(1)
	Pro	1.01(1)	0.94(1)	1.02(1)	0.88(1)	0.95(1)	0.98(1)	0.97(1)
	Pheol[b]	1	1	1	1	1	1	1

[a] Uncorrected. [b] Determined by the NMR spectra. [c] The Iva peak overlaps with the Val peak completely in the routine program of the amino acid analyser used. However, as the reactivity of Iva to ninhydrin reagent is extremely low (6% sensitivity compared with that of Gly), the values given above can be regarded as the values of Val.

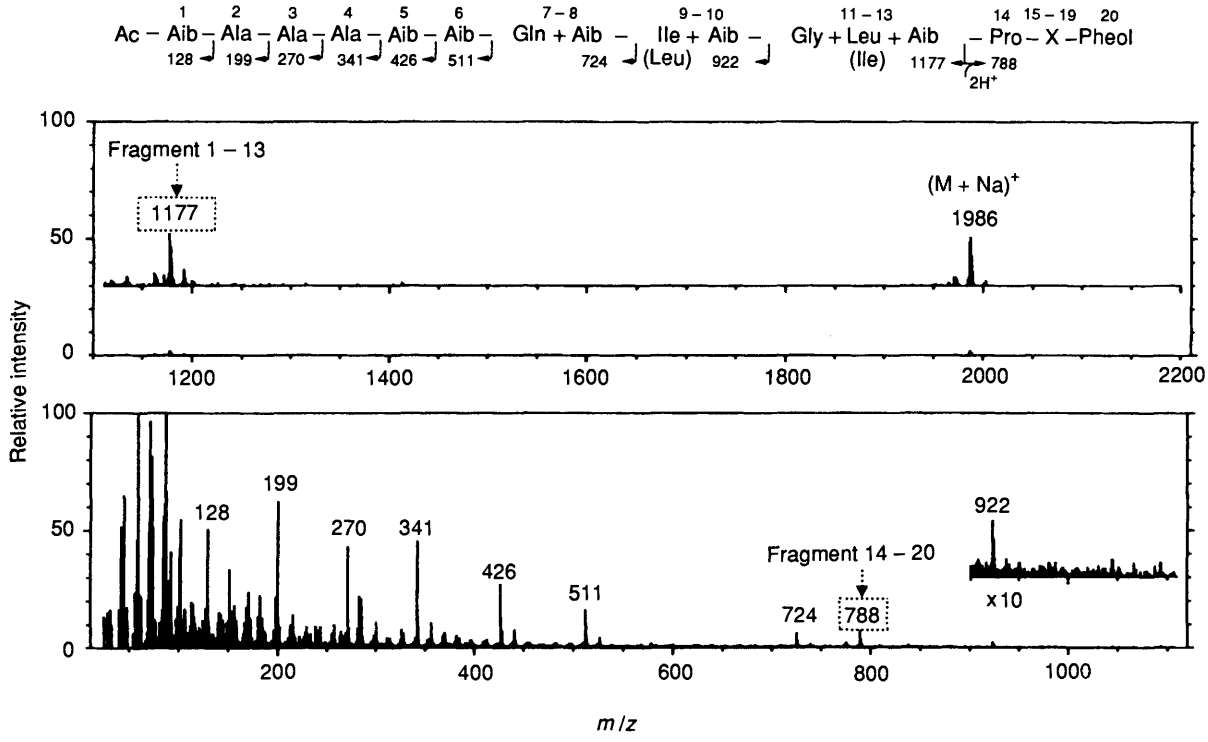

Fig. 2. Positive-ion FAB MS spectrum of trichosporin-B-VIb. X stands for Aib + 2Gln + Iva + Val.

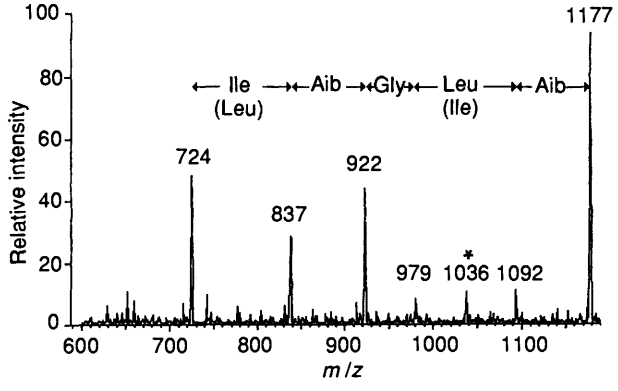

Fig. 3. CAD spectrum of *m/z* 1177 ion. * May arise from the elimination of the Ile or Leu side-chain.

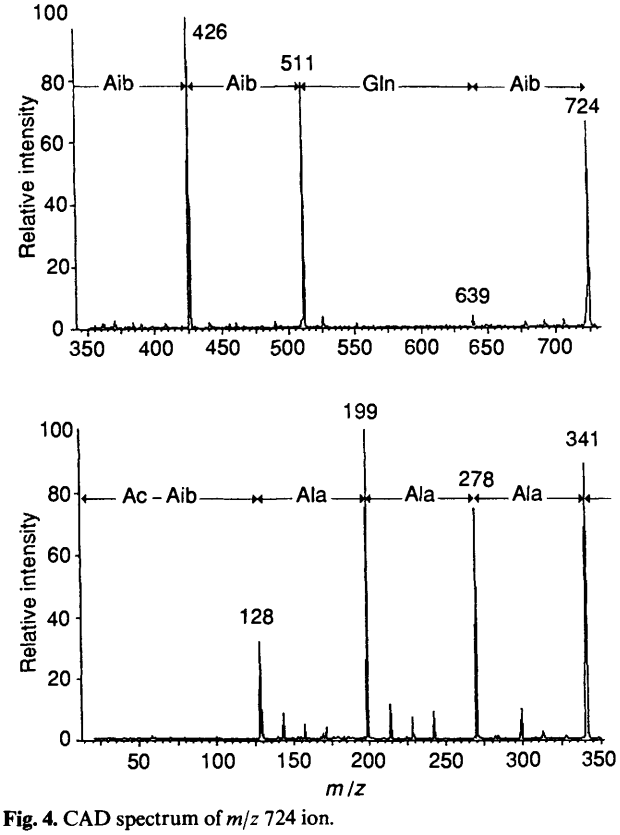

Fig. 4. CAD spectrum of *m/z* 724 ion.

in the expectation of the observation of the acylium ion with *m/z* 98 which would result from the loss of Val or Iva from the ion of *m/z* 197. The spectrum did not give the ion of *m/z* 98, but showed the significantly abundant B-type ion of *m/z* 70, which is deduced to originate from the loss of CO as shown in Fig. 6. The observation of this ion demonstrates that Pro is located at the *N*-terminal of the *C*-terminal oligopeptide. Therefore, the amino-acid sequence of the *C*-terminal oligopeptide of TS-B-VIb is as follows: Pro–Val(Iva)–Aib–Iva(Val)–Gln–Gln–Pheol.

The remaining problem was the differentiation of the isomeric amino acids, Ile and Leu, and Iva and Val. The differentiation of Ile and Leu by FAB MS/MS using a double-focusing mass spectrometer has already been reported.[17,18] However, the isomeric amino acids of TS-B-VIb could not be distinguished in this experiment. This problem was solved by correlated spectroscopy[19,20] (COSY), nuclear Overhauser enhancement spectroscopy[21,22] (NOESY) and ^{13}C, ^{1}H-correlated spectroscopy *via* long-range coupling[23–25] (COLOC) spectra of this peptaibol (detailed assignments are omitted here).

First, Ile and Leu were differentiated by the NOESY spectrum. Unfortunately, the Gly NH group shows connectivities with neither the Ile NH nor the Leu NH in the NOESY spectrum. However, the Ile NH has cross-peaks with the Aib^{b} and the Aib^{c} NH groups. Furthermore, the Aib^{c} NH shows

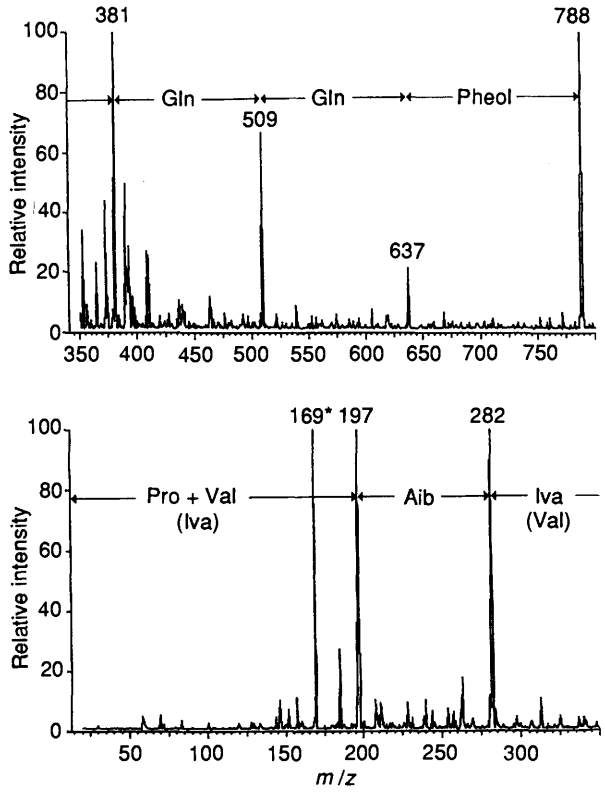

Fig. 5. CAD spectrum of *m/z* 788 ion. * B-type ion generated from the loss of CO from *m/z* 197 ion.

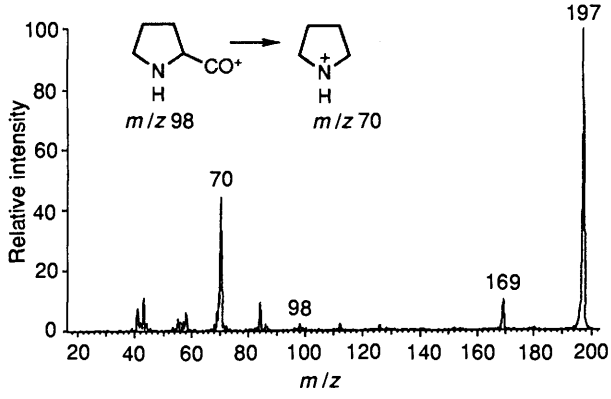

Fig. 6. CAD spectrum of *m/z* 197 ion.

connectivity with the Gln[a] NH (Fig. 7). These connectivities mean that Ile occupies position 9. Thus, position 12 should be occupied by Leu. Consequently, positions 9–12 in the *N*-terminal sequence were determined as Ile–Aib–Gly–Leu.

Next, the differentiation of Iva and Val was achieved through the observation of the ^{13}C, ^{1}H-COLOC spectrum, although NH–NH cross-peaks from the Iva or Val NH to other NHs were not observed in the NOESY spectrum. Fig. 8 shows the connectivity from the Val CO group to the Aib[d] NH group. This correlation means that a Val–Aib segment exists in this molecule. On the other hand, the Iva CO group (δ_C 179.34) shows a correlation with the Gln[b] NH group (δ_H 7.76). This is evidence for the existence of an Iva–Gln segment. Therefore, the correct sequence of the *C*-terminal oligopeptide is Pro–Val–Aib–Iva–Gln–Gln–Pheol.

On the basis of the findings obtained by mass spectrometry and NMR spectroscopy, the entire molecule of TS-B-VIb was determined as follows: Ac–Aib–Ala–Ala–Ala–Aib–Aib–

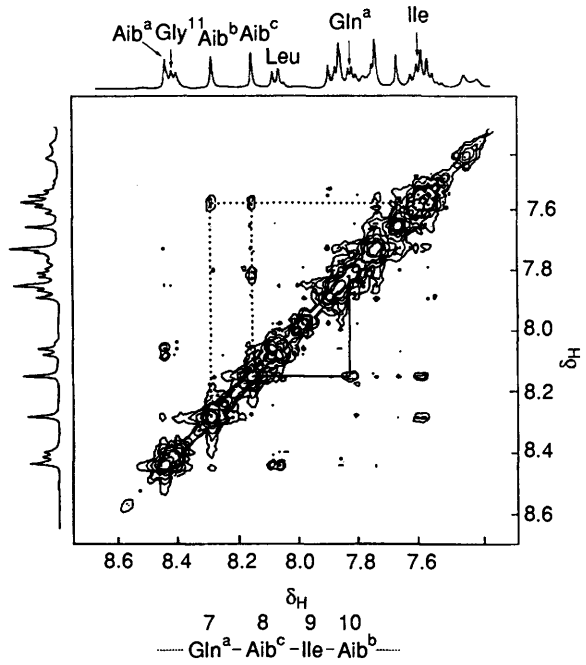

Fig. 7. NH–NH region of the NOESY spectrum of trichosporin-B-VIb. Continuous lines reveal the connectivity from the Gln[a] NH to the Aib[c] NH. Dotted lines indicate the couplings of the Ile NH with the Aib[b] and Aib[c] NHs.

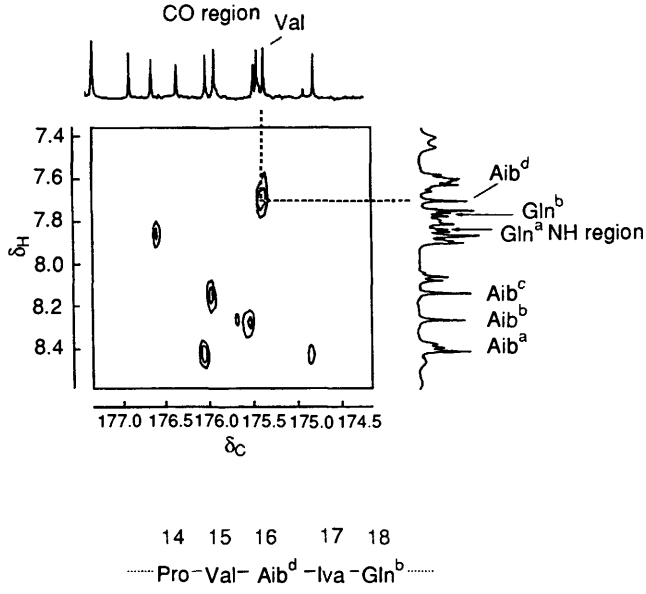

Fig. 8. Part of the CO–NH region of the ^{13}C, ^{1}H-COLOC spectrum of trichosporin-B-VIb. The connectivity from the Val CO to the Aib[d] NH is shown by dotted lines.

Gln–Aib–Ile–Aib–Gly–Leu–Aib–Pro–Val–Aib–Iva–Gln–Gln–Pheol.

Structures of the Other Trichosporin-Bs.—The structures of the remaining peptides were also determined in the same manner as that of TS-B-VIb. The FAB MS spectra of TS-B-IIIb, -IIIc, -IVb, -IVc, -IVd and VIa showed the $(M + H)^+$ and/or $(M + Na)^+$ ions between *m/z* 1900 and 2000, and no fragment ions between *m/z* 1200 and 1900. In addition, the fragmentation patterns were found to be very close to that of TS-B-VIb. These observations imply that their amino-acid sequences are extremely similar to that of TS-B-VIb. However, the dif-

Table 2a. Primary structures of trichosporin-Bs.

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
TS-B-Ia[2]	Ac-	Aib-	Ala-	Ser-	Ala-	Aib-	Aib-	Gln-	Aib-	Leu-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-IIIa[2]	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Leu-	Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-IIIb	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Ile	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Ala-	Gln-	Gln- Pheol
TS-B-IIIc	Ac-	Aib-	Ala-	Ala-	Ala-	Ala-	Aib-	Gln-	Aib-	Ile	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-IIId[2]	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Val	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-IVb	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Leu	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
TS-B-IVc	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Aib-	Gln-	Aib-	Val	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-IVd	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Val	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol
TS-B-V[2]	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Ile	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-VIa	Ac-	Aib-	Ala-	Aib-	Ala-	Aib-	Aib-	Gln-	Aib-	Ile	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Aib-	Gln-	Gln- Pheol
TS-B-VIb	Ac-	Aib-	Ala-	Ala-	Ala-	Aib-	Aib-	Gln-	Aib-	Ile	-Aib-	Gly-	Leu-	Aib-	Pro-	Val-	Aib-	Iva	-Gln-	Gln- Pheol

ferentiation of isomeric amino acids, Ile and Leu, and Iva and Val, could not be accomplished as well as for TS-B-VIb. This problem was solved in comparison with the sequences of the known TS-Bs and the partial sequences of the new peptides which do not contain Ile or Iva. The amino-acid sequences and the diagnostic ions of these peptides are summarized in Tables 2a and 2b.

The *C*-terminal sequences of the peptides except for TS-B-IVb and -IVd containing IVa could be determined by FAB MS/MS. Among these peptides, position 17 of TS-B-IIIb was found to be occupied by Ala. The sequences of the remaining peptides (TS-B-IIIc, -IVc and -VIa) were recognized to be the same Pro–Val–Aib–Aib–Gln–Gln–Pheol as that of the known TS-Bs. The sequence-specific ions obtained from the CAD spectra of the *m/z* 788 ion of TS-B-IVb and -IVd were the same as that of TS-B-VIb. This means that two *C*-terminal sequences are possible. One is the same as that of TS-B-VIb. The other is a sequence in which positions 15 and 17 are occupied by Iva and Val, respectively. In surveying the *C*-terminal sequences of the other peptide, it was found that position 15 is commonly occupied by Val. In addition, replacement in the *C*-terminal oligopeptides of TS-B-IIIb and -VIb occurs at position 17. Therefore, their *C*-terminal sequences could be deduced to be Pro–Val–Aib–Iva–Gln–Gln–Pheol.

The *N*-terminal sequences of TS-B-IVb, -IVc and -IVd could be determined easily. In comparison with the known TS-Bs, the sequences in the *N*-terminal segments of TS-B-IVb and -IVd were found to be identical with that of TS-B-IIIa and -IIId, respectively. Position 3 of TS-B-IVc was found to be occupied by Aib compared with that of TS-B-IVd. TS-B-IIIb, -IIIc and -VIa have isomeric amino acids, Ile and Leu, in the same way as do TS-B-V and -VIb. Thus, two possible *N*-terminal sequences for these peptides are considered. However, the other peptides commonly have Leu at position 12. Furthermore, replacement by Ile, Leu or Val had occurred at position 9. Therefore, positions 9 and 12 of TS-B-IIIb, -IIIc and -VIa could be deduced to be occupied by Ile and Leu, respectively.

Experimental

General Procedures.—All m.p.s were measured on a Yanagimoto micro melting point apparatus and are uncorrected. All NMR experiments were carried out by using a Bruker AC-300 or a Bruker AM-400 spectrometer at room temperature. Samples were dissolved in CD_3OH (0.5 cm^3) containing tetramethylsilane as internal standard. CD spectra were recorded on a Jasco J-600 spectropolarimeter at room temperature. Peptides (0.11–0.21 mg) were dissolved in methanol (2.1–3.5 cm^3).

Reversed-phase HPLC Separation of Trichosporins TS-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and VIb.—HPLC was performed on a Shimadzu LC-6 A system. First, separation of the TS-B mixture[2] (3.0 g) was performed repeatedly with a Nacalai Cosmosil ODS column (20 mm i.d. × 250 mm) to afford TS-B-III (0.68 g), -IV (0.17 g) and -VI (0.11 g). The preparative conditions were as follows: eluant, methanol–water (85:15, v/v); flow rate 5 cm^3 min^{-1}; detector, UV (220 nm); column temperature, 40 °C. Further fractionation was carried out repeatedly with a Nacalai Cosmosil phenyl column (20 mm i.d. × 250 mm) to give pure TS-B-IIIb (23 mg), -IIIc (14 mg), -IVb (7.2 mg), -IVc (9.9 mg), -IVd (10.3 mg), -VIa (13 mg), and -VIb (32.4 mg). The HPLC conditions were as follows: eluant (methanol–water, v/v) and flow rate, 85:15 and 2.5 cm^3 min^{-1} for IVb–d, 83:17 and 2.5 cm^3 min^{-1} for IIIb and d, and 80:20 and 9 cm^3 min^{-1} for VIa and b; detector, UV (220 nm); column temperature, 40 °C.

Identification and Absolute Configuration of Amino Acids and a Phenylalaninol.—For amino acid analyses, samples (0.5–1 mg) were hydrolysed in 6 mol dm^{-3} HCl at 110 °C for 24 h. The hydrolysates were analysed by an amino acid analyser. The absolute configuration of amino acids and a phenylalaninol of each peptide was established in the following manner. A acid hydrolysate (*ca.* 0.5 mg) was refluxed in absolute methanol–thionyl chloride (10:1; 2 cm^3) for 3 h. After removal of the solvent and reagent, the residues were treated with a solution of 3,5-dinitrobenzoyl chloride (*ca.* 1 mg) and triethylamine (one drop) in ethyl acetate (2 cm^3) while being stirred. The resulting 3,5-dinitrobenzoates were analysed by HPLC with a column which had an optically active stationary phase (Sumipax OA-4100; 4 mm i.d. × 250 mm), and retention times were compared with those of the derivatives of standard amino acids and a phenylalaninol. The standard analytical conditions were as follows: eluant, A:hexane, B:1,2-dichloroethane–ethanol (5:1, v/v); flow rate (isocratic), A:1 cm^3 min^{-1}, B:0.1 cm^3 min^{-1}; detector, UV (254 nm); column temperature, 30 °C.

FAB MS and FAB MS/MS Spectra.—FAB MS spectra were obtained by using a JEOL JMS-HX100 mass spectrometer. Samples were bombarded with 8 keV xenon atoms. For FAB MS/MS experiments, a Finnigan MAT 70 triple-stage quadrupole mass spectrometer was used. Xenon or argon was used as the collision gas at a pressure of *ca.* 0.2 mTorr.* The collision energy was 40 eV. For FAB MS and FAB MS/MS experiments, glycerol–thioglycerol (1:1) or dithiothreitol–dithioerythritol (3:1) was used as a matrix.

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* 1 Torr = 133.322 Pa.

Table 2b. Diagnostic ions[a] observed in the FAB mass and the CAD spectra of trichosporin-B-IIIb, -IIIc, -IVb, -IVc, -IVd, -VIa and -VIb.

	Acylium ions, *m/z*																			
	←——— *N*-Terminal oligopeptide ———→													←——— *C*-Terminal oligopeptide ———→						
Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	$[M + X]^+$
TS-B-IIIb	128	199	270	341	426	511	**639**	724	**837**	922	**979**	**1092**	1177		**197**	**282**	**353**	**481**	**609**	760	1936[b] 1958[c]
TS-B-IIIc	128	199	270	341	412	497	**625**	710	**823**	908	**965**	**1078**	1163		**197**	**282**	**367**	**495**	**623**	774	1958[c]
TS-B-IVb	128	199	270	341	426	511	**639**	724	**837**	922	**979**	**1092**	1177		**197**	**282**	**381**	**509**	**637**	788	1986[c]
TS-B-IVc	128	199	284	355	440	525	**653**	738	**837**	922	**979**	**1092**	1177		**197**	**282**	**367**	**495**	**623**	774	1950[b] 1972[c]
TS-B-IVd	128	199	270	341	426	511	**639**	724	**823**	908	**965**	**1078**	1163		**197**	**282**	**381**	**509**	**637**	788	1972[c]
TS-B-VIa	128	199	284	355	440	525	**653**	738	**851**	936	**993**	**1105**	1191		**197**	**282**	**367**	**495**	**623**	774	1986[c]
TS-B-VIb	128	199	270	341	426	511	**639**	724	**837**	922	**979**	**1092**	1177		**197**	**282**	**381**	**509**	**637**	788	1964[b] 1986[c]

[a] Bold numbers are ions clearly observed in the CAD spectra. [b] X = H. [c] X = Na.

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