

## Fusacintone A, a New Polyketide from the Endophytic Fungus *Fusarium tricinctum* from *Fritillaria monantha*

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**Abstract:** A previously undescribed polyketide (**1**) and four known compounds (**2-5**) isolated from the endophytic fungus *Fusarium tricinctum* from *Fritillaria monantha*. Compound **1** showed anti-inflammatory activity by inhibiting NO production with an IC<sub>50</sub> value of 17.6±0.46 μM.

**Keywords:** *Fusarium tricinctum*; endophytic fungus; polyketide; structural elucidation; anti-inflammatory. © 2025 ACG Publications. All rights reserved.

### 1. Fungal Source

The endophytic fungus *Fusarium tricinctum* was isolated from fresh and healthy *Fritillaria monantha* Migo collected from Sunhe Township, Enshi Tujia and Miao Autonomous Prefecture, Hubei Province. This isolate was identified according to the ITS sequence (GenBank Accession No. KX058063.1, query cover 100%, maximum identity 99%). Subsequently it was deposited in the Research Group of Medicinal Fungi and Ethnic Medicine, School of Pharmacy, South Central Minzu University.

### 2. Previous Studies

Endophytic fungi, commonly residing within healthy plant tissues, not only refrain from inducing diseases in their host plants but also engage in a symbiotic relationship that is mutually advantageous [1]. These fungi are integral to the global ecosystem, significantly contributing to the advancement of plant and animal life, as well as other microorganisms, fostering co-evolution and enhancing biodiversity [2]. Primarily existing in a symbiotic state, plant endophytic fungi adapt to their distinctive environments by synthesizing a diverse array of secondary metabolites characterized by novel structures and potent biological activities [3]. Consequently, research into plant endophytic fungi and their secondary metabolites holds substantial importance [4].

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*Fusarium* is one of the most important species of fungi, which exists in soil and plant tissues through parasitism and saprophytosis etc. *Fusarium* sp. are rich in variety of morphology and widely distributed, which has high research value. The secondary metabolites of *Fusarium* such as deoxynivalenol (DON) and zearalenone (ZEN) are the most important mycotoxins in the world [5-6]. To date, a variety of components, including terpenoids [7], alkaloids [7], pyranone [8] and naphthoquinone dimers [9], have been isolated from *Fusarium* sp. As part of our ongoing efforts to discover bioactive metabolites derived from endophytic fungi, a chemical investigation on the cultural broth of *F.tricinctum* was carried out.

### 3. Present Study

This study reports the isolation, structural elucidation, and biological activities of the isolates were reported. As a result, a new polyketone, fusacintone A (**1**), with four known compounds (**2-5**) were obtained (Figure 1). Herein, we report the isolation, structural elucidation and biological activities of the isolates.

The fermentation broth was extracted five times with 2-fold volume of EtOAc, and concentrated under reduced pressure to obtain 54 g of crude extract. The EtOAc extract was treated with 200-300 mesh normal-phase silica gel and eluted with a gradient of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (100:0-0:100, v/v) to obtain six fractions (A-F).

Fraction B (12.0 g) was subjected to C18 MPLC using MeOH-H<sub>2</sub>O (20:80-100:0, v/v), yielding nine subfractions (B-1~B-9). Fraction B-3 (180 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtOAc (10:1-0:1, v/v), resulting in six fractions (B-3-1~B-3-6). Fraction B-3-1 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN-H<sub>2</sub>O (57:43-62:38, v/v) to obtain **1** (7.9 mg, *t<sub>R</sub>* = 15.3 min). Fraction B-3-6 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN-H<sub>2</sub>O (65:35-70:30, v/v) to obtain **2** (4.1 mg, *t<sub>R</sub>* = 18.4 min). Fraction B-5 (79 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtOAc (10:1-0:1, v/v), resulting in five fractions (B-5-1~B-5-5). Fraction B-5-2 was isolated and purified by semipreparative C18 HPLC with a gradient of MeOH-H<sub>2</sub>O (68:32-73:27, v/v) to obtain **5** (3.9 mg, *t<sub>R</sub>* = 20.5 min).

Fraction C (11 g) was subjected to C18 MPLC using MeOH-H<sub>2</sub>O (20:80-100:0, v/v), yielding seven subfractions (C-1~C-7). Fraction C-7 (121 mg) by normal-phase silica gel column chromatography in a continuous gradient of petroleum ether-EtOAc (10:1-0:1, v/v), resulting in seven fractions (C-7-1~C-7-7). Fraction C-7-2 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN-H<sub>2</sub>O (68:32-73:27, v/v), to obtain **4** (5.4 mg, *t<sub>R</sub>* = 19.3 min). Fraction C-2-1 was isolated and purified by semipreparative C18 HPLC with a gradient of MeCN-H<sub>2</sub>O (63:37-62:38, v/v), to obtain **3** (5.2 mg, *t<sub>R</sub>* = 16.9 min).

**Equipment:** The HPLC model is an Agilent 1260 liquid chromatograph with a DAD detector and a Zorbax SB-C18 preparative column (4.6 mm×150 mm, 5 μm). The model of medium-pressure liquid chromatography was Biotage SP1 (Biotage, Sweden) with an RP-18 column (Fuji Silysia Chemical Ltd., Japan). Column chromatography was conducted using silica gel (80-100 mesh and 200-300 mesh, Qingdao Marine Chemical Factory, China), Sephadex LH-20 (Pharmacia Fine Chemical Co, Sweden). Thin-layer chromatography (TLC) was performed on GF254 plates (Qingdao Marine Chemical Factory, China). 1D and 2D spectra were produced by a Bruker spectrometer (Bruker, Germany, model AM600). HR-ESI-MS data were collected by A Q Exactive HF (Thermo Fisher Scientific, USA).

**Fusacintone A (1):** yellow powder; UV (MeOH): 230 (3.49); HR-ESI-MS (pos.): 233.04445 [M + H]<sup>+</sup> (calcd. for C<sub>12</sub>H<sub>9</sub>O<sub>5</sub><sup>+</sup>, 233.04443); <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>): δ (ppm) = 6.89 (1H, s, H-4), 6.21 (1H, s, H-8), 5.30 (3H, s, H-12), 2.45 (2H, s, H-10); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>): δ (ppm) = 161.6 (C, C-1), 107.1 (C, C-2), 153.5 (C, C-3), 100.5 (CH, C-4), 160.5 (C, C-5), 110.2 (C, C-6), 183.3 (C, C-7), 110.4 (CH, C-8), 168.4 (C, C-9), 20.6 (CH<sub>3</sub>, C-10), 167.5 (C, C-11), 68.8 (CH<sub>2</sub>, C-12).

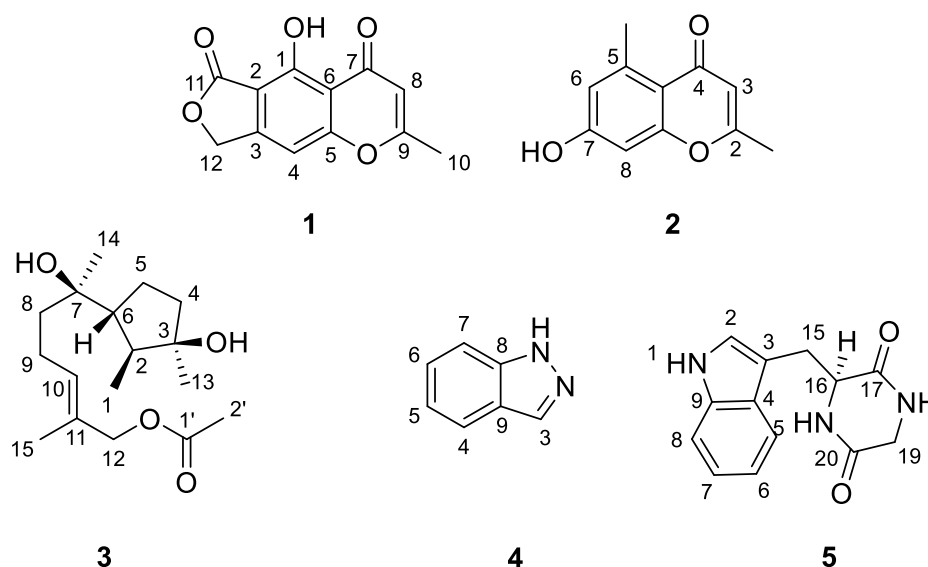
## Fusacintone A, a new polyketide

*2,5-Dimethyl-7-hydroxychromone (2)*: white amorphous powder;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 2.32 (3H, d,  $J = 0.7$  Hz, 2- $\text{CH}_3$ ), 2.71 (3H, s, 5- $\text{CH}_3$ ), 6.00 (1H, d,  $J = 0.8$  Hz, H-3), 6.62 (1H, dd,  $J = 1.0$  Hz, H-6), 6.64 (1H, d,  $J = 2.4$  Hz, H-8);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 182.1 (C, C-4), 166.7 (CH, C-2), 163.2 (C, C-7), 161.5 (C, C-8a), 143.7 (C, C-5), 118.0 (CH, C-6), 115.6 (C, C-4a), 111.4 (CH, C-3), 101.7 (CH, C-8), 23.2 (5- $\text{CH}_3$ ), 19.9 (C-2- $\text{CH}_3$ ). The above data is consistent with literature data [10].

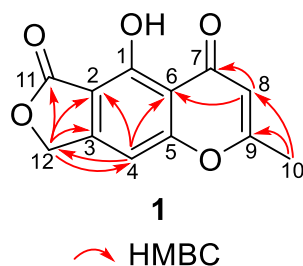
*(10Z)-12-Acetoxy-cyclonerodiol (3)*: Yellow oil;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 1.01 (3H, d,  $J = 6.9$  Hz, H-1), 1.63 (1H, m, H-2), 1.73 (1H, m, H-4a), 1.58 (2H, m, H-4b/5b), 1.83 (2H, m, H-5a/6), 1.55 (2H, m, H-8), 2.14 (2H, m, H-9), 5.44 (1H, m, H-10), 4.61 (2H, d,  $J = 7.9$  Hz, H-12), 1.29 (3H, s, H-13), 1.22 (3H, s, H-14), 1.69 (3H, s, H-15), 2.03 (3H, s, H-2');  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 173.4 (C, C-1'), 132.5 (C, C-11), 131.6 (CH, C-10), 82.6 (C, C-3), 76.00 (C, C-7), 64.6 ( $\text{CH}_2$ , C-12), 56.1 (CH, C-6), 46.0 (CH, C-2), 42.5 ( $\text{CH}_2$ , C-4), 41.9 ( $\text{CH}_2$ , C-8), 26.6 ( $\text{CH}_3$ , C-13), 25.7 ( $\text{CH}_2$ , C-5), 25.1 ( $\text{CH}_3$ , C-14), 24.0 ( $\text{CH}_2$ , C-9), 22.1 ( $\text{CH}_3$ , C-15), 21.3 ( $\text{CH}_3$ , C-2'), 15.9 ( $\text{CH}_3$ , C-1). The above data is consistent with literature data [11].

*Indazole (4)*: white amorphous powder;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 8.13 (1H, d,  $J = 7.7$  Hz, H-4), 7.89 (1H, s, H-3), 7.41 (1H, dd,  $J = 7.4, 1.5$  Hz, H-7), 7.17 (1H, m, H-6), 7.15 (1H, m, H-5);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 138.2 (C, C-8), 132.5 (CH, C-3), 127.9 (CH, C-6), 123.2 (C, C-9), 122.3 (CH, C-4), 121.9 (CH, C-5), 112.7 (CH, C-7). The above data is consistent with literature data [12].

*Cyclo(glycyltryptophyl) (5)*: white amorphous powder;  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 7.60 (1H, m, H-8), 7.32 (1H, d,  $J = 8.1$  Hz, H-5), 7.10 (1H, dd,  $J = 8.2, 1.2$  Hz, H-7), 7.05 (1H, s, H-2), 7.01 (1H, s, H-6), 4.20 (1H, t,  $J = 4.2$  Hz, H-19b), 3.47 (dd,  $J = 14.7, 3.9$  Hz, H-19a), 3.14 (1H, dd,  $J = 14.7, 4.5$  Hz, H-15a), 2.50 (1H, dd,  $J = 17.6, 1.1$  Hz, H-15b);  $^{13}\text{C}$  NMR (150 MHz,  $\text{CD}_3\text{OD}$ ):  $\delta$  (ppm) = 171.1 (C, C-17), 168.9 (C, C-20), 137.9 (C, C-4), 128.7 (C, C-9), 126.1 (CH, C-2), 122.6 (CH, C-6), 120.2 (CH, C-8), 119.7 (CH, C-7), 112.2 (CH, C-5), 109.0 (C, C-3), 57.4 (CH, C-16), 44.8 ( $\text{CH}_2$ , C-19), 31.1 ( $\text{CH}_2$ , C-15). The above data is consistent with literature data [13].



**Figure 1.** The chemical structures of compounds 1–5



**Figure 2.** Key HMBC correlations of compound **1**

Fusacintone A (**1**) was obtained as a yellow powder. The molecular formula  $C_{12}H_8O_5$  was indicated by the HR-ESI-MS at  $m/z$  233.04443  $[M + H]^+$  (calcd for  $C_{12}H_8O_5H^+$  233.04445), indicating a molecular unsaturation index of nine. The  $^1H$  NMR spectrum of this compound shows one methyl proton [ $\delta_H$  2.45 (s)], two olefinic protons [ $\delta_H$  6.21 (s), 6.89 (s)], and two oxygenated methylene protons [ $\delta_H$  5.30 (s)].  $^{13}C$  NMR and DEPT data revealed twelve carbons, which included one methyl ( $\delta_C$  20.6), one methylene ( $\delta_C$  68.8), two olefinic carbons ( $\delta_C$  100.5, 110.4), eight quaternary carbons ( $\delta_C$  107.1, 110.2, 153.5, 160.5, 161.6, 168.4, 167.5, 183.8). Above-mentioned NMR pattern of **1** was similar to those of the known synthetic compound 5-hydroxy-2-methylchromone-6,7-di-carboxylic anhydride [14]. The major difference was that the C-12 ester group in reported compound was replaced by a methylene group at the same position in **1**. This presumption was confirmed by HMBC correlations from H-12 to C-2, C-4 and C-11, and H-4 to C-12. Furthermore, the existence of intermediate aromatic ring linked to the pyranone ring was established through HMBC correlations from H-4 to C-2, C-6 and C-5, as well as H-8 to C-6 and C-7. The HMBC correlations of H-10 with C-8 and C-9, and of H-8 to C-10 confirmed a methyl group was linked at C-9 (Figure 2).

In vitro anti-inflammatory activity: [15] NO production was assessed indirectly by analyzing the supernatant. RAW264.7 cells were plated in 96-well plates and incubated for 24 hours. Afterward, the medium was replaced, and the cells were exposed to sample solutions at concentrations of 1, 5, 10, 20, 30, and 40  $\mu M$  for 1 hour. The  $IC_{50}$  values of the tested compounds were calculated by harvesting the supernatant following a 12-hour incubation period with LPS, which was utilized to quantify NO production. The absorption at 540 nm was measured by a microplate reader at room temperature. The  $IC_{50}$  values were calculated by GraphPad Prism 6 software.

$$\text{NO Relative Expression Level (\%)} = \frac{(\text{Experimental group} - \text{Control group})}{(\text{LPS-treated group} - \text{Control group})} \times 100 \%$$

Compound **1** was assessed for its anti-inflammatory properties by inhibiting NO release in LPS-activated RAW264.7 cells. As a result, compound **1** showed inhibitory activity against NO production with an  $IC_{50}$  value of  $17.6 \pm 0.46 \mu M$ .

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## Supporting Information

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

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