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SHORT REPORT

A new antifungal α-pyrone secondary metabolite isolated from the tobacco-derived fungus *Aspergillus aculeatus* Aa-1

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Abstract: This paper presents a chemical study of the tobacco-derived fungus *Aspergillus aculeatus* Aa-1. Three pyrones including a new α-pyrone namely asperacuone A (**1**), the α-pyrone saadamycin (**2**), and the 2-benzyl-γ-pyrone (**3**) were isolated from the EtOAc extract of this fungal strain. The chemical structures of these compounds were precisely determined via nuclear magnetic resonance spectroscopy (1D/2D NMR) as well as high-resolution electrospray ionization mass spectrometry (HRESIMS). The antifungal activities of compounds **1**–**3** were then evaluated against six agricultural pathogenic fungi. While compound **1** revealed strong inhibitory potency against *Alternaria alternata* (minimum inhibitory concentration [MIC] = 8 μg/mL), compound **2** showed broad-spectrum inhibitory activities against the tested pathogens (MIC = 4–16 μg/mL).

Keywords: Tobacco-derived fungus, *Aspergillus aculeatus*, secondary metabolite, α-pyrone, antifungal activity

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1 Fungal Source

The strain *Aspergillus aculeatus* Aa-1 used in the present study was isolated in July 2022 from the leaves of healthy tobacco cultivated in Nanning City, Guangxi Province, China. The strain had been preserved in the China General Microbiological Culture Collection Center since November 14, 2022 (deposition number: CGMCC 40424). Species identification was performed using a molecular biological technique comprising the DNA amplification and sequencing of the internal transcribed spacer area of the strain's ribosomal DNA (GenBank accession No: PX688150). Further BLAST search results demonstrated that the sequence obtained exhibited high similarity (99.8%) to the strain *Aspergillus aculeatus* (GenBank accession No. OQ629860).

2 Previous Studies

Secondary metabolites constitute a particularly fascinating and effective class of compounds involved in the vast and complex biochemical processes of fungi (Keller, 2019; Brakhage, 2013; Gerke & Braus, 2014). In addition to their role in metabolism, they also have significant application value in the fields of pesticides and medicine (Dong et al., 2025; Enyi et al., 2025). As more metabolic products continue to be discovered, the study of fungal biology has attracted significant attention (Naour-Vernet et al., 2025). Plant endophytic fungi, in particular, have garnered widespread interest (Yuan et al., 2024), as they exhibit unique secondary metabolic activities and yield abundant metabolic products with rich chemical diversity (Xu et al., 2025; Wang et al., 2025). Furthermore, these generated metabolic products possess a wide range of biological activities (Dinglasan et al., 2025). Prior research on the *Aspergillus* species has documented prolific secondary metabolite diversity, such as characteristic polyketides, indole-diterpenoids, and bioactive cyclic peptides (Li et al., 2024; Ren et al., 2024; Fu et al., 2024; Wang et al., 2024; Sun et al., 2024). Available reports concerning *A. aculeatus* have predominantly identified common structural classes, including lovastatin analogues and sesquiterpenoids from terrestrial strains (Liu et al., 2024; Gao et al., 2015; Chaiyosang et al., 2022). Conversely, our tobacco-derived strain Aa-1 produces structurally distinct compounds (**1**–**3,** as described in Section 3), revealing chemotaxonomic divergence and strain-specific chemodiversity influenced by the strain's unique endophytic niche of plants, thereby establishing novelty beyond existing species-level metabolite profiles of *Aspergillus*.

3 Present Study

This study details a chemical investigation of the tobacco-derived endophytic fungus *Aspergillus aculeatus* Aa-1. The strain was isolated from tobacco, along with other fungal

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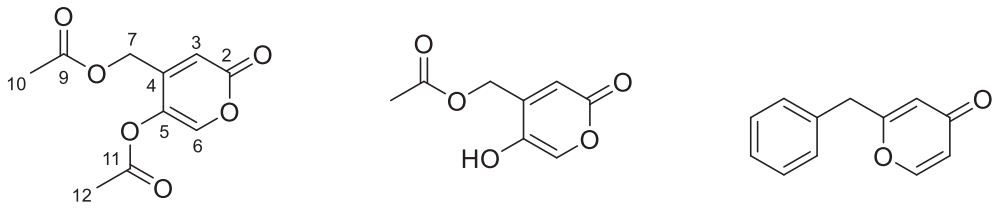

Figure 1. Chemical structure of compounds **1**–**3**

strains. Our previous bioactivity screening revealed that the Aa-1 strain possessed strong antifungal effects against some agricultural pathogenic fungi. This fungus was therefore selected as the target strain to discover biologically active secondary metabolites. After repeated column chromatography separation, three pyrones were isolated and identified from the EtOAc extract of this fungal strain: the new α-pyrone asperacuone A (**1**), the α-pyrone saadamycin (**2**), and the 2-benzyl-γ-pyrone (**3**) were isolated and identified from the EtOAc extract of this fungal strain (Figure 1).

A. aculeatus Aa-1 was cultured in solid rice medium using 100 × 1 L conical flasks containing 100 g of rice and 100 mL of distilled water. The entire fermentation process was carried out under static conditions at 28°C for 30 days. Then, to terminate fermentation, EtOAc (1:1, v/v) was added to the broth. The entire fermented broth was extracted three times with EtOAc (3 × 10 L). The EtOAc solute was combined and concentrated to yield 10.8 g of crude extract.

Using a stepwise gradient mixture of CH_2Cl_2 and MeOH (50:1–1:1, v/v), the crude extract (10.8 g) was subjected to vacuum-liquid chromatography on a silica gel column. Ultimately, six fractions (Fr.1–6) were obtained according to the polarity of different elution solutions. Bioassay-guided isolation revealed that Fr.4 (1.2 g, eluted with CH_2Cl_2–MeOH 20:1) possessed the strongest antifungal activity. Therefore, Fr.4 was selected for further separation. It was subjected to ODS reverse-phase silica gel column chromatography and eluted with MeOH–H_2O from 10% to 100%. Eight subfractions, Fr.4.1–4.8, were further acquired. Fr.4.3 was separated using semipreparative HPLC eluted with 55% MeCN–H_2O to obtain compound **2** (6.2 mg, t_R = 11.3 min). Fr.4.4 was purified via semipreparative HPLC eluted with 58% MeOH–H_2O to obtain compound **1** (3.0 mg, t_R = 12.8 min). Compound **3** (9.7 mg, t_R = 9.2 min) was acquired from Fr.4.5 through semipreparative HPLC with 55% MeOH–H_2O.

*Asperacuone A (**1**):* yellowish white solid; mp 102–104°C; UV (MeOH): λ_{max} 210 (4.59) and 270 (3.68) nM; ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data, measured in $CDCl_3$, see Table 1; (+)-HRESIMS: *m/z* 249.0372 $[M + Na]^+$ (calcd for $C_{10}H_{10}NaO_6^+$, 249.0370).

Antifungal assay: The antifungal activities of the isolated pyrone compounds **1**–**3** against six agricultural pathogenic fungi (*Fusarium oxysporum*, *Fusarium equiseti*, *Rhizoctonia cerealis*, *Alternaria alternata*, *Valsa mali*, and *Colletotrichum gloeosporioides*) were assessed by the broth microdilution method (Wang et al., 2016). Commercially available azoxystrobin and carbendazim usually used in agriculture were used as positive controls. The tested compounds were dissolved in MeOH with the culture medium for each pathogenic fungi and tested with the final concentrations ranging from 1 to 64 μg/mL. The minimum inhibitory concentration (MIC) values were calculated to indicate the

Table 1. ^{1}H and ^{13}C NMR data for compound **1** and **2** (δ in ppm)

No.	1 ($CDCl_3$)		2 ($CDCl_3$)		2 (DMSO-d_6)		Saadamycin (DMSO-d_6) (El-Gendy & El-Bondkly, 2010)	
	δ_H mult. (*J* in Hz)	δ_C type	δ_H mult. (*J* in Hz)	δ_C type	δ_H mult. (*J* in Hz)	δ_C type	δ_H mult. (*J* in Hz)	δ_C type
2		172.4 C		174.2 C		173.6 C		173.9 C
3	6.48 s	115.3 CH	6.50 s	111.5 CH	6.47 s	112.5 CH	6.34 s	109.8 CH
4		141.3 C		146.1 C		146.0 C		145.7 C
5		162.5 C		162.9 C		161.6 C		168.1 C
6	7.87 s	148.0 CH	7.85 s	138.2 CH	8.09 s	139.7 CH	8.03 s	139.2 CH
7	4.90 br s	61.0 CH_2	4.92 s	61.5 CH_2	4.93 s	61.3 CH_2	4.29 s	59.5 CH_2
9		169.8 C		170.0 C		169.8 C		Unreported
10	2.14 s	20.6 CH_3	2.15 s	20.7 CH_3	2.11 s	20.4 CH_3	Unreported	Unreported
11		167.8 C						
12	2.31 s	20.5 CH_3						

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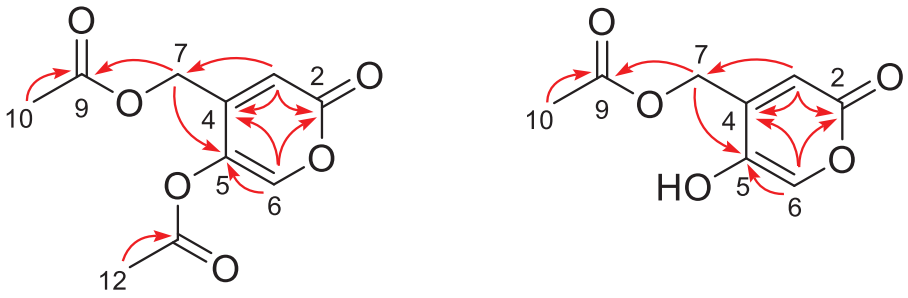

Figure 2. Key HMBC correlations for **1** and **2**

growth inhibition of each diluted concentrations of the tested compounds.

Asperacuone A (**1**) was acquired as a yellowish white solid. The molecular formula of compound **1** was unambiguously established as $C_{10}H_{10}O_6$ based on the observation of its high-resolution electrospray ionization mass spectroscopy (HRESIMS) positive-ion peak at *m/z* 249.0372 $[M + Na]^+$ (calcd for $C_{10}H_{10}NaO_6^+$, 249.0370) and the ^{13}C nuclear magnetic resonance (NMR) data, indicating the presence of six double bond equivalents. The UV spectrum showed characteristic absorption at 210 and 270 nM, indicating the presence of the α-pyrone chromophore (El-Gendy & El-Bondkly, 2010; Ayer et al., 1989). The 1D (^{13}C and ^{1}H) NMR spectra of **1** showed simple but straightforward resonance signals. The ^{1}H NMR data of **1** (shown in Table 1) exhibited characteristic signals for two olefinic protons [δ_H 6.48 (1H, s, H-3) and 7.87 (1H, s, H-6)], one oxygenated methylene resonating at δ_H 4.90 (2H, br s, H_2-7), and two methyl groups [δ_H 2.14 (3H, s, H_3-10) and 2.31 (3H, s, H_3-12)]. Analysis of the ^{13}C NMR data (Table 1) of **1**, aided by heteronuclear singular quantum correlation (HSQC) spectrum, clearly indicated the presence of two methyls [resonating at δ_C 20.5 (C-12) and 20.6 (C-10)], one methylene resonating at δ_C 61.0 (C-7), two methines [δ_C 115.3 (C-3) and 148.0 (C-6)], and five nonprotonated carbons [including three ester groups at δ_C 167.8 (C-11), 169.8 (C-9), and 172.4 (C-2), as well as two olefinic carbons at δ_C 141.3 (C-4) and 162.5 (C-5)]. The aforementioned information suggested that compound **1** was a lactone compound, and the 1D NMR data revealed strong similarities to those of saadamycin (compound **2**) (El-Gendy & El-Bondkly, 2010). The key HMBC correlation (Figure 2) from H_3-12 (δ_H 2.31) to C-11 (δ_C 167.8) confirmed the attachment of an acetyl group. Notably, the observed upfield shifts of C-4/C-5/C-6 were due to acetylation at O-5 in compound **1** (vs. OH-5 in compound **2**); this can be mechanistically explained by the p-π conjugation effect of the acetyl group, which alters electron density by replacing the hydroxyl at C-5. The planar structure of **1** is shown in Figure 1.

The other two known pyrones, namely the α-pyrone saadamycin (**2**) (El-Gendy & El-Bondkly, 2010) and the 2-benzyl-γ-pyrone 2-benzylpyrone (**3**) (Tan et al., 2022), were determined by analyzing their NMR data and comparing them to reported data. The 1D NMR data of **2** showed similar characteristic signals compared to those of **1**, except for the absence of the acetyl group (Table 1). However, the 1D NMR data of **2** were in full disagreement with the reported data of saadamycin by El-Gendy and El-Bondkly (2010). The 1D (^{1}H and ^{13}C) NMR data for **2** compared with data of saadamycin are given in Table 1 (both measured in the same solvent DMSO-d_6). For example, El-Gendy et al. reported ^{13}C NMR data for C-5 at δ_C 168.1 (Table 1), while it is very difficult for C-5 carbon to resonate at 168.1 ppm in an aromatic system. The observed key HMBC correlation in Figure 2 from H-3 (δ_H 6.47) to C-4 (δ_C 146.0), from H-6 (δ_H 8.09) to C-4 and C-5 (δ_C 161.6), and from H_2-7 (δ_H 4.93) to C-5 confirmed the assignment of C-5. Moreover, ^{13}C NMR data for C-9 and C-10 were unreported by El-Gendy and El-Bondkly (2010). Based on the analysis of HMBC correlations in Figure 2, the spectroscopic data of compound **2** was revised herein. Nonetheless, we cannot propose a structure suitable for the structure reported by El-Gendy and El-Bondkly (2010) due to the lack of more useful spectroscopic information.

The antifungal activities of the isolated pyrone compounds **1**–**3** were evaluated against six common agricultural pathogenic fungi (*F. oxysporum*, *F. equiseti*, *R. cerealis*, *A. alternata*, *V. mali*, and *C. gloeosporioides*). Azoxystrobin and carbendazim, which are generally used in agricultural production, served as positive controls. The antifungal results are presented in Table S1 (see supporting information). Compared to positive controls (azoxystrobin, minimum inhibitory concentration [MIC] = 16 μg/mL; carbendazim, MIC = 8 μg/mL), compound **1** exhibited strong inhibitory potency against *A. alternata* (MIC = 8 μg/mL). Compound **1** was inactive against other five pathogenic fungi (MIC > 64 μg/mL), signifying its high selectivity in the growth inhibitory effects. Conversely, the analogous α-pyrone **2** exhibited broad-spectrum inhibitory activities against *F. oxysporum*, *R. cerealis*, *A. alternata*, *V. mali*, and *C. gloeosporioides* (MIC = 4–16 μg/mL) (Table S1 (see supporting information)). Notably, compound **2** possessed the strongest inhibitory effect against *R. cerealis* (MIC = 4 μg/mL). In previous studies, El-Gendy and El-Bondkly (2010) demonstrated that compound **2** isolated from endophytic *Streptomyces* sp. possessed robust broad-spectrum antifungal efficacy against tested clinical fungi, including *Trichophyton rubrum* (MIC = 5 μg/mL), *Trichophyton mentagrophytes* (MIC = 1.5 μg/mL), *Microsporum gypseum* (MIC =

1.25 μg/mL), *Epidermophyton floccosum* (MIC = 1.0 μg/mL), *Aspergillus niger* (MIC = 1.0 μg/mL), *Aspergillus fumigatus* (MIC = 1.6 μg/mL), *Fusarium oxysporum* (MIC = 1.2 μg/mL), *Candida albicans* (MIC = 2.22 μg/mL), and *Cryptococcus humicolus* (MIC = 5.16 μg/mL) (El-Gendy & El-Bondkly, 2010). The discrepancy in the reported MIC of saadamycin against *F. oxysporum* (1.2 μg/mL in the literature vs. 8 μg/mL in this study) could be ascribed to methodological differences. While El-Gendy and El-Bondkly (2010) employed the paper-disc diffusion assay, optimizing compound diffusion and hyphal contact, we utilized the broth microdilution method, with limited solubility of saadamycin resulting in reduced observed antifungal efficacy. Our research concurs with previous studies, supporting the conclusion that compound **2** may serve as a broad-spectrum agricultural fungicide.

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Author Contributions

Zhong Fan: Formal analysis, Investigation, and Writing original manuscript; Zhi-Zhong Hu: Methodology, Investigation, and Data Curation; Yu-Feng Chen: Methodology and Formal analysis; Dong-Ye Huang: Data Curation; Yi Shi: Methodology and Formal analysis; Yu-Mei Qian: Writing-review & editing, Funding, and Supervision.

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.

Ethics Approval

This study only involved in vitro biochemical assays. No human participants or live animals were involved.

Conflicts of Interest

The authors declare that they have no competing financial interests.

Supporting Information

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

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