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SHORT REPORT

Paxillene A, a new meroterpenoid isolated from the mushroom *Paxillus ammoniavirescens* Contu & Dessi

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Abstract: In this study, a new meroterpenoid, namely paxillene A (**1**), along with six known compounds (**2**–**7**) were isolated from the mushroom *Paxillus ammoniavirescens* Contu & Dessi. Their structures were established through extensive spectroscopic analyses, including HR-ESI-MS, 1D and 2D NMR. All isolated compounds were evaluated for their anti-inflammatory activity against LPS-induced NO production in RAW 264.7 macrophages. Compound **1** exhibited moderate inhibitory activity with an IC_{50} value of 38.55 ± 3.72 μM.

Keywords: *Paxillus ammoniavirescens* Contu & Dessi, meroterpenoid, anti-inflammatory activity

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1 Mushroom Source

The fresh fruiting bodies of *Paxillus ammoniavirescens* Contu & Dessi were collected from Baishan city, Jilin Province, China, in September 2024 and identified based on morphological characteristics and molecular analysis. This species is characterized by a yellowish-brown to olive-green pileus, decurrent lamellae, and a robust, solid stipe. A voucher specimen (accession number: PA20240906) has been deposited in the Applied Mycology Laboratory, Hebei Normal University.

2 Previous Studies

The genus *Paxillus* is classified under the family Paxillaceae, order Boletales and class Agaricomycetes (Jargeat et al., 2014). Most species within this genus are toxic and inedible. These fungi commonly form ectomycorrhizae with trees and are regarded as one of the key model organisms for ectomycorrhizal research (Jargeat et al., 2016; Zmitrovich et al., 2019). Currently, 40 species of *Paxillus* have been recorded in the fungal database Index Fungorum. While numerous studies have focused on *P. involutus*, other congeners remain largely underinvestigated. Our previous research on the chemical constituents and biological activities of *P. involutus* yielded a series of structurally diverse diarylcyclopentenones (Lv et al., 2021a), furanopaxins (Lv et al., 2021b), pyrones (Lv et al., 2022), and coumarins (Zhang et al., 2020). Most of these metabolites exhibited potent antioxidant and antihyperglycemic activities.

Paxillus ammoniavirescens Contu & Dessi, is an ectomycorrhizal fungus mainly distributed in Heilongjiang, Jilin, Liaoning, and Hebei provinces, in China (Rose et al., 2024; Richardson et al., 2024), and its secondary metabolites remain uncharacterized. Inspired by the structural diversity of natural products from the genus *Paxillus*, we performed a phytochemical study on the fruiting bodies of *P. ammoniavirescens*. As a result, a new meroterpenoid, Paxillene A (**1**), as well as six known compounds (**2**–**7**) were obtained from *P. ammoniavirescens* (Figure 1). Herein, we describe the isolation and structural characterization of compounds **1**–**7** and evaluate their anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages.

3 Present Study

The air-dried fruiting bodies of *P. ammoniavirescens* (10.5 kg) underwent three cycles of 90% ethanol–water extraction (50 L, 3 days each) at room temperature, affording 1177 g crude extract. Afterwards, the extract was dispersed in 1 L water, and then fractionated sequentially with *n*-hexane (3 × 1.0 L) and ethyl acetate (3 × 1.0 L).

The *n*-hexane crude fraction was loaded onto a silica gel column (200–300 mesh, 500 g) for chromatographic separation. Stepwise elution was carried out with a petroleum ether-ethyl acetate system at volumetric ratios of 50:1, 20:1,

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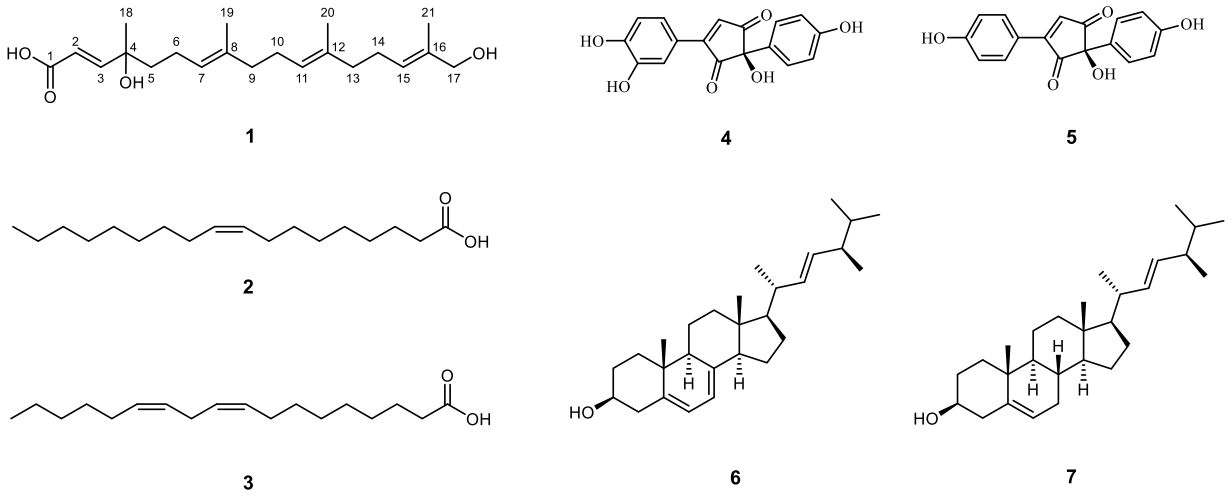

Figure 1. Structures of compounds **1**–**7**

10:1, 5:1, 3:1, 1:1, and 0:1, which generated a total of 10 distinct subfractions designated as Fr.1 to Fr.10. Fr. 2 was further purified via high-performance liquid chromatography with a YMC-Pack ODS-A (250 × 10 mm, 5 μM) column (YMC Co., Ltd., Kyoto, Japan). The chromatographic conditions were as follows: mobile phase acetonitrile/water (90:10, v/v), flow rate 3.0 mL/min, and detection wavelength 210 nM. Three pure compounds were acquired after separation: compounds **1** (6.2 mg, retention time = 7.5 min), **2** (11.3 mg, retention time = 15.2 min) and **3** (9.4 mg, retention time = 19.8 min). Fr. 5 was subjected to silica gel column chromatography eluted with petroleum ether-ethyl acetate (8:1) to give compounds **6** (37.8 mg) and **7** (25.3 mg). Fr. 9 was subjected to a Sephadex LH-20 column by eluting with gradient mixtures of methanol/water (1:1) to obtain compounds **4** (6.6 mg) and **5** (4.1 mg).

Paxillene A (***1***): White amorphous powder. UV (MeOH) λ_{max} 207 (4.13), 228 (3.89) nM; $[\alpha]_D^{21.0}$ 0 (c 0.1, MeOH); ^{1}H and ^{13}C NMR data see Table 1; HR-ESI-MS (*m/z* 349.2384 $[M–H]^-$, calcd. 349.2384).

Compound **1**, white amorphous powder, had a molecular formula of $C_{21}H_{34}O_4$ by the negative HR-ESI-MS (*m/z* 349.2384 $[M–H]^-$, calcd. 349.2384), requiring five degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) in methanol-d_4 of **1** exhibited the following easily identifiable signals: a pair of *trans* olefinic proton doublets at δ_H 6.95 (1H, d, *J* = 15.7 Hz, H-3) and 5.96 (1H, d, *J* = 15.7 Hz, H-2), three characteristic olefinic proton broad triplets assignable to three prenyl groups, an oxygenated methylene singlet at δ_H 3.91 (2H, br s, H-17), three olefinic methyl broad singlets, as well as an aliphatic methyl singlet in the high-field region. The ^{13}C NMR spectrum (Table 1) showed a total of 21 carbon signals, including a carboxyl carbon at δ_C 170.3, eight olefinic carbons due to four double-bond groups, two oxygenated sp^3 carbon at δ_C 73.4 (s, C-4) and 69.0 (t, C-17), as well as 10 aliphatic carbons (6 × CH_2 and 4 × CH_3). Analysis of the degrees of unsaturation indicated that it was a non-cyclic structure. The HMBC correlations (Figure 2) from the oxygenated methylene singlet to C-15 [δ_C 126.6 (d)] and C-21 [δ_C 13.7 (q)] revealed that a methyl group of the terminal prenyl moiety was hydroxylated. While the other end of this chain structure was determined to be a (*E*)-4-hydroxy-4-methylpent-2-enoic acid moiety by the HMBC correlations from the *trans* olefinic protons to the carboxyl carbon, and from the aliphatic methyl singlet [δ_H 1.30 (3H, s, H-18)] to C-3 [δ_C 156.4 (d)], C-4 [δ_C 73.4 (s)] and C-5 [δ_C 43.0 (t)]. And the analysis of the ^{1}H, ^{1}H-COSY correlations (Figure 2) further established the connection of these substructural units. Based on the coupling constant of 15.7 Hz between H-2 and H-3, the $\Delta^{2,3}$ double bond was assigned an *E*-configuration. And the NOESY correlations of H-7/H-9, H-11/H-13, and H-15/H-17, confirming that the $\Delta^{7,8}$, $\Delta^{11,12}$, and $\Delta^{15,16}$ double bonds all adopt *E*-configurations. Given the absence of optical rotation and CD signal, compound **1** occurred as an inseparable racemic mixture. Therefore, the structure of **1** was established as shown in Figure 1, and named as paxillene A.

By comparing their NMR spectroscopic data with the literature, six known compounds (**2**–**7**) were identified as oleic acid (**2**) (Ashrafi et al., 2022), linoleic acid (**3**) (Luyen et al., 2023), involutone (**4**) (Antkowiak et al., 2003), talaromycesins A (**5**) (Zheng et al., 2024), ergosterol (**6**) (Thanh et al., 2018), and ergosta-5,22-dien-3β-ol (**7**) (Gao et al., 2001).

Cytotoxicity of compounds **1**–**7** against RAW 264.7 macrophages (purchased from the National Collection of Authenticated Cell Cultures) was evaluated at concentrations up to 80 μM. None of the compounds showed significant cytotoxicity at the tested concentrations, with all cytotoxicity values >80 μM. Subsequently, the inhibitory effects of compounds **1**–**7** on LPS-induced NO production were determined using aminoguanidine (purchased from Rhawn Co., Ltd., Shanghai, China) as the positive control. IC_{50} value is the concentration that inhibits the production of NO by 50%. And it was calculated using GraphPad Prism 5.03 software. As shown in Table 2, compound **1** exhibited the strongest activity with an IC_{50} value of 38.55 ± 3.72 μM, followed by compounds **4** (52.27 ± 4.01 μM), **5** (65.21 ± 3.38 μM),

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Table 1. ^{1}H-NMR (600 MHz) and ^{13}C-NMR (150 MHz) data of **1** in CD_3OD

Position	^{1}H NMR (integral, multiplicity, *J* in Hz)	^{13}C NMR
1	—	170.3
2	5.96 (dd, *J* = 15.7, 1.6 Hz, 1H)	119.6
3	6.95 (dd, *J* = 15.7, 1.6 Hz, 1H)	156.4
4	—	73.4
5	1.63 – 1.55 (m, 2H)	43.1
6	2.10 – 2.04 (m, 1H); 2.02 – 1.96 (m, 1H)	23.5
7	5.13 (t, *J* = 7.9 Hz, 1H)	125.6
8	—	136.3
9	2.02 – 1.96 (m, 2H)	40.8
10	2.09 (q, *J* = 7.3 Hz, 2H)	27.5
11	5.13 (t, *J* = 7.9 Hz, 1H)	125.4
12	—	135.8
13	2.05 – 1.99 (m, 2H)	40.5
14	2.13 (q, *J* = 7.9 Hz, 2H)	27.4
15	5.39 (t, *J* = 7.2 Hz, 1H)	126.6
16	—	135.9
17	3.91 (s, 2H)	69.0
18	1.30 (s, 3H)	27.6
19	1.61 (s, 3H)	16.1
20	1.61 (s, 3H)	16.0
21	1.64 (s, 3H)	13.7

and **6** (69.89 ± 4.47 μM). Compounds **2**, **3**, and **7** showed weak activity with IC_{50} values > 80 μM. The positive control aminoguanidine displayed potent activity with an IC_{50} value of 10.64 ± 1.06 μM. And the IC_{50} of aminoguanidine was no obvious difference compared to the literature, demonstrating the accuracy of the anti-inflammatory experiments.

In this study, multiple natural product separation and purification techniques, including ethanol maceration, liquid-liquid extraction, silica gel column chromatography, Sephadex LH-20 gel filtration chromatography and reversed-phase high-performance liquid chromatography, were applied to isolate seven pure compounds from the fruiting bodies of *P. ammoniavirescens*. Compound **1** represents a previously undescribed meroterpenoid, whose full chemical structure is elucidated for the first time in this work on the basis of comprehensive spectroscopic analyses. Compounds **2** and **3** are common unsaturated fatty acids widely found in numerous fungi and plants. Compounds **4** and **5** are diarylcyclopentenone compounds, originally discovered from the mushroom *P. involutus*, which might be important chemotaxonomic biomarkers for *Paxillus* genus. Compounds **6** and **7** are known steroidal compounds. Sterols are essential membrane components in fungi, plants and animals, widely distributed in fungi with diverse pharmacological activities. In vitro anti-inflammatory assays revealed that compound **1** exerted moderate inhibitory activity against LPS-induced NO production in macrophages. These findings not only expanded the chemical diversity of meroterpenoids but also enriched the secondary metabolites of *P. ammoniavirescens*. Importantly, paxillene A showed favorable anti-inflammatory activity, highlighting its prospects for anti-inflammatory lead compound screening.

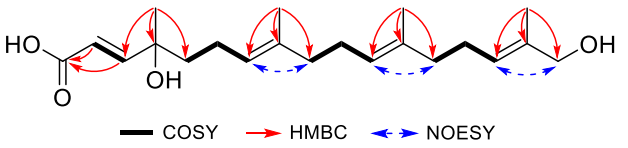

Figure 2. Key HMBC, ^{1}H–^{1}H COSY, and NOESY correlations of compound **1**

Table 2. Inhibition of LPS-induced NO production in RAW 264.7 cells of compounds **1**–**7**

Compounds	IC_{50} (μM)	Cytotoxicity (μM)
1	38.55 ± 3.72	>80
2	>80	>80
3	>80	>80
4	52.27 ± 4.01	>80
5	65.21 ± 3.38	>80
6	69.89 ± 4.47	>80
7	>80	>80
Aminoguanidine[a]	10.64 ± 1.06	>80

Note: [a]aminoguanidine was used as a positive control.

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Author Contributions

Juyou Liang performed the isolation and bioactivity assays. Chenglong Chuai assisted with compound purification and data analysis. Jinxiu Zhang contributed to mushroom collection and species identification. Lan Yao and Jianhua Lv designed the research, supervised the work, and finalized the manuscript. All authors read and approved the final version of the manuscript.

Availability of Data and Materials

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflicts of Interest

The authors declare that they do not have any conflict of interest.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journals/records-of-natural-products.

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