

A New Pair of Pentaketide Diastereoisomers from *Aspergillus melleus* YIM PHI001

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(Received April 3, 2017; Revised July 13, 2017; Accepted August 23, 2017)

Abstract: Two new aspinotriol derivatives (**1-2**) determined as melleusin A (**1**), B (**2**) and other seven known compounds were isolated from a soil-borne fungus *Aspergillus melleus*. The configurations of **1**, and **2** were determined by their analogues, aspilactonol B, C isolated previous in *Aspergillus*. Mellein (**8**) showed broad antibiotic activity against the test pathogens. Aspinonene, aspinotriols A and B can be used as the marker molecule in chemotaxonomy of *Aspergillus*.

Keywords: *Aspergillus melleus*; aspinotriol derivatives; diastereoisomer; spectroscopic analyses; antibiotic. © 2018 ACG Publications. All rights reserved.

1. Introduction

The genus *Aspergillus* is rich in species and serves as a reservoir of bioactive secondary metabolites [1]. The potential of finding even further new bioactive drug candidates in *Aspergillus* is evident, despite the fact that many secondary metabolites have already been structure elucidated and chemotaxonomic studies have shown that many new secondary metabolites have yet to be characterized [2]. Aspyrone, a weak broad spectrum antibiotic produced by the *Aspergillus melleus*, is a polyketide derived from five C₂ units [3]. During ongoing search for new natural products from microbes living in untapped niches, *Aspergillus melleus* YIM PHI001 isolated from an India soil sample was screened and two new aspinotriol derivatives (**1-2**) were obtained from its culture extract (Figure 1). Herein, we present the isolation, structure elucidation and bioactivities of the two new compounds.

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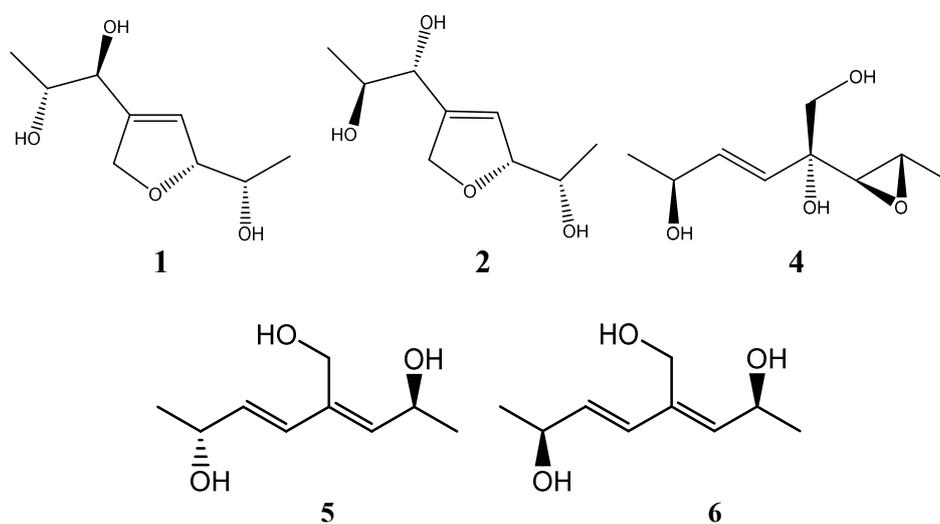


Figure 1. Structures of compounds **1** and **2**, and **4-6**

2. Materials and Methods

2.1. General Experimental Procedures

Silica gel (200-300 mesh; Qingdao Marine Chemical Group Co.), MCI (The Kaiteki Co.), ODS (Beijing Greenherbs and Technology and Development Co.) and Sephadex LH-20 (GE Healthcare Co.) were used for CC. 1D and 2D NMR spectra were obtained on a Bruker 400, 500, 600 MHz NMR instrument (Bruker). MS spectra were recorded with an Agilent G3250AA (Agilent) and AutoSpec Premier P776 spectrometer (Waters).

2.2. Fungal Material

The fungus strain was isolated from the soil in New Delhi, India. The species was identified as *Aspergillus melleus* (YIM PHI001) based on morphological, genetic (ITS) and pathogenicity analyses. A voucher specimen was deposited at the Yunnan Institute of Microbiology, Kunming, P.R. China.

2.3. Fermentation

Aspergillus melleus YIM PHI001 was maintained on the PDA medium. Small agar plugs (approximately 5 mm×5 mm) of the fungus were cultured in 0.5 L Erlenmeyer flasks containing 100 mL of modified martin medium at 130 rpm and 28°C for 3 days as a seed culture. Then a 50 mL seed culture was transferred into a 1L Erlenmeyer flask containing 200 mL of potato dextrose broth (PDB) and incubated at 130 rpm and 28°C for 15 days. A total 55 L fermentation broth was collected.

2.4. Extraction and Isolation of Compounds

The production broth (55 L) was centrifuged to separate the mycelia from the suspension. The broth was exhaustively extracted with EtOAc, yielding 16.8 g of extract. The mycelia were extracted three times with methanol with ultrasonic processing for 30 min each time. The methanol extract was removed under vacuum, and the resulting aqueous layer was extracted three times with an equal

volume of EtOAc to yield a crude extract (30.5 g). The extracts of the fermentation broth and the mycelia were separated after TLC and HPLC analyses. The organic extract of mycelia was first subjected to CC over silica gel eluted with stepwise $\text{CHCl}_3/\text{MeOH}$ gradient (100 : 0, 50 : 1, 20 : 1, and 5 : 1, v/v) to yield Fractions 1-7. Fr. 1 was fractioned by column chromatography on silica gel eluted with petroleum ether/ CHCl_3 (5:1, 1:1) to yield Fr.1.1- Fr.1.3. Fr. 1.3 was eluted upon Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}=1:1$) to obtain **8** (8 mg). Fr. 2 was fractioned by column chromatography on silica gel eluted with petroleum ether/ CHCl_3 (1:1) to yield Fr. 2.1-Fr. 2.3. Fr. 2.2 was fractioned by ODS eluted with acetone/ H_2O (20% to 100%) to obtain **7** (11 mg). Fr. 2.3 was fractioned by ODS eluted with acetonitrile/ H_2O (60% to 100%) to obtain **9** (15 mg). Fr. 5 was fractioned by column chromatography on silica gel eluted with petroleum ether/acetone (6:4) to obtain Fr.5.1-Fr.5.3. The sub-fraction Fr. 5.3 was eluted upon Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}=1:1$) and further purified by ODS eluted with acetonitrile/ H_2O (10%-30%, v/v) to afford compounds **1**, **2** (8 mg), **4** (6 mg), **5** (7 mg), **6** (11 mg). Fr. 6 was fractioned by column chromatography on silica gel eluted with $\text{CHCl}_3/\text{MeOH}$ gradient to yield Fr. 6.1-Fr. 6.2. Fr. 6.2 was eluted upon Sephadex LH-20 ($\text{CHCl}_3/\text{MeOH}=1:1$) to obtain **3** (6 mg).

2.5. Antimicrobial Activity Assays

Compounds **1-9** were evaluated for antimicrobial activities. The assays were performed in 96-well sterilized microplates using a microdilution method [4]. Kanamycin and nystatin (Taicheng Pharmaceutical Co., purity > 99%) were introduced as the positive controls. Each concentration was tested in triplicate. Microbial growth was observed with a CX21BIM-set 5 microscope (Olymps Corp). MICs were determined as the lowest concentrations that produced complete growth inhibition of the microorganisms tested.

3. Results and Discussion

HR-ESIMS analysis of compounds **1**, and **2** as stereoisomerism mixture (**2** for minor constituent) revealed quasi-molecular ion peak at $211.0936[\text{M}+\text{Na}]^+$, calcd. 211.0946, respectively. The ^1H and ^{13}C NMR spectra data (Table 1), including DEPT, clearly showed two groups of signals about **1** and **2** including two methyls, one methylene, five methines (one for olefinic bond), one olefinic quaternary carbon, which showed the skeleton of pentaketides. Aspilactonol B, C [5], aspinonene (**4**), aspinotriols A (**5**) and B (**6**) [6] sharing the similar structure with **1** and **2** were also produced by *Aspergillus*. The signals of ^1H -NMR and ^{13}C -NMR were assigned according to the correlations in the 2D NMR spectra and the chemical shift values. The fragments of H-1 to H-4 and H-7 to H-9 was established on the basis of the COSY correlations, and the HMBC correlations from H-1 to C-2, 3, from H-9 to C-7, 8, from H-6 to C-4, 5, from H-4 to C-3, from H-7 to C-6 confirmed the common skeleton of compounds **1**, **2** (Figure 2). The key connection of C-3 and C-6 was confirmed by the NMR data at C-3, and C-6 (Table1). The structures of **1**, **2** and the known compounds, aspilactonol B, C were almost the same, except for a methylene in **1**, **2** changed to a carbonyl in aspilactonol B, C. The stereochemistry of these new compounds in C-2, C-3, C-7, C-8 was determined the same as aspilactonol B, C for *S, R, S, R* in **1** and *S, R, R, S* in **2** by comparing the NMR data and biogenesis (Figure 1). So these two stereoisomers were determined as melleusin A (**1**), B (**2**). Metabolites **1**, **2** were derived from a common biosynthetic precursor, **A-3**, which originates from the intermediate **A**, the ultimate product of polyketide synthesis (PKS), by post-PKS modifications (Figure 3). Reduction of the aldehyde in **A-3** into the primary alcohol give **A-4**, and formation of **1**, **2** by nucleophilic attack of the primary alcohol on either site of the carbons in one of the two epoxide groups and hydration in the rest epoxide group, and the similar biosynthetic conversion was also reported in aspilactonol B, C [5].

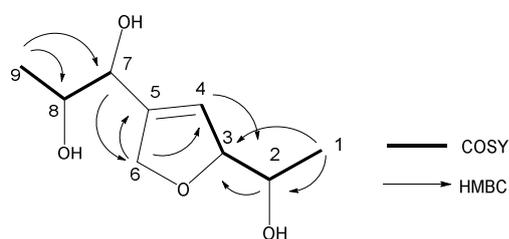


Figure 2. The key COSY and HMBC correlations of compounds **1** and **2**.

Table 1. ^1H and ^{13}C NMR Data of **1**, **2** in MeOD (δ in ppm, J in Hz)

Position	1		2	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	1.16 (d, 5.0)	17.3	1.22 (d, 6.0)	17.6
2	3.74(m)	69.2	3.74(m)	69.3
3	4.67 (m)	90.5	4.67 (m)	90.5
4	5.80 (s)	121.9	5.80 (s)	121.9
5		143.1		143.2
6	4.64, 4.73 (m)	74.9	4.64, 4.73 (m)	74.9
7	4.17 (d, 5.5)	72.6	4.17 (d, 5.5)	72.7
8	3.74 (m)	69.6	3.74 (m)	69.7
9	1.17 (d, 6.0)	17.4	1.21 (d, 6.0)	17.5

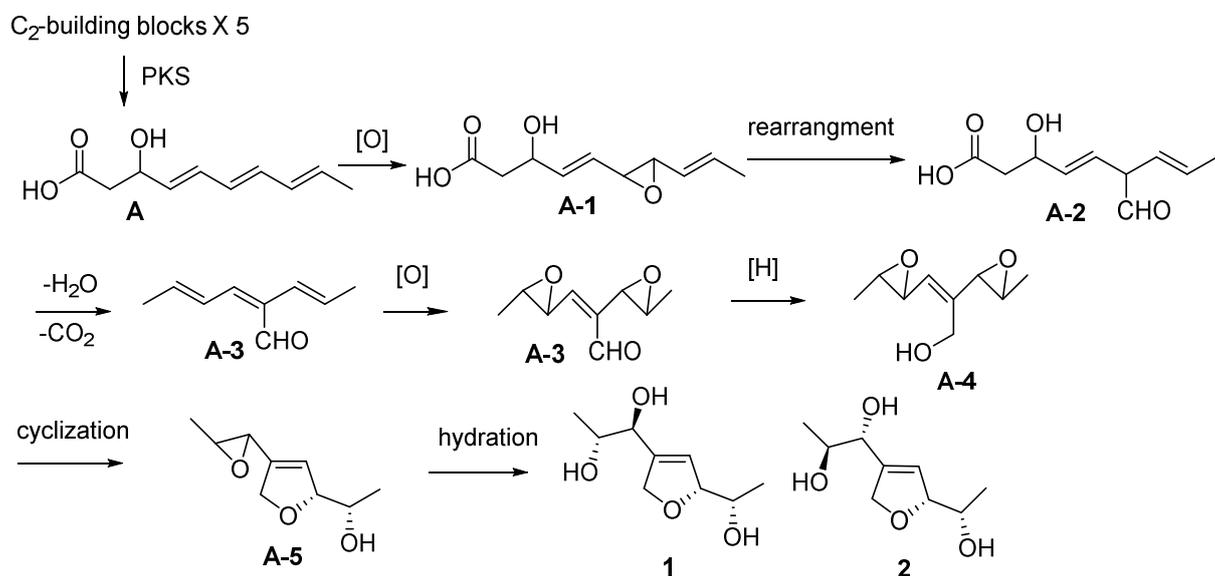


Figure 3. The possible biosynthetic pathway of new compounds **1-2**.

Other known compounds were isolated and determined as chrysogeside D (**3**) [7], aspinonene (**4**) [6], aspinotriols A (**5**) and B (**6**) [6], 22*E*,24*R*-ergosta-7,22-diene-3 β ,5 α ,6 β -triol (**7**) [8], mellein (**8**) [9], 4-hydroxymellein (**9**) [10].

The mixture of compound **1** and **2**, and other isolated compounds (**3-9**) were evaluated for

antimicrobial activity against the pathogenic bacteria and fungi, *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, *Fusarium oxysporum*, *Fusarium solani*. Compound **8** indicated the broad spectrum antibiotic activity against all five pathogens with MICs at 128-256 $\mu\text{g/mL}$. As positive controls, kanamycin had antibacterial activity against *E. coli*, *B. subtilis* with MICs of 32 $\mu\text{g/mL}$, nystatin had antifungal activity against *C. albicans*, *F. oxysporum*, and *F. solani* with MICs of 16, 32, and 16 $\mu\text{g/mL}$. Control of harmful microorganism in food, aquaculture, pharmaceuticals, agriculture, hospitals and recreation water pools are great global concern. Some infections maynot caused by single pathogen, but multi-pathogens, so the metabolites with broad antibiotic activity had significance on control of these multi-pathogens. In this research, we found mellein had antibacterial and antifungal activities, and it can be used as a biocontrol against disease caused by bacteria, and fungi. Previous works showed that aspinonene, aspinotriols A and B were mainly found in *Aspergillus*, so these compounds can be used as marker molecule in chemotaxonomy of *Aspergillus*.

Acknowledgments

This work was partly supported by the National Natural Science Foundation of China [grant number 81360480], [grant number 21562045], [grant number 81460536].

Supporting Information

Supporting Information accompanies this paper on <http://www.acgpubs.org/RNP>

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References

- [1] H. W. Zhang, Y. F. Tang, C. F. Ruan and X. L. Bai (2016). Bioactive secondary metabolites from the endophytic *Aspergillus* genus, *Rec. Nat. Prod.* **10**, 1-16.
- [2] J. C. Frisvad and T.O. Larsen (2015). Chemodiversity in the genus *Aspergillus*, *Appl. Microbiol. Biotechnol.* **99**, 7859–7877.
- [3] A. M. Hill, A. Jacobs and J. Staunton (1995). Investigation of the stereochemistry of the tri- and tetra-ketide hydroxyacyl intermediates in the biosynthesis of the polyketide aspyrone in *Aspergillus malleus* using deuterium labelling and deuterium NMR spectroscopy, *J. Chem. Soc. Chem. Commun.* **8**, 859-860.
- [4] H. Zhou, L. X. Zhao, W. Li, Y. B. Yang, L. H. Xu, and Z. T. Ding (2015). Anti-*Mycobacterium tuberculosis* active metabolites from an endophytic *Streptomyces* sp. YIM65484, *Rec. Nat. Prod.* **9**, 196-200.
- [5] X. W. Chen, C. W. Li, C. B. Cui, W. Hua, T.J. Zhu and Q. Q. Gu (2014) Nine new and five known polyketides derived from a deep sea-sourced *Aspergillus* sp. 16-02-1, *Mar. Drugs* **12**, 3116-3137.
- [6] K. Kito, R. Ookura, S. Yoshida, M. Namikoshi, T. Ooi and T. Kusumi (2007). Pentaketides relating to aspinonene and dihydroaspyrone from a marine-derived fungus, *Aspergillus ostianus*, *J. Nat. Prod.* **70**, 2022–2025.
- [7] Y. Y. Li, P. Liu, Y. Tang, H. M. Li, Y. L. Tang, X. H. Liang and Y. J. Tang (2013). Novel cerebrosides isolated from the fermentation mycelia of *Tuber indicum*, *Helv. Chim. Acta* **96**, 702-709.
- [8] W. Li, X. Q. Yang, Y. B. Yang, S. H. Qin, Q. L. Li, L. X. Zhao and Z. T. Ding (2015). A new natural nucleotide and other antibacterial metabolites from an endophytic *Nocardia* sp., *Nat. Prod. Res.* **29**, 132–136.

- [9] N. Kamal, C. V. Viegelmann, C. J. Clements and R. A. Edrada-Ebel. Metabolomics-guided isolation of anti-trypanosomal metabolites from the endophytic fungus *Lasiodiplodia theobromae*, *Planta Med.* DOI: 10.1055/s-0042-118601.
- [10] C. H. Lu, S. S. Liu, J. Y. Wang, M. Z. Wang and Y. M. Shen (2014). Characterization of eight new secondary metabolites from the mutant strain G-444 of *Tubercularia* sp. TF5, *Helv. Chim. Acta*, **97**, 334-344.

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