Fungal Metabolites. Part 6.[1] Nuclear Magnetic Resonance Study of Antibiotic Peptides, Trichosporin Bs, from *Trichoderma polysporum*

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Sequence-specific ^{1}H- and ^{13}C-nuclear magnetic resonance assignments of an antibiotic peptide, trichosporin B-V isolated from fungus *Trichoderma polysporum*, were achieved in methanol by using two-dimensional NMR techniques. The ^{1}H NMR spectra recorded at various concentrations (1–60 mmol dm^{-3}) suggested that the peptide behaves predominantly as a monomer in methanol and that its conformation is not affected by its concentration. Furthermore, complete or partial ^{1}H-resonance assignments of trichosporin B-Ia, -IIIa, -IVd and -VIb were carried out similarly. Comparison of the ^{1}H NMR parameters for the amide groups of trichosporin Bs suggested that their backbone conformations are very similar to each other. It is proposed that the significant differences in the biological activity of trichosporin Bs result from the lipophilicity of the individual molecules.

Antibiotic peptides, trichosporin (TS)-Bs,[2,3] were isolated from a culture broth of the fungus *Trichoderma polysporum*, which does serious damage to cultivations of *Lentinus edodes* (a kind of edible mushroom) in Japan. TS-Bs belong to the class of peptaibols such as alamethicin[4] and suzukacillin,[5] and predominantly adopt a helical form in methanol.[3,6] TS-Bs have uncoupling activity against oxidative phosphorylation in rat liver mitochondria, as do hypelcins[7] and alamethicin,[8] and induce Ca^{2+}-dependent catecholamine release from adrenal medullary chromaffin cells,[9,10] as does alamethicin.[11] Considering the structural similarity to alamethicin and hypelcins, these activities are likely to result from the formation of ion channels in cell membranes[12–14] or the perturbation of membrane organization.[15] Upon further examination of catecholamine release,[16] five tested TS-B components (TS-B-Ia, -IIIa, -IVd, -V and -VIb) showed significant differences in their activity, although their primary structures are very similar. Therefore, it is of interest and importance to examine the conformations of the peptides and to compare them with each other in order to obtain clues to the solution of the mechanisms of the above activities. In the case of peptaibols, the presence of α-aminoisobutyric acid (Aib), which does not have an α-proton, makes complete ^{1}H and ^{13}C NMR signal assignments difficult, although their relative molecular masses are up to 2000 daltons. So far many NMR studies of peptaibols and their synthetic analogues have been reported,[17–19] but there have been very few reports in which both ^{1}H and ^{13}C NMR signal assignments of natural peptaibols have been achieved in detail and simultaneously.

In this paper, we report on the assignments of the ^{1}H and ^{13}C NMR signals of a main component, TS-B-V, by two-dimensional NMR techniques, and on the effect of sample concentrations on its ^{1}H NMR spectra in methanol. Furthermore, we describe the effect of the replacement of one or two amino acid residues on the conformations of the other peptides, TS-B-Ia, -IIIa, -IVd and -VIb on the basis of a comparison of the ^{1}H NMR parameters for the amide groups.

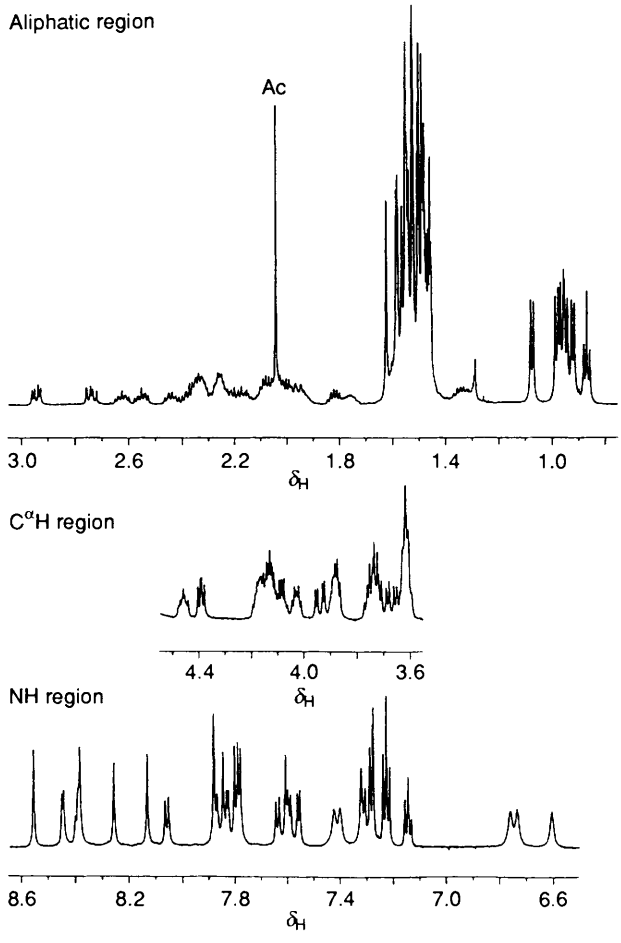

Fig. 1 600 MHz ^{1}H NMR spectrum of trichosporin B-V at 27 °C in CD_3OH (10 mmol dm^{-3}). The *y*-axis scale of the top spectrum is one-half that of the middle and bottom ones.

Results and Discussion

Assignment of the ^{1}H *NMR Signals of Trichosporin B-V.*— Fig. 1 shows a 600 MHz ^{1}H NMR spectrum of TS-B-V in CD_3OH; the signals are well resolved in the solvent. Most of the ^{1}H NMR signals were assigned by homonuclear two-dimensional NMR techniques, correlated spectroscopy (COSY),[20,21] relayed coherence transfer spectroscopy (RCT),[22,23] nuclear Overhauser enhancement spectroscopy (NOESY)[24,25] and homonuclear Hartmann–Harn spectroscopy (HOHAHA).[26] The chemical shifts of the ^{1}H NMR signals are summarized in Table 1.

The spin systems of the amino acids and Pheol except for

Position	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Ts-B-Ia	Ac	Aib	Ala	Ser	Ala	Aib	Aib	Gln	Aib	Leu	Aib	Gly	Leu	Aib	Pro	Val	Aib	Aib	Gln	Gln	Pheol
TS-B-IIIa	Ac	Aib	Ala	Ala	Ala	Aib	Aib	Gln	Aib	Leu	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	Ac	Aib	Ala	Ala	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

(Aib = α-aminoisobutyric acid, Iva = isovaline, Pheol = phenylalaninol)

Primary structures of trichosporin B-Ia, -IIIa, -IVd, -V and -VIb. The absolute configuration of Iva contained in trichosporin-B-IVd and -VIb is the *R* form and the other optically active constituents have the *S* form. Trichosporin B-VIb showed the highest catecholamine-release activity. The activities of Ts-Bs-Ia, -IIIa, -IVd and -V were ~4, 30, 50 and 40% of that of TS-B-VIb, respectively.

Table 1 Proton chemical shifts[a] of trichosporin B-V in CD_3OH at 27 °C (10 mmol dm^{-3})

	δ_H					
Residue	NH	α	β[b]	γ	δ	Others
Ac		2.040				
Aib^1	8.553		1.456			
Ala^2	8.443	4.091	1.474			
Ala^3	7.78	4.126	1.48			
Ala^4	7.556	4.130	1.49			
Aib^5	7.886		1.531			
Aib^6	7.846		1.579			
Gln^7	7.828	3.881	2.18, 2.25	2.38, 2.552		7.400, 6.734 (ε)
Aib^8	8.130		1.589			
Ile^9	7.591	3.74	2.10	1.36, 1.76	0.868	0.962 (γ^2)
Aib^{10}	8.256		1.568			
Gly^{11}	8.39	3.669, 3.938				
Leu^{12}	8.058	4.458	1.63, 1.98	1.93	0.919, 0.942	
Aib^{13}	8.382		1.539, 1.623			
Pro^{14}		4.390	1.83, 2.32	2.00, 2.08	3.75, 3.89	
Val^{15}	7.637	3.77	2.35	0.981, 1.073		
Aib^{16}	7.606		1.545			
Aib^{17}	7.801		1.553			
Gln^{18}	7.78	4.028	2.26	2.448, 2.619		7.421, 6.760 (ε)
Gln^{19}	7.88	4.18	2.01, 2.06	2.31, 2.34		7.32, 6.604 (ε)
$Pheol^{20}$	7.31	4.17	2.736, 2.941			3.62 (CH_2O)
						7.280 (*o*)
						7.225 (*m*)
						7.143 (*p*)

[a] Chemical shifts obtained from two-dimensional spectra are expressed with $\Delta\delta$ ±0.01 ppm. [b] Assignment of some Aib $C^\beta H_3$ signals was not achieved.

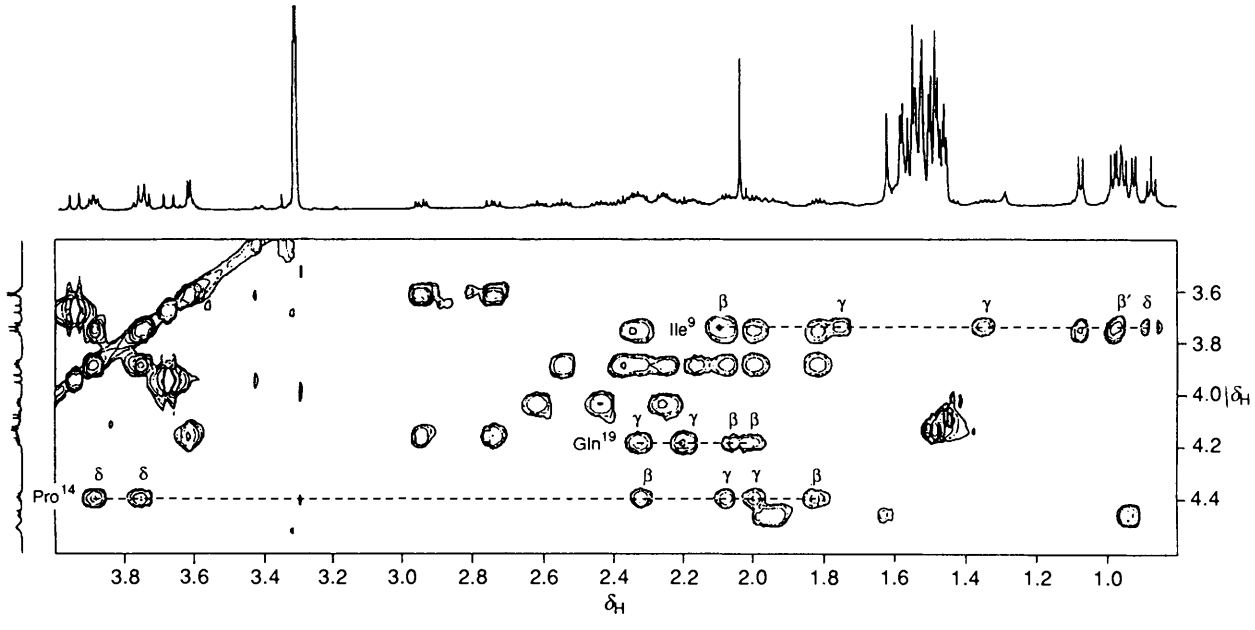

Fig. 2 Part of the C^α–H aliphatic region in a 600 MHz HOHAHA spectrum of trichosporin B-V at 27 °C in CD_3OD (10 mmol dm^{-3}). The spin systems originating from the C^α–H protons of Pro^{14}, Gln^{19} and Ile^9 are indicated by broken lines.

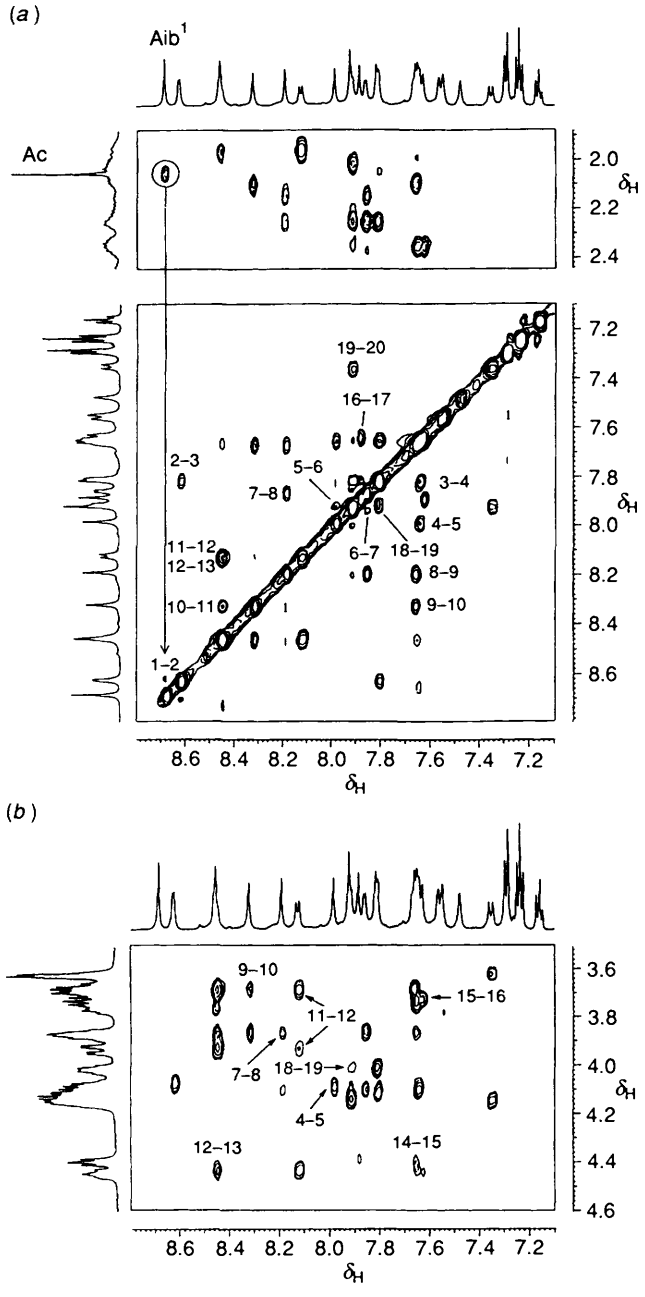

Fig. 3 Parts of a 600 MHz NOESY spectrum of trichosporin B-V at 10 °C in CD_3OH (30 mmol dm^{-3}). Sequential NH–NH (a) and $C^{\alpha}H$–NH cross-peaks (*b*) [d_{NN} (*i*, *i* + 1) and $d_{\alpha N}$ (*i*, *i* + 1)] are shown. NOESY spectra were recorded at concentrations of 10, 30 and 60 mmol dm^{-3} in CD_3OH at −5, 10 and 27 °C. At −5 and 10 °C, the spectra showed almost the same NOE patterns with efficiently intense cross-peaks, whereas the spectrum of the 10 mmol dm^{-3} sample recorded at 27 °C was inferior to those of the 30 and 60 mmol dm^{-3} samples in the number and intensity of cross-peaks.

Aib were unambiguously identified by the COSY, RCT and HOHAHA (Fig. 2) spectra recorded in CD_3OH or CD_3OD. In this stage, the sequence-specific assignment of particular amino acids (Ile[9], Gly[11], Leu[12], Pro[14], Val[15]) and Pheol, which appear only once in the primary sequence, were achieved. Some Aib C^{β}–H_3 signals were assigned by relayed connectivities between NH protons and C^{β}–H_3 protons, but their assignment is not complete because of the loss of cross-peaks.

The sequence-specific assignment of the signals of the backbone NH protons was almost completely carried out by using inter-residue NOE connectivities [$d_{\alpha N}$ (*i*, *i* + 1) and d_{NN} (*i*, *i* + 1)] according to the procedures outlined by Wagner and Wüthrich.[27] Fig. 3 shows parts of the NOESY spectrum. The lowest-field singlet NH proton shown had a cross-peak with the acetyl $C^{\beta}H_3$ protons. Thus, this NH proton was assigned to the Aib[1] NH proton. Starting from this proton, NH–NH connectivities [d_{NN} (*i*, *i* + 1)] extended from Aib[1] to Aib[13] and Gln[18] to Pheol[20] [Fig. 3(*a*)]; at this stage, the NH signals of three Ala, three Gln and six Aib residues were identified sequence-specifically. In Fig. 3(*a*), there is one more NOE cross-peak originating from the Aib[16] and Aib[17] NH protons, but their signals could not be distinguished sequence-specifically because of the loss of sequential NH–NH connectivities between Val[15] and Gln[18]. Identification of both Aib residues was achieved by $d_{\alpha N}$ (*i*, *i* + 1)-type NOE cross-peaks. One of six $C^{\alpha}H$–NH cross-peaks observed in the fingerprint region [Fig. 3(*b*)] was assigned to that between the Val[15] C^{α}–H proton and the Aib[16] NH proton. Thus, all backbone NH protons were identified sequence-specifically. Also the Gln side-chain ε–NH_2 signals could be assigned by NOESY, since three singlet NH protons observed in the lower-field region (Fig. 1) showed intraresidual NOE cross-peaks with the C^{γ}–H_2 protons (not shown here). However, the C^{β}–H_3 signal assignment could not be achieved unambiguously even with the NOESY spectra for the same reason as in the case of the RCT spectra.

Assignment of the ^{13}C *NMR Signals of Trichosporin B-V.*—A ^{13}C NMR spectrum of TS-B-V in CD_3OH at 150 MHz is shown in Fig. 4. The ^{13}C NMR signal assignments, listed in Table 2, were carried out by using distortionless enhancement by polarization transfer (DEPT), ^{13}C,^{1}H-COSY[29] and ^{13}C,^{1}H-correlated spectroscopy *via* long-range coupling (COLOC)[30–32] NMR techniques on the basis of the results of ^{1}H NMR signal assignment. As each C^{α} carbon except for the Aib residues was shown to be coupled with the corresponding C^{α}–H proton in the ^{13}C,^{1}H-COSY spectrum, the assignment of these carbons were accomplished easily. All C^{α} carbons of the Aib residues exhibited couplings with their own C^{β}–H_3 and NH protons in the ^{13}C,^{1}H-COLOC spectra. However, the cross-peaks between the C^{α} carbons and the C^{β}–H_3 protons overlapped and those between the C^{α} carbons and the NH protons were weak. Thus, the assignment of the Aib C^{α} carbons is a little uncertain.

TS-B-V has 23 CO carbons in the molecule. Among these signals, identification of 20 backbone CO signals was achieved through the observation of CO–C^{α}–H cross-peaks ($^{2}J_{CH}$) and/or CO–NH cross-peaks ($^{2}J_{CH}$ or $^{3}J_{CH}$) in the ^{13}C,^{1}H-COLOC spectra recorded by exchanging the delay Δ_1. Parts of the spectrum measured in the delay Δ_1 = 36 ms are shown in Fig. 5. The CO signals originating from the amino acids which have an α-proton had cross-peaks with their own C^{α}–H protons ($^{2}J_{CH}$) and, at the same time, showed CO–NH connectivities ($^{2}J_{CH}$) with the adjacent amino acids. Similarly, seven Aib CO carbons except for Aib[13] had CO–NH cross-peaks ($^{2}J_{CH}$) with the adjacent amino acids. On the other hand, the Aib[1] and Aib[13] CO carbons had intraresidual CO–NH cross-peaks ($^{3}J_{CH}$); the Aib[1] CO carbon showed both inter- and intra-residual CO–NH connectivities. In addition, the Aib[10] CO carbon had a cross-peak with one of the Gly[11] C^{α}–H_2 protons ($^{3}J_{CH}$). Thus, 20 backbone CO signals were all assigned sequence-specifically. Among three Gln side-chain CO signals, one (δ_C 177.61) had cross-peaks with the Gln[7] C^{γ}–H_2 protons. Thus, this signal comes from Gln[7] (not shown here). The Gln[18] and Gln[19] side-chain CO signals could not be distinguished one from another.

Most of the signals arising from the side-chain carbons were assigned easily through the observation of $^{1}J_{CH}$ connectivities. However, some of the Aib C^{β} signals could not be distinguished clearly because of overlap of the cross-peaks. Thus, some ambiguity still remains about the assignment of these carbon signals.

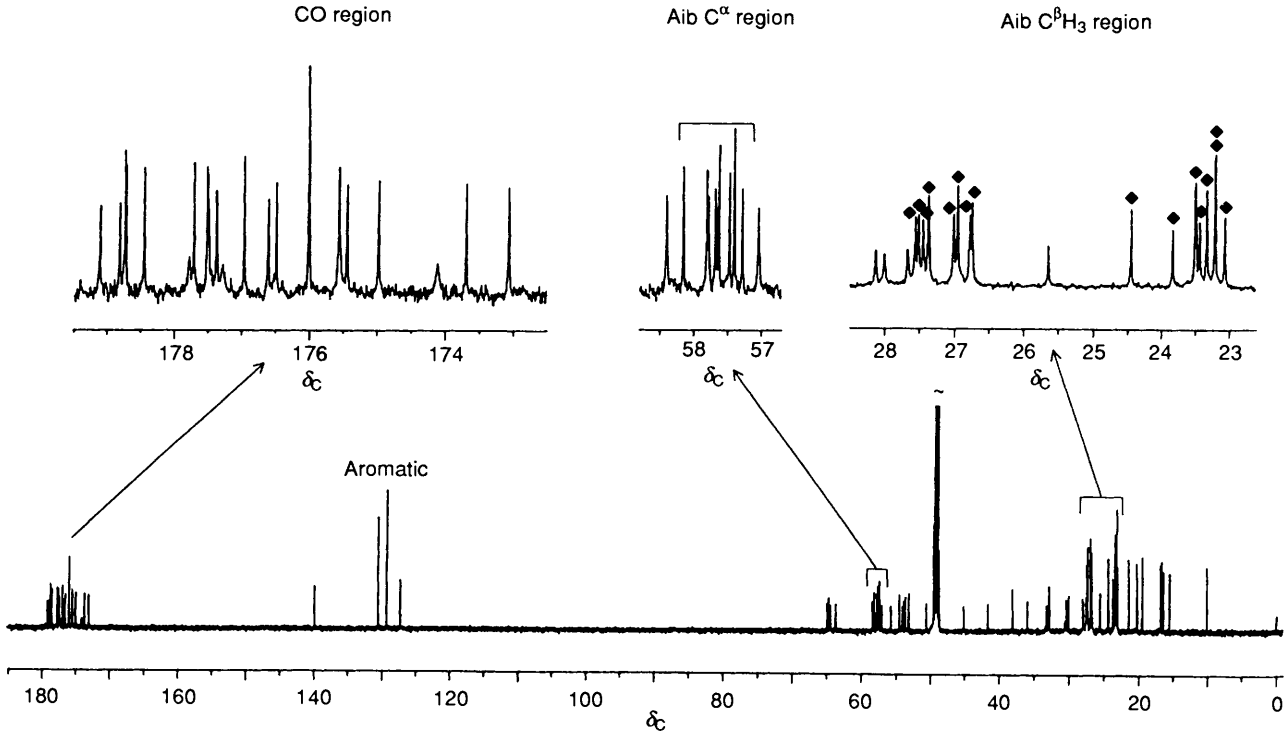

Fig. 4 150 MHz ^{13}C NMR spectrum of trichosporin B-V at 27 °C in CD_3OH (60 mmol dm^{-3}). The CO, Aib quarternary C^{α} and $C^{\beta}H_3$ carbon regions are expanded. The $C^{\beta}H_3$ signals are indicated by ◆. ~ Indicates solvent peak.

Table 2 Carbon chemical shifts[a] of trichosporin B-V in CD_3OH at 27 °C (10 mmol dm^{-3})

	δ_C			
Residue	α	β	CO	Others[b]
Ac	23.22		173.88	
Aib^1	57.86	24.43, 26.73	178.84	
Ala^2	53.65	16.89	177.67	
Ala^3	53.14	16.45	177.46	
Ala^4	53.90	16.70	177.09	
Aib^5	57.55	23.22, 27.00	176.69	
Aib^6	57.36	23.06, 27.38	178.84	
Gln^7	58.35	27.68	176.06	32.95 (γ), 177.61 (δ)
Aib^8	57.77	23.33, 27.57	178.54	
Ile^9	63.59	36.00	175.60	27.38 (γ^1), 15.58 (γ^2), 10.21 (δ)
Aib^{10}	57.87	23.50, 27.50	179.30	
Gly^{11}	45.14		173.16	
Leu^{12}	54.13	41.67	176.00	25.67 (γ), 21.48, 23.47 (δ)
Aib^{13}	58.20	23.83, 26.80	175.07	
Pro^{14}	64.73	30.13	176.58	26.95 (γ), 50.63 (δ)
Val^{15}	64.25	30.55	175.49	19.54, 20.24 (γ)
Aib^{16}	57.70	23.50, 27.43	177.76	
Aib^{17}	57.06	23.30, 27.00	178.96	
Gln^{18}	57.01	28.00	175.60	33.22 (γ)
Gln^{19}	55.75	28.14	174.12	32.82 (γ)
$Pheol^{20}$	54.53	38.09		64.92 (CH_2O), 139.79 (γ), 130.51 (δ), 129.26 (ε), 127.29 (ξ)

[a] Assignments of some Aib C^{α} and C^{β} carbons are tentative. [b] The signals arising from the $Gln^{18,19}$ C^{δ} carbons are observed at δ_C 177.44 and 177.94.

Concentration-dependence of the ^1H *NMR Parameters of the Amide Groups of Trichosporin B-V.*—For the conformational analysis of TS-B-V in solution, it is important to examine whether or not the conformation is affected by peptide aggregation if this would happen under the experimental conditions, or to discuss the effect of peptide concentration on the conformation if aggregation does not occur. Therefore, the NH chemical shifts, their temperature coefficients ($d\delta/dT$) and $^3J_{NH-C_{\alpha}H}$-values were monitored as a function of concentration (1–60 mmol dm^{-3}).

Concentration dependence of the NH chemical shifts. Apolar peptides like peptaibols are not expected to aggregate easily in methanol since the peptide molecules would prefer peptide–solvent interactions to peptide–peptide ones; methanol is a strongly hydrogen-bonding solvent like dimethyl sulfoxide[32] and dissolves TS-B-V very easily. Line broadening was not recognized in the one-dimensional ^1H NMR spectra measured at concentrations of 1, 5, 10, 30 and 60 mmol dm^{-3}. However, the possibility of aggregation in methanol cannot be ruled out as reported previously with respect to alamethicin.[17] It is believed that large shifts of NH signals show the presence of intermolecular hydrogen bonds.[32] Fig. 6 shows a comparison

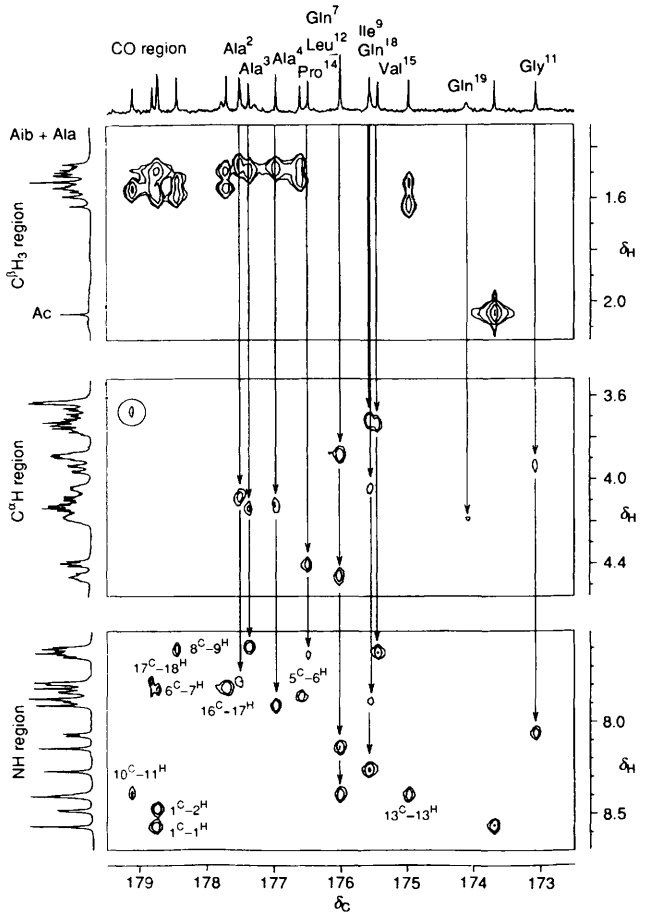

Fig. 5 Parts of a $^{13}C,^{1}H$-COLOC spectrum at 27 °C in CD_3OH (60 mmol dm $^{-3}$). Cross-peaks with the CO carbons of the amino acids other than Aib residues are indicated by arrows; the $^{2}J_{CH}$-connectivities between the Gln[19] CO carbon and the Pheol[20] NH proton is not shown here. Numbers near cross-peaks which are due to long-range couplings with the Aib CO carbons represent residual numbers. The superscript C and H stand for CO carbons and NH protons, respectively. The cross-peak surrounded by a circle is a $^{3}J_{CH}$-connectivity between the Aib[10] CO carbon and one of the Gly[11] $C^{\alpha}H_2$ protons.

of the NH chemical shifts at concentrations of 1 and 60 mmol dm^{-3}. The NH chemical shift of each residue is found to be almost insensitive to peptide concentration. Even in Ala[4], which shows the largest shift, the change of the chemical shift ($\Delta\delta$) is 0.032 ppm. This result indicates the absence of intermolecular hydrogen bonds between the backbone NH protons and CO carbons. However, the possibility of aggregation still remains since the Gln side-chain NH protons can take part in hydrogen bonding.[33] Hence, the concentration dependence of these NH chemical shifts was examined. The change of the chemical shifts was almost identical with that of Ala[4] ($\Delta\delta < 0.035$). From these observations, therefore, it was shown that the backbone and side-chain NH protons do not participate in intermolecular hydrogen bonding and TS-B-V does not aggregate at concentrations up to 60 mmol dm^{-3} in methanol.

Temperature coefficients and $^{3}J_{NH-C\alpha H}$*-values as a function of concentration.* Temperature coefficients ($d\delta/dT$) of NH chemical shifts provide information on NH protons participating in intramolecular hydrogen bonding. If the conformation is influenced by peptide concentration, the values would be changed. Fig. 7 shows the values determined at concentrations of 1 and 60 mmol dm^{-3}. From the data, the Aib[1] and Ala[2] NH protons are considered to be exposed to solvent. On the other hand, the Ala[3,4], Val[15] and Aib[16] NH protons are found to be solvent-shielded or to participate in strong intramolecular hydrogen bonding. However, there is no significant concentration dependence in the temperature coefficients. This result indicates that TS-B-V adopts the same intramolecular hydrogen-bonding patterns up to 60 mmol dm^{-3}. In addition, the $^{3}J_{NH-C\alpha H}$-values of the corresponding amino acid residues at respective concentrations were very similar (Table 3). As already reported,[34] $^{3}J_{NH-C\alpha H}$ coupling constants reflect solution conformations of peptides. Therefore, TS-B-V is most likely to take an almost constant conformation irrespective of the peptide's concentration in methanol.

Table 3 Concentration dependence of the $^{3}J_{NH-C\alpha H}$-values (Hz) at 27 °C

	Concentration (mmol dm^{-3})				
Residue	1	5	10	30	60
Ala[2]	4.7[a]	4.1	4.1	4.0	4.0
Ala[3,b]	n.d.	n.d.	n.d.	n.d.	n.d.
Ala[4]	5.8	6.0	6.0	5.9	6.0
Gln[7]	4.7	4.7	4.7	4.7	4.5
Ile[9]	6.2	6.1	6.1	6.1	6.3
Gly[11]	5.1	5.0	5.0	5.1	5.0
Leu[12]	8.0	7.8	7.7	7.8	7.7
Val[15]	7.6	7.8	7.8	7.7	7.8
Gln[18,b]	n.d.	n.d.	n.d.	n.d.	n.d.
Gln[19]	n.d.	n.d.	7.5[c]	7.7.	7.5
Pheol[20]	n.d.	n.d.	9.2[c]	9.2[c]	9.2[c]

[a]This value suggests that the *N*-terminal helical structure is somewhat flexible at this concentration. [b] The Ala[3] and Gln[18] NH chemical shifts are almost identical at 27 °C. [c] Obtained from *J*-resolved spectra. n.d.: Not determined.

Chemical Shifts of the NH Protons and $^{3}J_{NH-C\alpha H}$ *Coupling Constants of Trichosporin B-Ia, -IIIa, -IVd and -VIb.*—The ^{1}H NMR signals of TS-B-Ia, -IIIa, -IVd and -VIb were assigned similarly. Their primary structures differ from that of TS-B-V by the replacement of only one or two amino acid residues. Thus, TS-Bs are deduced to adopt very similar backbone conformations. On the other hand, the differences in their catecholamine-release activity suggest the possibility of significant conformational changes due to the replacement of amino acids. In that case, it is expected that the conformations of TS-B-Ia and -VIb would be considerably different from each other and those of the remaining peptides are very similar to each other, judging from the activity of each peptide. Hence, the effect of the replacement on their conformations was discussed. For brevity, Table 4 summarizes only the NH chemical shifts and $^{3}J_{NH-C\alpha H}$-values.

In TS-B-Ia, in which Ser occupies position 3, the NH proton of Ala[4] shows a low-field shift ($\Delta\delta > 0.1$), but its $^{3}J_{NH-C\alpha H}$-value is not significantly different from those of the other TS-Bs. Furthermore, the Ala[2] and Ser[3] $^{3}J_{NH-C\alpha H}$-values are almost identical with those of Ala[2] and Ala[3] of the other TS-Bs. When position 9 is Ile (as in TS-B-V and -VIb) or when position 17 is Iva (as in -IVd and -VIb), the Aib[10] and Aib[16] NH chemical shifts seem to be affected: the signals shift slightly to the low-field region ($\Delta\delta$ 0.05 and 0.07, respectively). However, there is no marked effect of the replacement on the $^{3}J_{NH-C\alpha H}$-values of the residues near both positions. In addition, the $^{3}J_{NH-C\alpha H}$-values of Ile, Leu and Val at position 9 are very close. From these observations, therefore, it is considered that the above replacement at positions 3, 9 and 17 does not contribute to conformational changes of TS-Bs and that they adopt very similar backbone conformations in methanol. Similar results can be seen in a conformational study of trichorzianines.[35] That report describes the differences in their biological activity, the

Table 4 NH Chemical shifts[a] and $^3J_{NH-CαH}$-values of trichosporin B-Ia, -IIIa, -IVd and -VIb in CD_3OH at 27 °C

Residue	Ia (5 mmol dm^{-3}) δ_H	J (Hz)	IIIa (5 mmol dm^{-3}) δ_H	J (Hz)	IVd (5 mmol dm^{-3}) δ_H	J (Hz)	VIb (5 mmol dm^{-3}) δ_H	J (Hz)
1	8.631		8.556		8.560		8.558	
2	8.476	4.3	8.446	4.1	8.450	4.1	8.451	4.0
3	7.891	6.6	7.79	n.d.	7.782	6.5	7.784	6.5
4	7.678	5.7	7.558	5.8	7.555	5.9	7.557	6.0
5	7.870		7.891		7.885		7.883	
6	7.79		7.832		7.840		7.846	
7	7.849	4.9	7.84	n.d.	7.851	4.5	7.828	4.6
8	8.106		8.129		8.101		8.131	
9	7.61	n.d.	7.636	6.0	7.536	5.8	7.592	5.8
10	8.172		8.188		8.208		8.256	
11	8.372	5.0	8.376	5.1	8.369	5.1	8.390	4.9
12	8.096	7.8	8.100	7.9	8.075	7.8	8.063	7.6
13	8.264		8.274		8.423		8.398	
14								
15	7.62	n.d.	7.613	7.8	7.605	7.7	7.603	7.8
16	7.597		7.599		7.675		7.680	
17	7.79		7.794		7.738		7.736	
18	7.78	n.d.	7.79	n.d.	7.759	5.5	7.758	5.4
19	7.870	7.3	7.870	7.3	7.869	7.6	7.867	7.6
20	7.31	9.0[b]	7.309	9.2	7.25	9.0[b]	7.26	9.0[b]

[a] Chemical shifts obtained from two-dimensional spectra are expressed with $\Delta\delta$ ±0.01 ppm. [b] Obtained from J-resolved spectra. n.d.: Not determined.

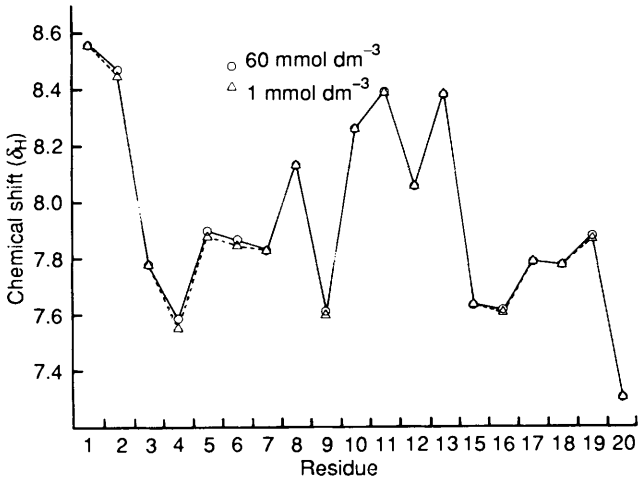

Fig. 6 Concentration dependence of the NH chemical shifts at 27 °C in CD_3OH. The NH chemical shifts obtained at concentrations of 5, 10 and 30 mmol dm^{-3} are also in good agreement with those shown here.

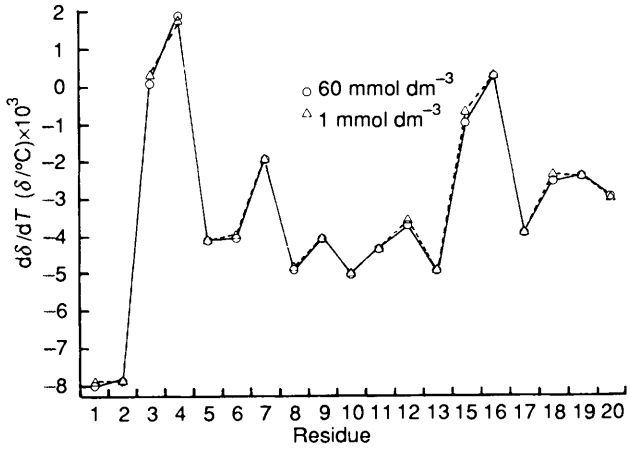

Fig. 7 Temperature coefficients of the NH chemical shifts obtained in the range 13–34 °C in CD_3OH. Positive $d\delta/dT$-values represent downfield shifts of the NH signals with increasing temperature. The values determined at concentrations of 5, 10 and 30 mmol dm^{-3} also show no concentration dependence.

growth inhibition and lysis of the amoeba *Dictyostelium*,[36] are a result of a negative charge on the Glu residue. In the case of TS-Bs, the molecules have no charge. Therefore, the differences in the catecholamine-release activity of TS-Bs is likely to result from lipophilicity of the molecules, judging from the lowest activity found in TS-B-Ia containing Ser and the highest one found in TS-B-VIb containing Ile and Iva.

Experimental

For NMR measurements, dried and purified samples were dissolved in CD_3OH or CD_3OD (0.35 cm^3), containing $SiMe_4$ as an internal standard. All NMR experiments were performed on a Bruker AM-600 spectrometer.

COSY and RCT spectra were measured in absolute-value mode, in the case of CD_3OH, with selective saturation of the OH resonance of the solvent only during the relaxation delay time. A total of 512 t_1-values were recorded, with 2048 data points and 16 or 32 scans for each t_1. The data matrices were filtered by sine-bell multiplication in the t_1 and t_2 dimensions; spectral width 6024 Hz; relaxation delay time 2.5 s; mixing time (for RCT) 34 ms.

NOESY spectra were measured in phase-sensitive mode in CD_3OH with selective irradiation of the OH resonance at all times except during the evolution and detection periods. A total of 512 t_1-values were recorded, with 2048 data points and 32, 48 or 128 scans for each t_1. The data matrices were filtered by shifted squared sine-bell multiplication in the t_1 and t_2 dimensions and zero-filled to 2048 data points in t_1 dimension; spectral width 6330 Hz; relaxation delay time 2.5 s; mixing time 300 ms with a random variation of 15 ms. The spectra were unsymmetrized.

A HOHAHA spectrum was recorded in phase-sensitive mode at 27 °C in CD_3OD. A total of 512 t_1-values were recorded, with 2048 data points and 16 scans for each t_1. The data matrix was filtered by shifted squared sine-bell multiplication in the t_1 and t_2 dimensions and zero-filled to 2048 data points in t_1 dimension; spectral width 6172 Hz; relaxation delay time 2.5 s; mixing time 102 ms. The spectrum was unsymmetrized.

^{13}C, ^{1}H-COLOC and ^{13}C,^{1}H-COSY spectra were recorded at 27 °C in CD_3OH. A total of 256 t_1-values were recorded, with 8192 data points and 128 scans for each t_1; spectral width 6000 Hz in F_1 and 29 410 Hz in F_2. Gaussian line broadening (7 Hz) and shifted sine-bell filter were used in the t_1 and t_2 dimensions, respectively; relaxation delay time 2.3 s; delay time (Δ_1,Δ_2) to obtain remote $^{2}J_{CH}$- and $^{3}J_{CH}$-connectivities for ^{13}C,^{1}H-COLOC (36 ms, 18 ms), (80 ms, 40 ms) and (120 ms, 60 ms).

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