The Journal of Antibiotics (2018) 71:682–684
https://doi.org/10.1038/s41429-018-0041-3

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Tolyprolinol, a new dipeptide from *Tolypocladium* sp. FKI-7981

Wataru Fukasawa[1] · Natsuki Mori[1] · Masato Iwatsuki[1,2] · Rei Hokari[2] · Aki Ishiyama[1,2] · Moe Nakajima[2] · Takahito Ouchi[2] · Kenichi Nonaka[1,2] · Hiroki Kojima[2] · Hirotaka Matsuo[1,2] · Satoshi Ōmura[2] · Kazuro Shiomi[1,2]

Received: 18 December 2017 / Revised: 5 February 2018 / Accepted: 5 February 2018 / Published online: 22 March 2018

Abstract

A new dipeptide, named tolyprolinol, was isolated from the static culture of a fungus, *Tolypocladium* sp. FKI-7981. The structure of tolyprolinol was elucidated as *N*-acetyl-L-phenylalanyl-L-prolinol. It showed moderate antimalarial activity but did not show cytotoxicity or any other antimicrobial property.

Fungal secondary metabolites are rich sources of unique compounds and a lot of useful compounds have already been discovered. However, it has been proposed that there is an immeasurable number of microbial metabolites not yet discovered [1]. Therefore, our group has continued to investigate fungal metabolites, which has already resulted in the discovery of novel compounds such as the virgaricins A and B, cinatrins D and E, and cladomarine [2–4]. Recent research led us to discover a new dipeptide, tolyprolinol (**1**), containing a L-phenylalanine and a L-prolinol, from a culture broth of *Tolypocladium* sp. FKI-7981. Prolinol is a rare moiety among natural products and this is the first report of a natural product containing a prolinol moiety isolated from *Tolypocladium* species. We detail here the taxonomy of producing strain, as well as the fermentation, isolation, structure elucidation, and some biological properties of **1**.

The fungal strain FKI-7981 had 95.2% similarity with the internal transcribed spacer sequence of CBS 869.73 (ex-type of *Tolypocladium album*). From this information, combined with morphological characteristics, FKI-7981 was identified to be a member of the genus *Tolypocladium* family [5, 6].

The EtOAc extract of a 14-day static cultured broth was subjected to column choromatographies and high-performance liquid chromatography purification to afford to **1** (22.3 mg). The detailed fermentation and isolation procedure for **1** is summarized in Schemes S1 and S2 in the Supplementary Information.

The physico-chemical properties of **1** are summarized in Table 1. It is soluble in methanol with ultraviolet (UV) absorption at 206 nm, as well as dimethyl sulfoxide

Table 1 Physico-chemical properties of tolyprolinol (**1**)

	Tolyprolinol (1)
Appearance	Pale yellow oil
Molecular formula	$C_{16}H_{22}N_2O_3$
Molecular weight	290
ESI-MS (*m/z*) positive	313 $[M + Na]^+$
ESI-MS (*m/z*) negative	335 $[M + HCOOH–H]^-$
HR-ESI-MS (*m/z*)	
Calcd.	313.1528
Found	313.1521
UV λ^{MeOH} nm (ε)	206
IR ν^{KBr} (cm^{-1})	3440, 3267, 1624, 1454
$[\alpha]_D^{23.5}$	14.7 ($c = 0.1$, MeOH)
Solubility	
Soluble	MeOH, DMSO, $CHCl_3$, H_2O
Slightly soluble	MeCN
Insoluble	*n*-Hexane

ESI-MS electrospray ionization mass spectrometry, *IR* infrared, *UV* ultraviolet

These authors contributed equally: Wataru Fukasawa, Natsuki Mori.

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0041-3) contains supplementary material, which is available to authorized users.

✉ Satoshi Ōmura
omuras@insti.kitasato-u.ac.jp

✉ Kazuro Shiomi
shiomi@lisci.kitasato-u.ac.jp

[1] Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

[2] Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan

Table 2 ^{1}H and ^{13}C NMR data of the major and minor conformers of tolyprolinol (**1**) in $CDCl_3$

	Position	Tolyprolinol (**1**)			
		Major conformer		Minor conformer	
		δ_C (ppm)	δ_H (ppm) (Int., multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (Int., multiplicity, *J* in Hz)
Prolinol	OH	—	4.38 (1H, br. s)	—	4.58 (1H, br. s)
	1	65.9	3.42 (1H, m)	64.7	3.32 (1H, m)
			3.48 (1H, m)		3.47 (1H, m)
	2	61.1	4.13 (1H, m)	59.6	3.30 (1H, m)
	3	27.6	1.48 (1H, m)	28.2	1.10 (1H, m)
			1.93 (1H, m)		1.50 (1H, m)
	4	24.1	1.65 (2H, m)	21.6	1.62 (2H, m)
	5	47.8	2.67 (1H, m)	45.4	3.36 (2H, m)
			3.65 (1H, m)		
Phe	1′	172.1		170.5	
	2′	52.3	4.93 (1H, ddd, 8.6, 8.6, 5.6)	52.9	5.09 (1H, ddd, 10.0, 6.8, 6.8)
	3′	39.4	3.02 (2H, m)	39.4	2.99 (2H, m)
	4′	135.9		135.9	
	5′, 5″	128.5	7.2–7.3 (5H, overlapped)	128.5	7.2–7.3 (5H, overlapped)
	6′, 6″	129.3		129.3	
	7′	127.1		127.1	
	NH	—	6.92 (1H, d, 8.0)	—	7.20 (1H)
	8′	169.7	—	170.8	—
	9′	22.9	1.98 (3H, s)	22.8	1.98 (3H, s)

NMR nuclear magnetic resonance spectroscopy

(DMSO), chloroform, and water, but insoluble in *n*-hexane. The characteristic infrared absorptions at 3440, 3267, 1624, and 1454 cm^{-1} suggested the presence of an amidocarbonyl group moiety.

The molecular formula of **1** was elucidated by HR-ESI-MS to be $C_{16}H_{22}N_2O_3$ with a molecular ion peak $[M + Na]^+$ at *m/z* 313.1521 (calcd. *m/z* 313.1528). Two conformers were observed with the ratio of 3:2 in ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) spectral data of **1** in DMSO-d_6 at room temperature (Figs. S2-1 and S2-2). The spectra did not change dramatically, even at 80 °C. Amide signals and α-proton signals in ^{1}H and ^{13}C NMR spectral data of **1** suggested **1** is a peptide compound. When **1** was measured in $CDCl_3$, two conformers were observed with the ratio of 5:2 at room temperature (Figs. S2-6 and S2-7). The structure of major and minor conformers of **1** in $CDCl_3$ was elucidated by 1D and 2D NMR, as shown in Table 2.

Combined analysis of the ^{1}H-^{1}H correlation spectroscopy (COSY) and heteronuclear multiple-bond correlation (HMBC) spectra identified phenylalanine (Phe) and prolinol residues in **1** (Fig. 1b), which were finally confirmed by their 1-fluoro-2,4-dinitrophenyl-5-D-leucineamide (FDLA) derivatives described below. In the major conformer of **1**, HMBC correlations observed from CH_3 (δ_H 1.98) and Phe NH (δ_H 6.92) to C-8′ (δ_C 169.7) indicated that the amino group in Phe in **1** was acetylated. The planar structure of **1** was established by HMBC correlations from prolinol H_2-5 (δ_H 2.67 and 3.65) to Phe C-1′ (δ_C 172.1) as *N*-acetylphenylalanylprolinol. Compound **1** has not been previously reported and we designated it as tolyprolinol. The minor conformer of **1** was also elucidated in the same manner (Table 2). This minor conformer is suggested as being derived from the regioisomeric amide bond of prolinol residue in **1**.

The absolute configuration of amino acids in **1** was elucidated by Advanced Marfey's method after acid hydrolysis [7]. Compound **1** was hydrolyzed and derivatized with FDLA and analyzed by an ultraperformance liquid chromatography coupled with ESI-MS. As the result of the comparison of retention time with FDLA derivatives of standard Phe and prolinol, both Phe and prolinol were elucidated to be the L configuration (Table S3 and Fig. S3).

Compound **1** was tested for antimalarial activity against both a chloroquine-resistant K1 strain and chloroquine-sensitive FCR3 strain of *P. falciparum*, as well as

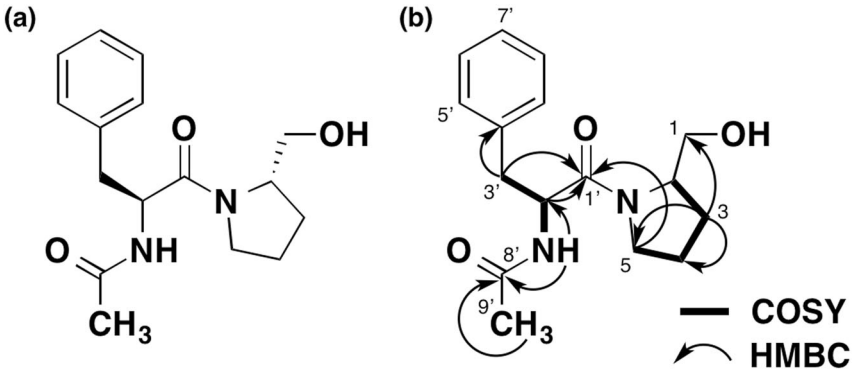

Fig. 1 **a** Structure of tolyprolinol (**1**). **b** Key correlations of ^{1}H-^{1}H COSY and HMBC in **1**. Bold lines show proton spin networks; arrows show ^{1}H-^{13}C long-range correlations

cytotoxicity against nine human cell lines. Compound **1** showed half-maximal inhibitory concentration values of 163 and 285 μM against the K1 strain and the FCR3 strain of *P. falciparum*, respectively. However, **1** did not display any cytotoxicity against the human MRC-5 cell at 345 μM and other eight human cancer cell lines, HL-60, Jarkat, THP-1, HeLa S3, A549, Panc1, HT29, and H1299 at 100 μM.

The antimicrobial activity of **1** was assessed against six microorganisms, *Bacillus subtilis* KB 211 (ATCC 6633), *Kocuria rhizophilia* KB 212 (ATCC 9341), *Escherichia coli* KB 213 (NIHJ), *Xanthomonas oryzae* pv. *oryzae* KB 88, *Candida albicans* KF 1 (ATCC 64548), and *Mucor racemosus* KF 223 (IFO 4581) using a disk diffusion assay with 8-mm paper disks, as previously described [8]. Compound **1** was inactive against all microorganisms tested, even at 50 μg per disk.

In summary, we have discovered a new dipeptide, named tolyprolinol, consisting of L-Phe and L-prolinol, from secondary metabolites of *Tolypocladium* sp. FKI-7981. We found **1** showed moderate antimalarial activity, but did not show cytotoxicity or other antimicrobial activity against the microbes tested. There have been a few reports of compounds containing prolinol, such as actinonin, viriditin, scalusamides, asperelines, and barmumycin [9–14]. Most of the reported compounds produced by genus *Tolypocladium* were cyclosporin-like polypeptides [15]. Therefore, the isolation of the new dipeptide **1** suggests that *Tolypocladium* may be a potential source of unique compounds that could prove to be interesting lead compounds for future drug discovery.

Acknowledgements This research was partially supported by the Platform Project for Supporting Drug Discovery and Life Science Research (Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)) from the Japan Agency for Medical Research and Development (AMED) as well as by the 24th Botanical Research Grant of the New Technology Development Foundation. We are grateful to Dr Kenichiro Nagai, Kitasato University, and Kazunari Sakai for useful suggestions concerning the experiments and collection of soil samples.

References

1. Ōmura S. Chemical screening. In: Ōmura S, editors. The search for bioactive compounds from microorganisms. New York: Springer; 1992. p. 263–280.
2. Ishii T, et al. Virgaricin produced by *Virgaria* sp. FKI-4860. J Antibiot. 2012;65:139–141.
3. Ishii T, et al. Cinatrins D and E, and virgaricin B, three novel compounds produced by a fungus, *Virgaria boninensis* FKI-4958. J Antibiot. 2015;68:633–7.
4. Takahashi K, et al. Cladomarine, a new anti-saprolegniasis compound isolated from the deep-sea fungus, *Penicillium coralligerum* YK-247. J Antibiot. 2017;58:911–4.
5. Gams W. *Chaunopycnis alba*, gen. *et* sp. nov., a soil fungus intermediate between *Moniliales* and *Sphaeropsidales*. Persoonia. 1980;11:75–79.
6. Quandt CA, et al. Phylogenetic-based nomenclatural proposals for *Ophiocordycipitaceae* (Hypocreales) with new combinations in *Tolypocladium*. IMA Fungus. 2014;5:121–34.
7. Harada K, et al. Application of d,l-FDLA derivatization to determination of absolute configuration of constituent amino acids in peptide by advanced Marfey's method. Tetrahedron Lett. 1996;37:3001–4.
8. Iwatsuki M, et al. Guadinomines, type III secretion system inhibitors, produced by *Streptomyces* sp. K01-0509. J Antibiot. 2008;61:222–9.
9. Gordon JJ, et al. Actinonin: an antibiotic substance produced by an actinomycete. Nature. 1962;18:819–25.
10. Omolo JO, et al. New variotin analogues from *Aspergillus viridinutans*. J Nat Prod. 2000;63:975–7.
11. Tsuda M, et al. Scalusamides A–C, new pyrrolidine alkaloids from the marine-derived fungus *Penicillium citrinum*. J Nat Prod. 2005;68:273–6.
12. Ren J, et al. Asperelines A–F, peptaibols from the marine-derived fungus *Trichoderma asperellum*. J Nat Prod. 2009;72:1036–44.
13. Ren J, et al. Sequential determination of new peptaibols asperelines G-Z_{12} produced by marine-derived fungus *Trichoderma asperellum* using ultrahigh pressure liquid chromatography combined with electrospray-ionization tandem mass spectrometry. J Chromatogr A. 2013;1309:90–95.
14. Lorente A, Pla D, Canedo LM, Albericio F, Alvarez M. Isolation, structural assignment, and total synthesis of barmumycin. J Org Chem. 2010;75:8508–15.
15. Rüegger A, et al. Cyclosporin A, a peptide metabolite from *Trichoderma polysporum* (Link ex Pers.) Rifai, with a remarkable immunosuppressive activity. Helv Chim Acta. 1976;59:1075–92.