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PEPTIDIC IMMUNOSUPPRESSANTS FROM THE FUNGUS *TRICHODERMA POLYSPORUM*

Akira Iida,[a] Tomohiro Mihara,[a] Tetsuro Fujita*,[a,†] and Yoshihisa Takaishi[b]

[a] *Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*
[b] *Faculty of Pharmaceutical Sciences, The University of Tokushima, Shomachi, Tokushima 770-0044, Japan*

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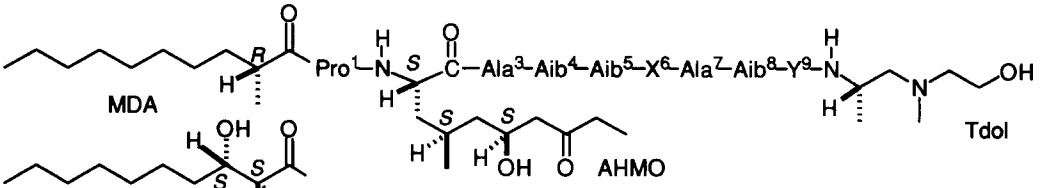
Abstract: Three new 10-residue lipopeptaibols, trichopolyns III–V have been isolated from the fungus *Trichoderma polysporum* together with the known trichopolyns I and II. Structure determination has been achieved on the basis of spectroscopic and chemical evidence. These peptides have been shown to suppress the proliferation of lymphocytes in mouse allogeneic mixed lymphocyte reaction.

The peptaibols, trichopolyns (TP) I and II, were previously obtained as a mixture from culture broth of the fungus *Trichoderma polysporum* (Link ex Pers.) Rifai (TMI 60146)[1] and exhibited powerful antagonism against fungi and Gram-positive bacteria.[2] They are highly lipophilic 10-residue peptides containing α-aminoisobutyric acid (Aib), (2*S*,4*S*)-2-amino-6-hydroxy-4-methyl-8-oxodecanoic acid (AHMO) and trichodiaminol (Tdol) as non-proteinic constituents (Scheme 1). In addition, the N-terminal residues of TP-I and II are protected by (2*R*)-methyldecanoic acid (MDA). Thus, TPs belong to the class of lipopeptides[3] such as leucinostatins[4] and helioferins.[5]

Scheme 1. Structures of trichopolyns I–V



Trichopolyn	Acyl	X^6	Y^9
TP I	MDA	Ile	Aib
II*	MDA	Val	Aib
III*	MDA	Ile	Ala

	Acyl	X^6	Y^9
TP IV*	MDA	Val	Ala
V*	HMDA	Ile	Aib

* See ref. 9.

In our continuing search of immunosuppressants[6] from fungi, we found that potent immunosuppressive activity is produced in the culture broth of *T. polysporum* and TPs are the active principles. Further purification of the TP mixture led to the isolation of new TP families, TP-III–V, in addition to pure TP-I and II. We now describe the structures of TP-I–V and their immunosuppressive activity in the mouse allogeneic mixed lymphocyte reaction (MLR).

The TP mixture (920 mg) was fractionated by recycle HPLC (YMC Packed ODS column with CH_3CN-

† Present address: Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Osaka 573-0101, Japan.

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0.05% TFA) to give pure TP-III (40 mg), IV (15 mg) and V (49 mg), together with TP-I and II, each as a colorless, amorphous powder.

The amino acid sequences of the new peptides were unambiguously determined through observation of a series of acylium ions by electrospray ionization (ESI)-MS and FAB-MS/MS as in the case of other peptaibols.[7,8,9] As shown in Scheme 1, the structures of TP-III and IV differ from TP-I and II only in the replacement of amino acid at position 9, respectively. Unlike TP-I–IV, TP-V, of which amino acid sequence is the same as that of TP-I,[10] was suggested to possess a hydroxylated MDA (HMDA) residue in the N-terminal based on the observation of the *m/z* 282 ion peak that can be assigned as the HMDA-Pro sequence (Figure 1). The presence of HMDA in TP-V was demonstrated by FAB-MS/MS affording the *m/z* 185 ion peak that is 16 mass units higher than the MDA ion (169 a.m.u.) in the corresponding spectra of TP-I (not shown). The structure of HMDA was established as 3-hydroxy-2-methyldecanoic acid by *J*-connectivities observed in the double-quantum filtered (DQF)-COSY (600 MHz, CD_2Cl_2) and heteronuclear multiple bond correlation (HMBC) spectra (150 MHz, CD_2Cl_2) of TP-V. The N-terminal region (HMDA-Pro) in TP-V was

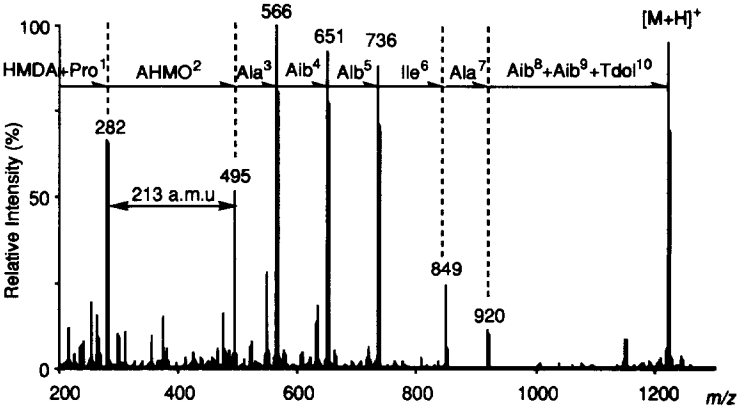
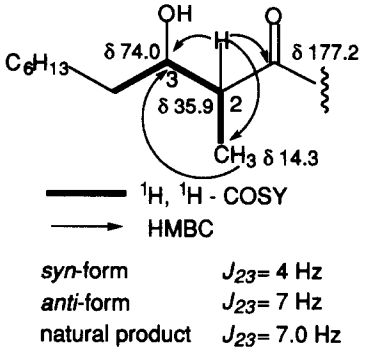
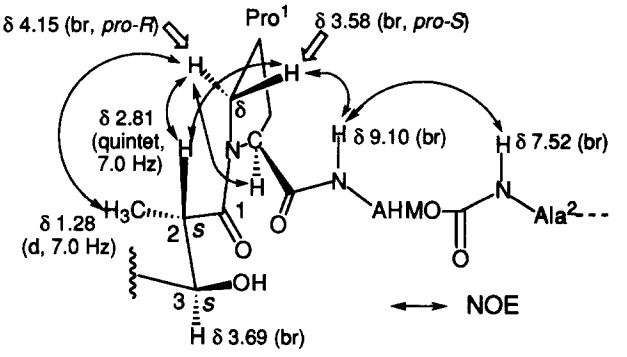


Figure 1. ESI-MS of trichopolyn V





found to take a conformation where the α-proton (= H-2, δ 2.81) of HMDA reveals NOE connectivities[3a] with the proline δ-H_2 and, furthermore, the *pro-R* δ-proton (δ 4.15) of the proline residue had a cross-peak with the α-methyl protons (δ 1.28) of HMDA. These NOE correlations demonstrate the *S*-configuration at C-2 (δ 35.9) in the HMDA moiety. In addition, the *anti* configuration of the hydroxyl group to the α-methyl was determined by the vicinal coupling constant (J = 7.0 Hz)[11] between H-2 and H-3 (δ 3.69), leading to the *S*-configuration at C-3 (δ 74.0) in the HMDA moiety. This absolute structure of HMDA is identical with that of trichospolide,[12] a cyclic depsipeptide isolated from the culture broth of *T. polysporum* together with TPs.

The fraction containing TPs suppressed the proliferation of lymphocytes in mouse allogeneic MLR. In

particular, TP-I and II exhibit quite potent and comparable activity with cyclosporin A (CsA). The effect of each TP and CsA on mouse allogeneic MLR is summarized in Table 1. Unambiguously, replacement of Ile^4 and/or Aib^9 to less hydrophobic Val and/or Ala contributes to decreasing the activity. However, the MDA moiety is most likely to play a crucial role in increasing the activity. In fact, MDA in TP-I induces approximately 60-fold higher activity than that of TP-V bearing HMDA, though TP-V has the same amino acid sequence as TP-I. On the other hand, none of TPs affect the production of IL-4, indicating that TPs are novel immunosuppressants of which mode of action is different from that of CsA.

Table 1. Effects of trichopolyns and cyclosporin A (CsA) on mouse allogeneic MLR and production of interleukin 4 (IL-4).

	IC_{50} (nM)	
	MLR	IL-4
trichopolyn I	5.2	>1000
II	10.7	>1000
III	20.5	>1000
IV	60.4	>1000
V	294.3	>1000
CsA	7.5	2.5

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8. The acid hydrolysates (6M HCl, 110°C, 24h) were analyzed by HPLC using a chiral column (SUMICHIRAL OA-5000) with 2 mM $CuSO_4$ as an eluent for identification and quantification of amino

acids (L-Ala, L-Ile, L-Val, L-Pro, Aib) together with the enantiomer analysis. The L-configuration of Tdol isolated from each acid hydrolysate was determined by HPLC of its N-3,5-dinitrobenzoate with a chiral column (SUMICHIRAL OA-4100). Data for TP-I ($C_{61}H_{111}N_{11}O_{13}$): HR-FABMS *m/z*, 1206.8475 (MH+, calcd mass=1206.8440); mp (°C), 108–110 (dec); $[\alpha]_D^{30}$ –31.2 (*c* 0.89, MeOH); IR (KBr) ν_{max} 3300, 1660, 1530 cm^{-1}; molecular ellipticity [δ] (° cm^2 dmol) –151200 (208 nm), –130100 (222 nm). Data for TP-II ($C_{60}H_{109}N_{11}O_{13}$): HR-FABMS *m/z*, 1192.8301 (MH+, calcd mass=1192.8284); mp (°C), 80–82 (dec); $[\alpha]_D^{30}$ –25.1 (*c* 0.67, MeOH); IR (KBr) ν_{max} 3300, 1660, 1530 cm^{-1}; molecular ellipticity [δ] (° cm^2 dmol) –127600 (208 nm), –109300 (222 nm). Data for TP-III ($C_{60}H_{109}N_{11}O_{13}$): HR-FABMS *m/z*, 1192.8285 (MH+, calcd mass=1192.8284); mp (°C), 87–88 (dec); $[\alpha]_D^{30}$ –31.3 (*c* 0.97, MeOH); IR (KBr) ν_{max} 3300, 1660, 1530 cm^{-1}; molecular ellipticity [δ] (° cm^2 dmol) –117100 (208 nm), –83160 (222 nm). Data for TP-IV ($C_{59}H_{107}N_{11}O_{13}$): HR-FABMS *m/z*, 1178.8108 (MH+, calcd mass=1178.8124); mp (°C), 98–100 (dec); $[\alpha]_D^{30}$ –24.8 (*c* 0.89, MeOH); IR (KBr) ν_{max} 3300, 1660, 1530 cm^{-1}; molecular ellipticity [δ] (° cm^2 dmol) –171800 (208 nm), –136600 (222 nm). Data for TP-V ($C_{61}H_{111}N_{11}O_{14}$): HR-FABMS *m/z*, 1222.8392 (MH+, calcd mass=1222.8390); mp (°C), 103–104 (dec); $[\alpha]_D^{30}$ –26.6 (*c* 0.94, MeOH); IR (KBr) ν_{max} 3300, 1660, 1530 cm^{-1}; molecular ellipticity [δ] (° cm^2 dmol) –162500 (208 nm), –146400 (222 nm).

9. In this study, the absolute confuguration at C-6 of the AHMO residue in TP-I, which had not been established, was determined as the *S*-configuration by the application of the modified Mosher's method.[13] The stereochemistry of the AHMO residue is the same as that of the Dec residue in leucinostatin A (Dec is identical with AHMO).[14] The absolute configuration at C-6 of AHMO in TP-II and at C-4 and -6 in TP-III–V have not been determined yet. The *S*-configuration at C-2 of the AHMO residue in TP-III–V was deduced from two experimental results that these peptides take a right-handed helical conformation based on CD spectra and the α-proton of the residue shows an inter-residual NOE [dαN (i,i+3)-type] with the Aib^5 NH.[15] (2*R*)-MDA isolated from the acid hydrolysates of TP-III and IV was derived to (2*R*)-MDA-L-Pro-OBzl. The retention time of the derivative on HPLC was compared with those of standards [Rt = 82.3 and 76.3 min for the (2*R*)- and (2*S*)-MDA derivatives, respectively (Cosmosil 5-Ph, CH_3CN/H_2O = 55/45, 1 ml/min)].
10. The ESI-MS of TP-V shows a series of sequence-specific acylium ions beginning at the *m/z* 1222, which loses $(Aib)_2$-Tdol, Ala, Ile, Aib, Aib, Ala, AHMO successively, affording the N-terminal acylated proline (*m/z* 282) described in the text. The sequence of the C-terminal tripeptide of which fragment ions are not observed in the ESI-MS shown in Figure 1 was confirmed by FAB-MS/MS giving ion peak at *m/z* 1090 and 1005.

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