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# Nodupetide, a potent insecticide and antimicrobial from *Nodulisporium* sp. associated with *Riptortus pedestris*

Hui Min Wu [a,1], Li Ping Lin [a,b,c,1], Qin Lan Xu [a], Wen Bo Han [a], Song Zhang [d], Ze Wen Liu [d], Ya Ning Mei [e], Zhu Jun Yao [a], Ren Xiang Tan [a,c,*]

[a] Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, China
[b] Jiangsu Center for Research & Development of Medicinal Plants, Institute of Botany, Jiangsu Province and Chinese Academy of Sciences, Nanjing 210014, China
[c] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China
[d] Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), College of Plant Protection, Nanjing Agricultural University, Nanjing 210095, China
[e] Department of Clinical Laboratory, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

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ABSTRACT

Nodupetide (**1**), a new cyclodepsipeptide unique in its incorporation of a 3-hydroxy-4-methylhexanoic acid (HMHA) derived motif, was discovered from *Nodulisporium* sp. IFB-A163, a fungus residing in the insect (*Riptortus pedestris*) gut. The nodupetide structure was elucidated by its MS/MS and 2D NMR spectra, and its absolute configuration by the X-ray crystallography and modified Marfey's method. Nodupetide is insecticidal against rice brown planthopper (*Nilaparvata lugens*) with an $LD_{50}$ value of 70 ng/larva, and inhibitory towards the drug-resistant human pathogenic bacterium *Pseudomonas aeruginosa* with its MIC value (5.0 μM) comparable to that (3.2 μM) of ciprofloxacin, a prescribed antibacterial agent co-assayed equally.



The newer generation of pesticides and agricultural antibiotics is always indispensable for the continuous improvement of crop productions to feed on the rapidly growing world's population.[1] However, the efficacy of currently applied insecticidal and antimicrobial agents keeps decreasing, or even vanishing, because of the drug resistance development of crop-threatening pests and microbes to most if not all of the marketed agrochemicals.[2] Accordingly, there is a pressing need to search for novel leads for insecticidal and antimicrobial agents, the latter being required as well for the "war" against human microbial pathogens.[3]

Owing to the intimate host-microbe interaction,[4] symbionts are being recognized as versatile producers of small molecule secondary metabolites with unforeseeable architectures and diverse bioactivities.[4,5] Widely spread in China are the herbivorous insects such as *Riptortus pedestris* (Hemiptera: Alydidae), which feed on crop species such as soybean.[6] From a symbiotic perspective, insects usually harbor a particular community of microorganisms, some of which might generate low-molecular-weight biomolecules to help counteract the challenges from bacterial infectors, snatcher and predators.[7] Based on these theories, we therefore hypothesized that *R. pedestris* might be among the reliable host of the microbes capable of producing insecticidal and/or antimicrobial chemicals. As a result, *Nodulisporium* sp. IFB-A163, an anamorph-form genus of Xylariaceae,[8] was isolated from the *R. pedestris* gut. Some *Nodulisporium* fungi were demonstrated to be efficient producers of bioactive secondary metabolites such as insecticidal indole terpenes,[9] and viridins which inhibit amyloid β-peptide aggregation involved in the Alzheimer's disease incidence.[10] Those evidences inspired the subsequent bioassay-guided fractionation of the extract derived from the scaled-up fermentation of the fungal strain IFB-A163. Then a structurally undescribed cyclodepsipeptide named nodupetide (**1**) was afforded, rather than indole terpenes and viridins, as the first cyclodepsipeptide member characterized from cultures of *Nodulisporium* fungi.

The fungal strain IFB-A163 was cultured in the malt extract (ME) medium (100 × 400 mL) for 14 days at 28 °C with an agitation of 200 rpm. Filtration of the culture gave the fermentation broth which was partitioned with EtOAc with the mycelium extracted with a methanol-dichloromethane mixture (1:1). The obtained extracts were combined and purified by column chromatographies over silica gel, octadecyl silane (ODS), followed by Sephadex LH-20 column with $CH_2Cl_2$-MeOH system (v/v 1:1)

* Corresponding author at: Institute of Functional Biomolecules, State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing 210093, China; School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China.
*E-mail address:* rxtan@nju.edu.cn (R.X. Tan).
[1] H.M. Wu and L.P. Lin contributed equally.

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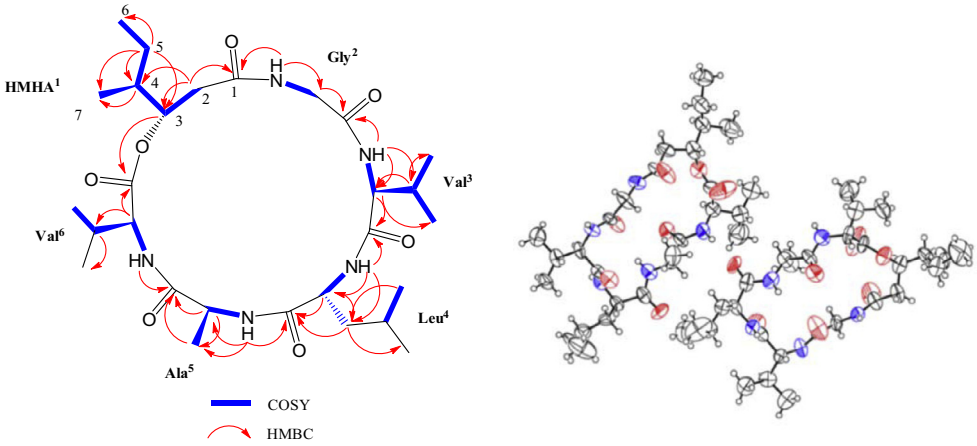


**Fig. 1.** Chemical and crystal structures of nodupetide (**1**).

purification to nodupetide (**1**, Fig. 1), which was evidenced to have a molecular formula of $C_{28}H_{49}N_5O_7$ from the $Na^+$-liganded molecular ion at *m*/*z* 590.3522 (calculated for $C_{28}H_{49}N_5O_7Na$:590.3524) in its high resolution electrospray ionization mass spectrometry (HR-ESI-MS).

Its depsihexapeptide nature was evidenced both from the five characteristic amide proton signals in the range of $\delta_H$ 7.44–8.66 of its $^1H$ NMR spectrum, and from the six carbonyl groups between $\delta_C$ 169 and 172 in its $^{13}C$ NMR spectrum (Table S1, Figs. S2–3). With the information in mind, the assignment of its $^1H$ and $^{13}C$ NMR signals to the amino acid residues was subsequently accomplished by interpreting its $^1H$-$^1H$ COSY, HSQC, and HMBC spectra (Table S1, Figs. S5–7), indicating that **1** was likely composed of glycine (Gly$^2$), valine (Val$^3$), leucine (Leu$^4$), alanine (Ala$^5$), and valine (Val$^6$) residues. Furthermore, a linear alkyl chain was indicated by the HMBC correlations of a diastereotopic methylene ($H_2$-2) signals at $\delta_H$ 2.54 and 2.25 with the carbonyl C-1 ($\delta$ 169.8) and oxymethine carbon C-3 ($\delta$ 75.3), and of H-3 ($\delta_H$ 4.92) of 3-hydroxy-4-methylhexanol acid (HMHA) moiety to Val$^6$-CO ($\delta$ 170.7), suggesting the attachment of this oxymethine C-3 to C-2. The absence of correlation between the CO (Val$^6$) and an amide proton associated with the HMBC correlation of the oxymethine carbon C-3 ($\delta$ 75.3) suggests that the ester linkage is at this position. And the rest of this alkyl chain is easily characterized with the COSY and HMBC correlations. Finally, these enabled the determination of the complete cyclic sequence as cyclo [HMHA$^1$-Gly$^2$-Val$^3$-Leu$^4$-Ala$^5$-Val$^6$] by the HMBC correlations of the amide protons of amino acid residues with the corresponding carbonyls, respectively. The sequence proposed for **1** was reinforced by its LC-MS/MS analysis which pinpointed the successive cleavage of amino acid moieties from its protonated molecular ion at *m*/*z* 568.3707 (Fig. 2).

The optical purity of **1** was secured by its specific rotation ($[\alpha]_D^{25} = -63.5°$, $c = 0.085$, MeOH) and its CD spectrum (Fig. S1) and the advanced Marfey's method was subsequently applied to address the absolute configuration of the cyclodepsipeptide.[11,12] Hydrolysis of **1** with 6 N HCl at 110 °C for 20 h gave a hydrolysate. After derivatised with 1-fluoro-2,4-dinitro-5-(L)-alanine amide (FDAA), the hydrolysate was demonstrated to contain Gly, L-Ala, L-Val, and D-Leu by the HPLC comparison with the authentic samples in an acetonitrile-water gradient (3:7 → 7:3). However, this effort was unable to clarify the absolute configuration of the 3-hydroxy-4-methylhexanoyl residue. We therefore resolved to obtain the single crystal of **1**, and fortunately succeeded. Relative to the established chirality of L-Ala, L-Val, and D-Leu, the 3*S*- and 4*S*-configurations were assigned for the 3-hydroxy-4-methylhexanoyl moiety of **1** through its X-ray

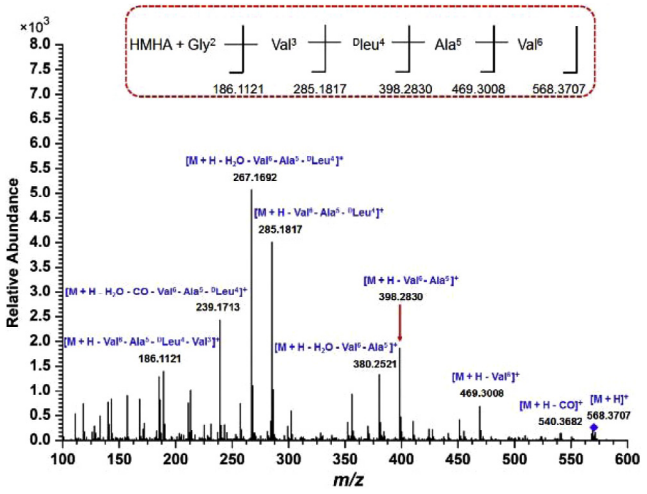


**Fig. 2.** The nodupetide (**1**) sequence indicated by the pronounced fragment ions in its ESI-MS/MS spectrum. HMHA, 3-hydroxy-4-methylhexanoic acid; Gly, glycine; Val, valine; Leu, leucine; Ala, alanine.

crystallographic analysis with the anomalous dispersion of Cu Ka radiation (Fig. 1). To our knowledge, **1** seems to be the first characterized fungal metabolite with the 3-hydroxy-4-methylhexanoyl motif, which is very rare in nature. Prior to the study, this substructure was only found in the secondary metabolites of the bacterium *Paenibacillus ehimensis*[13] and the plant *Quillaja saponaria*.[14]

Inspired by the biosynthetic pathways of emericellamide A from *Aspergillus nidulans*[15] and fusaristatin A from *Fusarium graminearum*,[16] nodupetide (**1**) perhaps was biosynthetically of mixed origins with polyketide and amino acid substructures. According to the basic common logic of natural polyketide biosynthesis,[17] HMHA might be assembled under the catalysis of fungal polyketide synthases (PKSs). The HMHA-based polyketide chain may subsequently serve as a precursor to directly assimilated by the downstream nonribosomal peptide synthetase,[16] or be transferred to a CoA thioester by an acyl-CoA ligase and then loaded onto an acyl-transferase, which subsequently shuttles the product to the NRPS.[15] Following the successive incorporation of the five amide acid residues (Gly, Val, Leu, Ala, and Val), nodupetide (**1**) is released through an intramolecular esterification reaction which seems to be shared by the biosynthesis of fungal cyclodepsipeptides (Fig. 3).

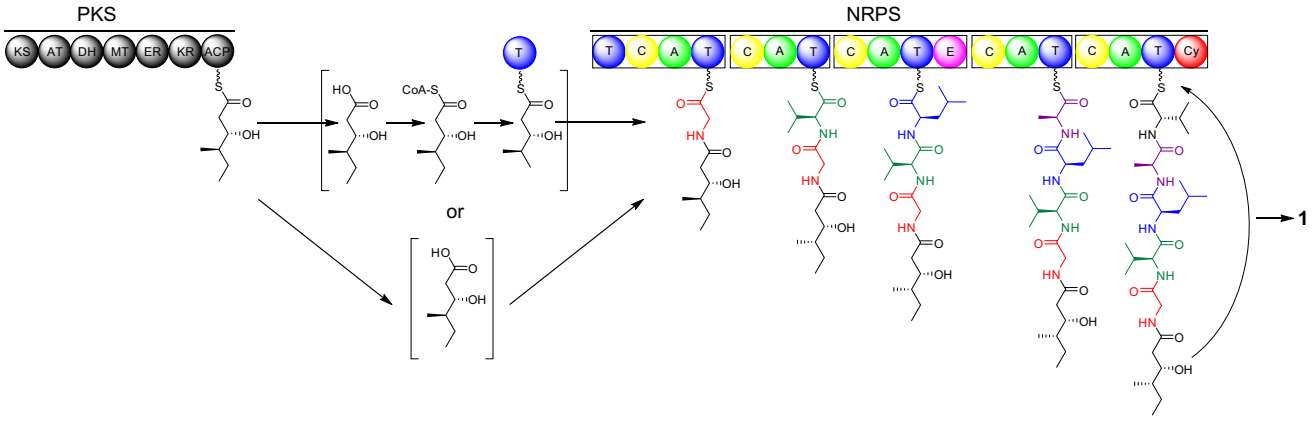


**Fig. 3.** Proposed biosynthetic pathway for nodupetide (**1**) in *Nodulisporium* sp.

Using the purified sample, nodupetide (**1**) was examined to be substantially insecticidal against the 3rd instar nymphae of brown rice planthopper *Nilaparvata lugens* with its median lethal dose ($LD_{50}$) being 70 ng/larva.[18,19] Furthermore, **1** was screened for its antibacterial activities against *Peptostreptococcus anaerobius*, *Clostridium prazmowski*, *Pseudomonas aeruginosa*, and *Bacteroides fragilis*. To our surprise, **1** displayed exclusively an inhibitory action towards *P. aeruginosa* with its MIC value (5.0 μM) close to that of ciprofloxacin (3.2 μM), a marketed antibacterial agent co-assayed identically as a positive reference in the study.[20]

In summary, one of the major objectives concerning natural product sciences is to address the biologically relevant (sub)structures enshrined mysteriously in the complex natural products. With inspiration from the intimate host-microbe interaction, the work unravels nodupetide (**1**) as a structurally new cyclodepsipeptide with insecticidal and antibacterial activities. The sequence and configuration of cyclodepsipeptide **1** was clarified by its NMR and MS/MS spectra, in conjunction with the advanced Marfey's method and its single-crystal X-ray crystallographic analysis. The nodupetide (**1**) architecture is unique in its possession of the linear polyketide motif derived from 3-hydroxy-4-methylhexanol acid, which is considerably rare in nature. Collectively, the findings provide a novel starting molecule that may be valuable for the discovery of newer generation of insecticidal and/or antibacterial agents.

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## A. Supplementary material

Supplementary data ($^1H$, $^{13}C$ NMR, DEPT, HSQC, HMBC, COSY, NOESY, and HR-ESI-MS spectroscopic data for compound **1**) associated with this article can be found in the online version.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2017.01.009.

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