Fungal Metabolites. XIII.[1] Isolation and Structural Elucidation of New Peptaibols, Trichodecenins-I and -II, from *Trichoderma viride*

Tetsuro FUJITA,*[,a] Shun-ichi WADA,[a] Akira IIDA,[a] Tetsuya NISHIMURA,[a] Michiko KANAI,[b] and Nobuo TOYAMA[c]

Faculty of Pharmaceutical Sciences, Kyoto University,[a] Sakyo-ku, Kyoto 606–01, Japan, Finnigan MAT Instruments, Inc.,[b] Chiyoda-ku, Tokyo 102, Japan, and Faculty of Horticulture, Minamikyushu University,[c] Takanabe-cho, Miyazaki 884, Japan. Received September 27, 1993; accepted November 29, 1993

Three new groups of peptaibols, trichodecenins, trichorovins and trichocellins, have been isolated from conidia of the fungus, *Trichoderma viride*. The structures of trichodecenins-I and -II were established by positive-ion fast-atom bombardment, collision-induced dissociation mass spectrometry and two-dimensional NMR spectroscopy. Trichodecenins-I and -II have a (*Z*)-4-decenoyl group, six amino acid residues and a leucinol moiety in the molecules. Trichodecenin-II was synthesized by the solution-phase method.

Keywords *Trichoderma viride*; peptaibol; α-aminoisobutyric acid; trichodecenin; trichorovin; trichocellin

Trichoderma spp. are imperfect fungi present in soil throughout the world. They are active as mycoparasites, and so represent potential biocontrol agents. Many antibiotic peptides belonging to the class of peptaibols, such as alamethicin,[2] trichopolyn,[3] trichosporin[4] and trichogin,[5] have been isolated from *Trichoderma* spp. The structural characteristics of peptaibols are as follows: the N- and C-terminal amino acids are protected by an acyl group and an aminoalcohol such as phenylalaninol (Pheol) or leucinol (Lol), respectively, and the molecules contain an abnormal amino acid, α-aminoisobutyric acid (Aib), in a high ratio. Peptaibols have unique biological activities such as voltage-dependent ion channel formation,[6] uncoupling activity against rat liver mitochondria[7] and induction of catecholamine secretion from adrenal chromaffin cells.[8]

In the course of our search for antibacterial principles,[9] three new groups of peptaibols, trichodecenins (TDs), trichorovins (TVs) and trichocellins (TCs), have been isolated from the methanolic extract of conidia of *T. viride*, which is a strain producing large amounts of cellulase.[10] TDs, TVs and TCs are peptaibols composed of 7, 11 and 20 amino acid residues, respectively. In this paper, we describe the isolation of these peptaibols, together with the structural elucidation and NMR assignments of TDs-I and -II, and the synthesis of TD-II.

Results and Discussion

Isolation of TDs, TVs and TCs The isolation scheme is shown in Fig. 1. Conidia of *T. viride* were extracted with methanol, and the methanol extract was partitioned between ethyl acetate (EtOAc) and water. The EtOAc-

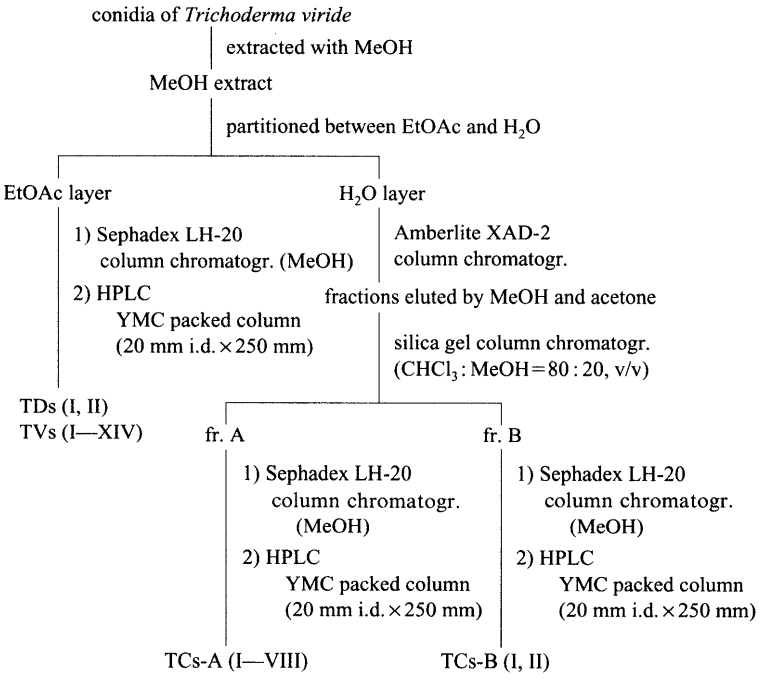

Fig. 1. Isolation Scheme for TDs, TVs and TCs

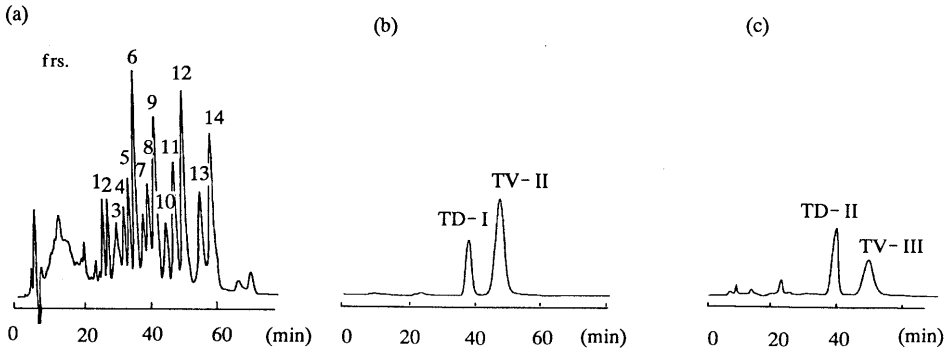

Fig. 2. Analytical HPLC Chromatograms of the Whole Peptide Mixture (a), Frs. 2 (b) and 3 (c)

Conditions: mobile phase, acetonitrile–H_2O (60:40, v/v) for (a), MeOH–H_2O (80:20, v/v) for (b) and (c); flow rate, 0.8 ml/min for (a), 0.6 ml/min for (b) and (c); detector, UV (220 nm); column, YMC packed column AM-313 (6 mm i.d. × 250 mm); column temperature, 40°C.

soluble fraction was chromatographed on Sephadex LH-20 to give a peptide mixture (Fig. 2a), which was fractionated by semi-preparative HPLC on a reversed-phase octadecyl silica (ODS) column to yield frs. 1 to 14. Unfortunately, all of them showed heterogeneity in their NMR spectra. Further separation of frs. 2 and 3 gave TD-I and TV-II, and TD-II and TV-III, respectively (Fig. 2b, c). The TD group has a molecular weight of 751, while the TV group has a molecular weight of *ca.* 1100. On the other hand, frs. 1 and 4—14, which could not be further purified, contained TVs-I and -IV—XIV. TDs-I and -II were obtained as pure compounds by further HPLC, while TVs-II and -III were still mixtures of closely related compounds.

The water-soluble fraction of the methanolic extract gave TCs-A-I—VIII and -B-I and -B-II. The above fraction was subjected to Amberlite XAD-2 and silica gel column chromatography to give two fractions, frs. A [*Rf* 0.60 ($CHCl_3$: MeOH : H_2O = 60 : 35 : 5, v/v/v)] and B (*Rf* 0.45). Each of frs. A and B was chromatographed on LH-20, followed by reversed-phase HPLC to give TCs-A and -B, respectively. TCs-A (Fig. 3a) and -B (Fig. 3b) showed eight and seven peaks, respectively, on their HPLC chromatograms. The components of these peaks were repeatedly purified by HPLC to give TCs-A-I—VIII from fr. A and, TCs-B-I and -B-II from fr. B, as pure compounds.

Characterization of TDs-I and -II TDs showed the following spectral data, indicating the presence of amide bonds; IR (KBr) 3300 (NH), 1650 (CO) and 1540 (NH) cm^{-1}; ^{1}H-NMR δ_H 7.2—8.3 (NH); ^{13}C-NMR δ_C 168—175 (CO). Furthermore TDs were negative to both the ninhydrin reaction and methylation with diazomethane, suggesting that the N- and C-terminal amino acids of TDs are both protected. Amino acid analyses of the complete acid hydrolysates revealed that TD-I contains Gly (3), Leu (1) and Ile (1), while TD-II has Gly (3) and Leu (2). The analysis of quaternary carbons (δ_C *ca.* 55.9) in both TDs-I and -II appearing in the distortionless enhancement by polarization transfer (DEPT) spectra suggested the presence of one Aib residue in each molecule. The absolute configuration of L-Ile and L-Leu in TDs was determined by HPLC analyses of the 3,5-dinitrobenzoate methyl esters of acid hydrolysates. At the same time, the presence of L-Lol and Aib was recognized. The DEPT spectra of TDs showed ten signals originating from CH_3 (1), CH_2 (6), –CH= (2) and CO (1), in addition to the signals arising from the amino acids and Lol, suggesting that both TDs have a linear decenoyl chain.

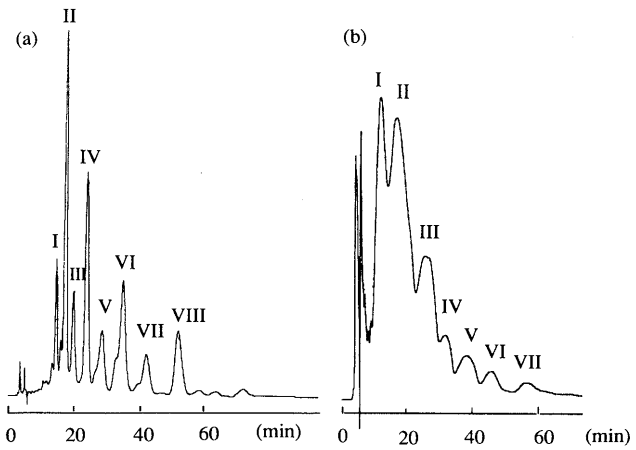

Fig. 3. Analytical HPLC Chromatograms of Trichocellins-A (a) and -B (b)

Conditions: mobile phase, acetonitrile–H_2O (60:40, v/v) for (a), acetonitrile–H_2O (55:45, v/v) for (b); flow rate, 0.8 ml/min for (a), 0.4 ml/min for (b); detector, UV (220 nm); column, YMC packed column AM-313 (6 mm i.d. × 250 mm) for (a), YMC Pack ODS-AM (4.6 mm i.d. × 150 mm) for (b); column temperature, 40°C.

These observations indicate that the N-terminal amino acids of TDs are protected by a decenoyl group and the C-terminal amino acids are linked with Lol. Considering the findings mentioned above, the molecular formulas of TDs should be $C_{38}H_{69}N_7O_8$ (MW 751).

Structures of TDs-I and -II The amino acid [Gly (3), Leu (1), Ile (1) and Aib (1)] sequence in TD-I was investigated by fast-atom bombardment (FAB) and collision induced-dissociation (CID)[11] mass spectrometry. Figure 4 shows the positive-ion FAB-MS of TD-I. The protonated molecular ion, $(M+H)^+$, is observed at *m/z* 752. In addition to the matrix-adduct and dehydration ions (*m/z* 860, 844, 734, respectively), some sequence-specific ions are observed. Three y-type ions[12] (ammonium ions) at *m/z* 600, 543 and 486 give the partial N-terminal structure, decenoyl-Gly^1–Gly^2–, while one b-type ion[12] (acylium ion) at *m/z* 635 confirms the presence of Lol at the C-terminus. Thus, the structure of TD-I is decenoyl-Gly^1–Gly^2–(Leu, Aib, Gly and Ile)$^{3-6}$–Lol^7.

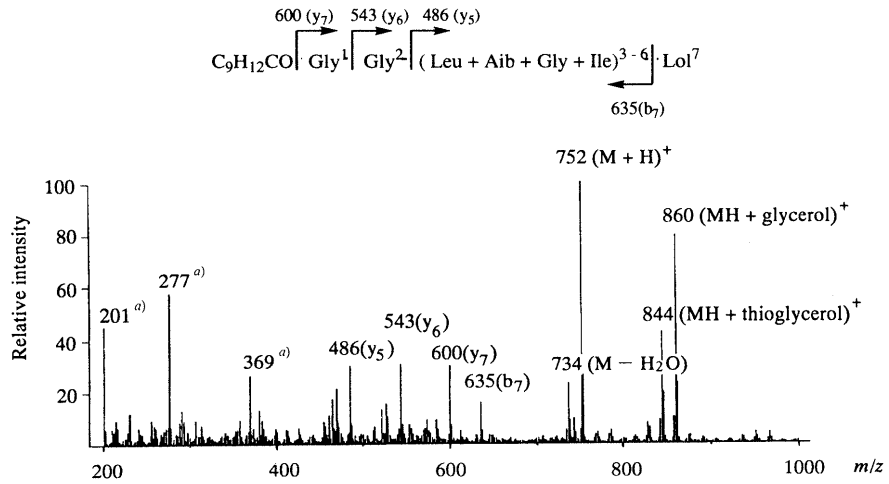

Fig. 4. Positive-Ion FAB-MS of TD-I

a) Matrix-related ions.

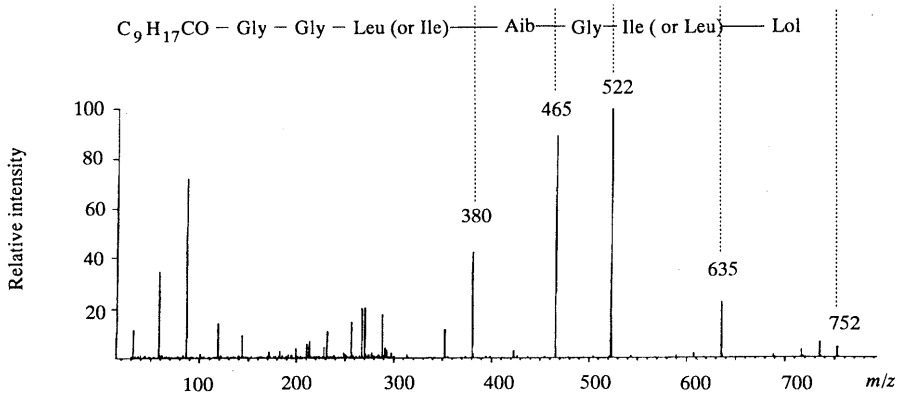

Fig. 5. Product Ion Spectrum of *m/z* 752 ion of TD-I

The sequence-specific ions between Gly^2 and Lol^7 are of minor abundance. The complete sequence between Gly^2 and Lol^7 was determined by CID of the $(M+H)^+$ ion at *m/z* 752. The acylium fragment ions at *m/z* 380, 465, 522, 635 and 752 in the product ion spectrum (Fig. 5) led to decenoyl-Gly^1–Gly^2–Leu (or Ile)^3–Aib^4–Gly^5–Leu (or Ile)^6–Lol^7 as the structure of TD-I.

The remaining problems are determination of the location of Ile, Leu and the double bond in the decenoyl group. All of them were solved by using two-dimensional (2D) NMR techniques, *i.e.*, ^1H,^1H-correlated spectroscopy (COSY),[13] relayed coherence transfer (RCT)-COSY,[14] nuclear Overhauser enhancement spectroscopy (NOESY)[15] and ^{13}C, ^1H-correlated spectroscopy *via* long-range coupling spectroscopy (COLOC).[16] First, the spin systems of amino acids[17] and Lol were identified by ^1H,^1H-COSY and RCT-COSY. Next, sequence-specific assignments[18] of the NH protons was carried out using NOESY. Figure 6 shows the NH–NH region of the NOESY spectrum, with successive NH–NH cross-peaks starting from Gly^2 to Lol^7. Moreover, the Gly^1–NH has a cross-peak with the Gly^2–αH in the finger region (not shown here). Therefore, the complete amino acid sequence of TD-I was determined to be as follows: Gly^1–Gly^2–Leu^3–Aib^4–Gly^5–Ile^6–Lol^7.

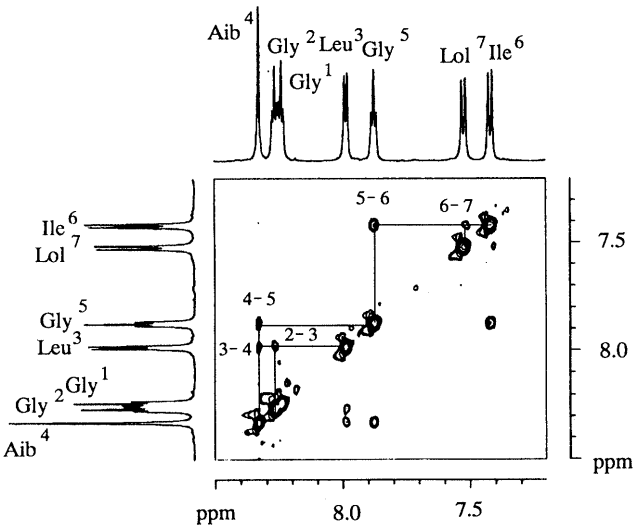

Fig. 6. NH–NH Region of the 600 MHz NOESY Spectrum of TD-I

In DMSO-d_6 at 23°C (mixing time: 1 s, unsymmetrized). Continuous lines reveal the NH–NH connectivities from Gly^2 to Lol^7.

The location and geometry of the double bond in the decenoyl group were established from the NMR connectivities shown in Fig. 7. In the ^{13}C, ^1H-COLOC spec-

trum, the decenoyl carbonyl carbon displayed connectivities with the Gly[1]–NH and 2′-protons. In the ^{1}H, ^{1}H-COSY spectra, the 3′-protons showed a cross-peak with one of the vinyl protons, which was assigned as the 4′-proton. Furthermore, the 3′-protons had an NOE cross peak with the 6′-protons. These results clarified that the acyl moiety is a (*Z*)-4-decenoyl group. The coupling constant, $^3J_{4',5'}$ (= 10.8 Hz, CD_3OH, 10 °C), supports the (*Z*)-configuration.

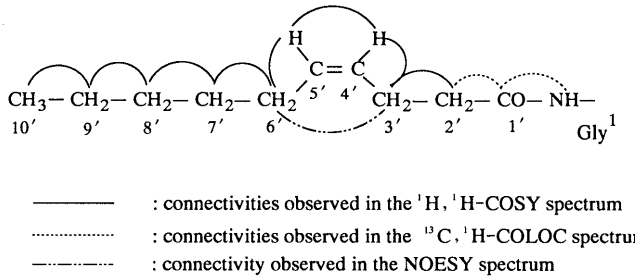

Fig. 7. Connectivities of (*Z*)-4-Decenoyl-Gly[1] Observed by 2D NMR
Only important connectivities are shown.

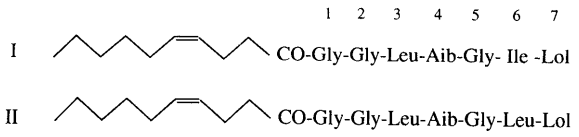

Fig. 8. Structures of TDs-I and -II

Therefore, the total structure of TD-I was concluded to be (*Z*)-4-decenoyl-Gly–Gly–Leu–Aib–Gly–Ile–Lol (Fig. 8). The proton and carbon chemical shifts assigned by using 1D and 2D NMR are listed in Tables I and II, respectively, together with those of TD-II. The structure of TD-II was also determined, in the same manner, to be as follows: (*Z*)-4-decenoyl-Gly–Gly–Leu–Aib–Gly–Leu–Lol (Fig. 8). TD-II differs from TD-I by replacement of Ile[6] by Leu.

Synthesis of TD-II The main component, TD-II, was synthesized by the solution-phase method, to obtain sufficient material for screening various biological activities. As protecting groups, the benzyloxycarbonyl group (Z) and methyl ester were used for the N- and C-terminal amino acids, respectively. For deprotection, the Z was removed by catalytic hydrogenation and the methyl ester was hydrolyzed with 1 N NaOH. The synthetic scheme is shown in Fig. 9. Peptide fragments [2] and [3] were synthesized stepwise from the C-terminals with *N*,*N*′-dicyclohexylcarbodiimide (DCC)–1-hydroxybenzotriazole (HOBt). The (*Z*)-4-decenoic acid [1] was synthesized according to the reported method,[19] *i.e.*, the (*Z*)-4-hexenolide was treated with lithium dibutylcuprate to give a mixture of fatty acids. Without purification, the mixture was condensed with the N-terminal deprotected peptide fragment [2] to afford three products. After purification by reversed-phase HPLC, the desired acylated tetrapeptide was subjected to alkaline hydrolysis. The tetrapeptide acid was coupled with H-Gly–Leu–Lol

TABLE I. Proton Chemical Shifts[a] and Coupling Constants of TDs-I and -II in DMSO-d_6 at 27 °C (600 MHz)

Residue	TD-I		TD-II	
	δ NH, mult, *J* (Hz)	δ other groups, mult, *J* (Hz)	δ NH, mult, *J* (Hz)	δ other groups, mult, *J* (Hz)
(*Z*)-4-Decenoyl-				
CH_3(10′)		0.852 t (6.73)		0.855 t (6.04)
CH_2(9′)		1.27 m		1.27 m
CH_2(8′)		1.25 m		1.22 m
CH_2(7′)		1.31 m		1.29 m
CH_2(6′)		1.99 m		1.98 m
CH (5′)		5.35 m		5.40 m
CH (4′)		5.32 m		5.31 m
CH_2(3′)		2.24 m		2.23 m
CH_2(2′)		2.17 m		2.17 m
Gly 1	8.234 t (5.60)	α 3.677 d (5.36)	8.196 d (5.65)	α 3.681 d (5.54)
Gly 2	8.266 dd (5.76, 5.76)	α 3.807 dd (16.60, 6.01), α′ 3.664 dd (15.27, 5.68)	8.241 dd (5.68, 5.68)	α 3.777 dd (16.40, 5.70) α′ 3.696 dd (20.29, 5.59)
Leu 3	7.971 d (6.72)	α 4.209 dt (6.50, 4.98), β 1.54 m, γ 1.62 m, δ_1 0.903 d (6.48), δ_2 0.854 d (6.44)	7.985 d (7.01)	α 4.22 m, β 1.53 m, γ 1.63 m, δ_1 0.898 d (6.52), δ_2 0.850 d (6.26)
Aib 4	8.276 s	β_1 1.358 s, β_2 1.327 s	8.169 s	β_1 1.352 s, β_2 1.331 s
Gly 5	7.871 dd (5.88, 5.77)	α 3.633 dd (16.85, 5.71), α′ 3.522 dd (16.77, 5.49)	7.912 dd (5.88, 5.83)	α 3.608 dd (16.82, 6.19), α′ 3.558 dd (16.84, 5.49)
Leu 6			7.491 d (8.17)	α 4.17 m, β_1 1.64 m, β'_1 1.45 m, γ 1.34 m, δ_1 0.870 d (6.03), δ_2 0.812 d (6.43)
Ile 6	7.405 d (8.59)	α 4.037 dd (8.37, 8.36), β 1.85 m, γ_1 1.47 m, γ'_1 1.11 m, γ_2 0.819 d (6.84), δ 0.787 t (6.53)		
Lol 7	7.459 d (8.76)	α 3.79 m, β_1 1.33 m, β'_1 1.26 m, β_2 3.32 m, β'_2 3.18 m, γ 1.60 m, δ_1 0.835 d (6.41), δ_2 0.782 d (6.27), –OH 4.599 dd (5.56, 5.56)	7.209 d (8.95)	α 3.77 m, β_1 1.30 m, β'_1 1.26 m, β_2 3.29 m, β'_2 3.19 m, γ 1.59 m, δ_1 0.829 d (6.67), δ_2 0.791 d (6.55), –OH 4.498 dd (5.70, 5.48)

a) Chemical shifts obtained from two-dimensional spectra are expressed in $\Delta\delta = \pm 0.01$.

TABLE II. Carbon Chemical Shifts of TDs-I and -II in DMSO-d_6 at 27 °C (75 MHz)

Residue	TD-I δ	TD-II δ
Decenoyl-		
CH_3(10′)	13.80	13.82
CH_2(9′)	21.88	21.88
CH_2(8′)	30.79	30.79
CH_2(7′)	28.65	28.65
CH_2(6′)	26.48	26.48
CH (5′)	130.07	130.13
CH (4′)	128.39	128.35
CH_2(3′)	22.77	22.82
CH_2(2′)	34.95	34.99
CO (1′)	172.54	172.46
Gly 1	α 42.36, CO 169.69	α 42.29, CO 169.91
Gly 2	α 42.05, CO 169.66	α 42.21, CO 169.62
Leu 3	α 51.82, β 39.82, γ 24.02, δ_1 22.77, δ_2 21.69, CO 172.62	α 51.85, β 39.54, γ 23.98, δ_1 22.82, δ_2 21.55, CO 172.85
Aib 4	α 55.89, β_1 25.25, β_2 24.35, CO 174.41	α 55.85, β_1 24.89, β_2 24.55, CO 174.79
Gly 5	α 42.97, CO 168.70	α 43.10, CO 168.79
Ile 6	α 57.25, β 35.71, γ_1 24.35, γ_2 15.22, δ 10.79, CO 170.65	
Leu 6		α 51.37, β 40.29, γ 23.98, δ_1 23.06, δ_2 21.18, CO 171.68
Lol 7	α 48.72, β_1 40.00, β_2 63.81, γ 24.02, δ_1 23.28, δ_2 21.60	α 48.58, β_1 39.73, β_2 63.86, γ 23.98, δ_1 23.28, δ_2 21.63

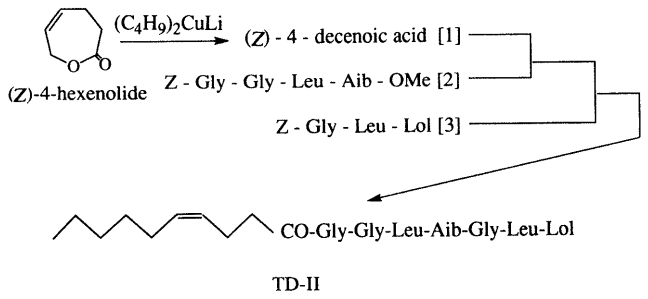

Fig. 9. Synthetic Scheme of TD-II

derived from fragment [3] to give TD-II. The synthetic TD-II was confirmed to be identical with the natural product by HPLC, FAB-MS, and ^{1}H- and ^{13}C-NMR. The physico-chemical constants (melting point and $[\alpha]_D$), as well as the spectral data were in good agreement with those of the natural TD-II.

Experimental

General Procedures All melting points were measured on a Yanagimoto micro melting point apparatus, without correction. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. IR spectra were obtained on a Shimadzu IR-435 with the KBr method. All NMR experiments were carried out by using JEOL JNM-FX200, Bruker AC-300 and Bruker AM-600 spectrometers. For FAB-MS and CID, a Finnigan MAT 70 triple-stage quadrupole mass spectrometer was used. Samples were bombarded with 8 keV xenon atoms. For CID experiments, argon was used as the collision gas. Samples were dissolved in 5% aqueous acetic acid solution or 0.1% trifluoroacetic acid (TFA) methanol solution and mixed with glycerol–thioglycerol (1 : 1, v/v) as a matrix. TLC was performed on silica gel (Kieselgel 60F_{254}, Merck). For gel filtration, Sephadex LH-20 (Pharmacia) was employed. HPLC was performed on a Shimadzu LC-6A system or an LC-8A system. Coupling reactions were performed by the DCC–HOBt method at room temperature for 24 h. Unless otherwise stated, work-up was carried out as follows: the mixture, after removal of *N,N′*-dicyclohexylurea (DCU) and the solvent, was extracted with EtOAc and the extract was washed with 1 N HCl, saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel chromatography or recrystallization from appropriate solvents. The benzyloxycarbonyl group, Z, was removed in MeOH containing 1 eq of 1 N HCl by the use of H_2 gas over 10% palladium-on-charcoal with stirring. After removal of the catalyst by filtration, the filtrate was concentrated. The residue was usually employed in the following step without further purification.

Isolation of TDs and TVs Conidia of *T. viride* (163 g) were extracted with MeOH (500 ml) at 60 °C for 30 min three times, followed by evaporation to give a residue (35.4 g). The residue was suspended in water (200 ml) and the suspension was extracted with EtOAc (300 ml) three times. The EtOAc-soluble layers were combined and concentrated. The residue (6.31 g) was subjected to gel filtration chromatography on Sephadex LH-20 with MeOH. The HBr–ninhydrin positive fractions (checked by TLC) were combined and the solvent was removed *in vacuo*. The residue containing peptides (1.23 g) was repeatedly subjected to preparative HPLC [conditions: mobile phase, acetonitrile–H_2O (60 : 40, v/v); flow rate, 6 ml/min; detector, UV (220 nm); column, YMC packed column SH-343-5 (20 mm i.d. × 250 mm, Yamamura Chemical Laboratories Co., Ltd.); column temperature, 40 °C] to give frs. 1—14. Further purification of frs. 2 and 3 was performed by repeated HPLC [conditions: mobile phase, MeOH–H_2O (80 : 20, v/v); flow rate, 5 ml/min; detector, UV (220 nm); column, YMC packed column SH-343-5 (20 mm i.d. × 250 mm); column temperature, 40 °C]. TDs-I (5.7 mg); mp 87—90 °C; $[\alpha]_D^{25}$ −18.2° (c=0.29, MeOH), -II (20.8 mg); 167—170 °C; $[\alpha]_D^{25}$ −17.2° (c=0.37, MeOH), TVs-I (13.9 mg), -II (7.8 mg), -III (9.5 mg), -IV (13.5 mg), -V (15.4 mg), -VI (34.9 mg), -VII (12.1 mg), -VIII (16.9 mg), -IX (42.9 mg), -X (18.8 mg), -XI (25.3 mg), -XII (41.2 mg), -XIII (37.0 mg) and -XIV (48.7 mg).

Isolation of TCs-A-I—VIII, -B-I and -B-II The water-soluble fraction of the methanolic extract (281 g) of the conidia (1.06 kg) was subjected to Amberlite XAD-2 column chromatography. The eluates with MeOH and acetone were combined and concentrated. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 80 : 20, v/v) to give two fractions, frs. A (1.88 g) and B (2.85 g), *Rf* 0.60 and 0.45 ($CHCl_3$: MeOH : H_2O = 60 : 35 : 5, v/v/v), respectively. Each of these fractions was subjected to LH-20 column chromatography (MeOH) to give TCs-A (1.69 g) and -B (2.05 g). TC-A was subjected to HPLC [conditions: mobile phase, acetonitrile–H_2O (60 : 40, v/v); flow rate, 99.9 ml/min; detector, UV (220 nm); column, YMC packed column R-355-15 (50 mm i.d. × 500 mm); column temperature, 40 °C] and further fractionation was carried out by recycling HPLC 2—6 times [conditions: mobile phase, acetonitrile–H_2O (60 : 40, v/v); flow rate, 10 ml/min; detector, UV (220 nm); column, Cosmosil 5C18 (20 mm i.d. × 250 mm); column temperature, 40°C]. TCs-A-I (7.5 mg), -A-II (36.0 mg), -A-III (7.5 mg), -A-IV (53.9 mg), -A-V (29.2 mg), -A-VI (21.1 mg), -A-VII (17.9 mg) and -A-VIII (36.6 mg).

TC-B was subjected to HPLC [conditions: mobile phase, MeOH–H_2O (80 : 20, v/v); flow rate, 50 ml/min; detector, UV (220 nm); column, YMC packed column R-355-15 (50 mm i.d. × 500 mm); column temperature, 40 °C] and further fractionation was carried out by HPLC [conditions: mobile phase, acetonitrile–H_2O (44 : 56, v/v) for TC-B-I, MeOH–H_2O (80 : 20, v/v) for TC-B-II; flow rate, 5 ml/min for TC-B-I, 6 ml/min for TC-B-II; detector, UV (220 nm); column, YMC packed column SH-343-5 (20 mm i.d. × 250 mm) for TC-B-I, Cosmosil 5 Phenyl column (20 mm i.d. × 250 mm) for TC-B-II; column temperature, 40 °C]. TCs-B-I (15.0 mg) and -B-II (64.0 mg).

Identification and Absolute Configuration of Amino Acids and Leucinol in TDs-I and -II For amino acid analyses, samples (0.5—1 mg) were hydrolyzed with 6 N HCl in sealed tubes at 110 °C for 24 h. Each hydrolysate was analyzed on an automatic amino acid analyzer. The acid hydrolysate was refluxed in absolute MeOH–thionyl chloride (10 : 1, v/v; 2 ml) for 3 h. After removal of the solvent and reagent, the residue was treated with a solution of 3, 5-dinitrobenzoyl chloride (*ca.* 1 mg) and triethylamine (one drop) in EtOAc (2 ml) and the mixture was stirred for 24 h. The resulting *N*-3,5-dinitrobenzoate methyl esters of amino acids and *N*-3,5-dinitrobenzoate of Lol were analyzed by HPLC with a column having an optically active stationary phase [conditions: mobile phase, hexane–dichloroethane–ethanol (90 : 8 : 2, v/v/v) for Ile and Leu, (80 : 17 : 3, v/v/v) for Lol; flow rate, 0.8 ml/min; detector, UV (254 nm); column, Sumipax OA-4100 (4 mm i.d. × 250 mm, Sumika Chemical Analysis Service Ltd.); column temperature, 35 °C]. Retention times were compared with those of the derivatives of standard amino acids and Lol. Observed t_Rs (min): TD-I, 20.9 (L-Ile), 30.1 (L-Leu), 49.8 (L-Lol); TD-II, 30.6 (L-Leu), 49.5 (L-Lol). t_Rs (min) of standard samples: 21.2 (L-Ile); 26.4 (D-Ile); 30.3 (L-Leu); 33.2 (D-Leu); 49.4 (L-Lol), 31.7 (D-Lol).

Z–Leu–Lol Z–Leu–OH (2.38 g, 8.97 mmol), HOBt (1.21 g, 1 eq) and DCC (1.85 g, 1 eq) were added successively to a solution of Lol (1.00 g, 1 eq) in EtOAc (50 ml) with stirring. The reaction mixture was worked up as usual. The residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 98 : 2, v/v) to afford Z–Leu–Lol; yield 2.89 g (66%), mp 93—95 °C, $[\alpha]_D^{25}$ −36.4° (c = 1.0, MeOH), electron impact (EI)-MS m/z: 364 (M^+), 248 (M^+ − Lol). *Anal.* Calcd for $C_{20}H_{32}N_2O_4$: C, 65.91; H, 8.85; N, 7.69. Found: C, 65.96; H, 8.80; N, 7.69.

HCl·H–Leu–Lol The above compound (2.50 g, 6.86 mmol) was hydrogenated in MeOH (30 ml) containing 1 N HCl (6.86 ml, 1 eq). After removal of the catalyst by filtration, the solvent was evaporated to give HCl·H–Leu–Lol; yield 1.78 g (97 %).

Z–Gly–Leu–Lol [3] Z–Gly–OH (1.18 g, 5.62 mmol), HOBt (0.76 g, 1 eq) and DCC (1.16 g, 1 eq) were added successively to a stirred solution of HCl·H–Leu–Lol (1.00 g, 1 eq) in dimethylformamide (DMF, 20 ml) containing triethylamine (TEA, 0.78 ml, 1 eq). After the usual work-up, the residue was purified by silica gel chromatography ($CHCl_3$: MeOH = 95 : 5, v/v) to afford Z–Gly–Leu–Lol; yield 1.10 g (46%), mp 129—130 °C, $[\alpha]_D^{25}$ −46.3° (c = 1.0, MeOH), EI-MS m/z: 421 (M^+), 305 (M^+ − Lol). *Anal.* Calcd for $C_{22}H_{35}N_3O_5$: C, 62.69; H, 8.37; N, 9.97. Found: C, 62.59; H, 8.39; N, 9.97.

HCl·H–Gly–Leu–Lol Z–Gly–Leu–Lol (1.00 g, 2.37 mmol) was hydrogenated in MeOH (15 ml) containing 1 N HCl (2.37 ml, 1 eq). After removal of the catalyst by filtration, the solvent was evaporated to afford HCl·H–Gly–Leu–Lol; yield 745 mg (97%).

Z–Leu–Aib–OMe, HCl·H–Leu–Aib–OMe and Z–Gly–Leu–Aib–OMe The title compounds were prepared according to the procedures described previously.[20]

HCl·H–Gly–Leu–Aib–OMe Z–Gly–Leu–Aib–OMe (5.00 g, 11.9 mmol) was hydrogenated in MeOH (40 ml) containing 1 N HCl (11.9 ml, 1 eq). After removal of the catalyst by filtration, the solvent was evaporated to give HCl·H–Gly–Leu–Aib–OMe; yield 3.65 g (95%).

Z–Gly–Gly–Leu–Aib–OMe [2] Z–Gly–OH (2.26 g, 10.8 mmol), HOBt (1.46 g, 1 eq) and DCC (2.23 g, 1 eq) were added successively to a stirred solution of HCl·H–Gly–Leu–Aib–OMe (3.50 g, 1 eq) in DMF (30 ml) containing TEA (1.50 ml, 1 eq). The reaction mixture was worked up as described above. The residue was recrystallized from EtOAc and *n*-hexane to afford Z–Gly–Gly–Leu–Aib–OMe; yield 4.79 g (92%), mp 61—62 °C, $[\alpha]_D^{25}$ −16.3° (c = 1.0, MeOH), EI-MS m/z: 478 (M^+). *Anal.* Calcd for $C_{23}H_{34}N_4O_7 \cdot 1/2H_2O$: C, 56.66; H, 7.03; N, 11.49. Found: C, 56.93; H, 7.25; N, 11.71.

HCl·H–Gly–Gly–Leu–Aib–OMe The above tetrapeptide (4.00 g, 8.36 mmol) was hydrogenated in MeOH (30 ml) containing 1 N HCl (8.36 ml, 1 eq) to give the title compound; yield 3.14 g (98%).

(*Z*)-4-Decenoyl–Gly–Gly–Leu–Aib–OMe A solution of (*Z*)-4-hexenolide (1.54 g, 13.8 mmol) in ether (15 ml) was added to a solution of lithium *n*-butylcuprate prepared from a 15% hexane solution of *n*-butyllithium (12.8 ml, 30.7 mmol) and copper(I) iodide (2.92 g, 15.3 mmol) in ether (125 ml) at −1 °C. The reaction mixture was stirred at the same temperature for 30 min, quenched with 2 N HCl, and extracted with ether. The ether solution was extracted with 3 N NaOH. The alkaline solution was acidified with 6 N HCl and extracted with ether, and evaporation of the organic solution gave a crude acid mixture (736 mg, 32%) containing (*Z*)-4-decenoic acid, (*E*)-4-decenoic acid and 4-butyl-5-hexenoic acid. The crude acid mixture (736 mg, 4.33 mmol), HOBt (585 mg, 1 eq) and DCC (893 mg, 1 eq) were added successively to a stirred solution of HCl·H–Gly–Gly–Leu–Aib–OMe (1.65 g, 1 eq) in DMF (30 ml) containing TEA (0.60 ml, 1 eq). After the usual work-up, the residue was purified by preparative HPLC [conditions: mobile phase, MeOH–H_2O (70 : 30, v/v); flow rate, 5 ml/ min; detector, UV (220 nm); column, YMC packed column SH-343-5 (20 mm i.d. × 250 mm); column temperature, 40 °C] to give the title compound; yield 490 mg (23%), mp 132—134 °C, $[\alpha]_D^{25}$ −20.5° (c = 1.0, MeOH), EI-MS m/z: 496 (M^+). *Anal.* Calcd for $C_{25}H_{44}N_4O_6$: C, 60.46; H, 8.93; N, 11.28. Found: C, 60.26; H, 9.06; N, 11.14.

(*Z*)-4-Decenoyl-Gly–Gly–Leu–Aib–OH The above acylated tetrapeptide (475 mg, 1.00 mmol) was hydrolyzed in 1 N methanolic NaOH (15 ml) at 40 °C. After complete saponification, neutralization with 1 N HCl and evaporation of the MeOH, the residual solution was acidified and extracted with EtOAc. The extract was washed with saturated NaCl, dried over Na_2SO_4 and concentrated to yield the (*Z*)-4-decenoyl-Gly–Gly–Leu–Aib–OH; yield 450 mg (97%). The acid was employed in the following step without further purification.

(*Z*)-4-Decenoyl-Gly–Gly–Leu–Aib–Gly–Leu–Lol [TD-II] (*Z*)-4-Decenoyl-Gly–Gly–Leu–Aib–OH (417 mg, 0.93 mmol), HOBt (117 mg, 1 eq) and DCC (178 mg, 1 eq) were added successively to a stirred solution of HCl·H–Gly–Leu–Lol (280 mg, 1 eq) in DMF (20 ml) containing TEA (0.12 ml, 1 eq). The reaction mixture was worked up in the usual manner. The product was purified by preparative TLC ($CHCl_3$: MeOH = 70 : 30, v/v) and preparative HPLC [conditions: mobile phase, MeOH–H_2O (70 : 30, v/v); flow rate, 5 ml/min; detector, UV (220 nm); column, YMC packed column SH-343-5 (20 mm i.d. × 25 mm); column temperature, 40 °C] to afford trichodecenin-II; yield 457 mg (65%), mp 167—170 °C, $[\alpha]_D^{25}$ −17.8° (c = 0.37, MeOH), t_Rs (min): 36.5 (synthesis), 36.8 (natural) [conditions: mobile phase, MeOH–H_2O (80 : 20, v/v); flow rate, 0.6 ml/min; detector, UV (220 nm); column, YMC packed column AM-313 (6 mm i.d. × 250 mm); column temperature, 40 °C], positive-ion FAB-MS: 752 (MH^+), 635 (MH^+ − Lol − H), 522 (635 − Leu), 465 (522 − Gly), 380 (465 − Aib), 267 (380 − Leu), 210 (267 − Gly), amino acid ratios (6 N HCl, 24 h): Gly 2.96 (3), Leu 2.00 (2).

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