SHORT *REPORT*

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Isopimarane diterpenoids and pyranones from the endophytic fungi *Xylaria curta* E10

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Abstract: Chemical studies on ethyl acetate extract of solid-state fermentation of *Xylaria curta* E10 resulted in isolation of two undescribed isopimarane diterpenoids (**1** and **2**) and two known pyranones (**3** and **4**). The structures of compounds **1** and **2** were established by spectral data analysis and quantum chemical calculations. The cytotoxic and anti-inflammatory activity of compounds **1** and **2** were evaluated.

Keywords: *Xylaria curta* E10; isopimarane diterpenoids; pyranones.

1. Fungi Source

Xylaria curta E10, an endophytic fungus, was isolated from the healthy potato plants collected in Lincang City, Yunnan Province, China. This isolate was identified according to the ITS sequence (GenBank Accession No. KJ883611.1, 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

Xylaria, a highly diverse genus within the family *Ascomycota*, plays significant ecological roles across various ecosystems [1]. Since 1978, over 300 *Xylaria* species have been identified globally, predominantly inhabiting temperate, tropical and subtropical regions [2]. Extensive research has revealed the genus' remarkable capacity to produce structurally diverse and biologically active secondary metabolites. Notable examples include sesquiterpenes exhibiting anti-inflammatory properties [3], anti-*Plasmodium falciparum* sesquiterpene lactones [4], antimicrobial pyrantel derivatives effective against *Escherichia* coli and *Staphylococcus aureus* [5], and tricyclic polyketides demonstrating antitumor

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potential [6]. Additionally, other compound classes such as diterpenes [7], ergosterols [8], anthraquinones [9], and alkaloids [10] have been characterized from this genus.

Building upon our research group's prior work isolating bioactive secondary metabolites from *Xylaria curta* E10 [11-13], we further investigated this strain's biosynthetic potential through the one strain many compounds (OSMAC) strategy. By employing solid-state fermentation, we aimed to induce novel secondary metabolite production. Here, we report the isolation, structural elucidation, and bioactivity assessment of two previously undescribed isopimarane diterpenoids and two known pyranones.

3. Present Study

In this study, four compounds including two new isopimarane diterpenoids (**1** and **2**) as well as two known pyranones (**3** and **4**) (Figure 1) were isolated from rice solid-state fermentation of *Xylaria curta* E10. This study reports the isolation, structural elucidation, and biological activities of the isolates were reported.

Xylaria curta E10 was inoculated into PDA agar medium and incubated at 25 ℃ for 3-5 days. Then the medium was cut into several small pieces, access to solid rice medium (50 g rice and 50 ml water into 500 ml culture bottles, 120 ℃ autoclaved for 30 minutes, a total of 200 bottles) in the dark culture at 25 ℃ fermentation 30 days. The rice medium was mashed, extracted five times with 2-fold volume of EtOAc, and concentrated under reduced pressure to obtain 220 g of crude extract. The EtOAc extract was treated with 80-100 mesh normal-phase silica gel and eluted with a gradient of CH_2Cl_2-MeOH (100:0-0:100, v/v) to obtain five fractions (A-E).

Fraction D (30.6 g) was subjected to C_{18} MPLC using MeOH–H_2O (10:90 – 100:0, v/v) yielding nine subfractions (D-1 – D-9). Subsequent petroleum ether-acetone (30:1-0:1, v/v), which was used as a normal-phase column eluent, divided fraction D-3 (8.8 g) into ten subfractions (D-3-1 – D-3-10). Then, the choice was made to continue the separation of D-3-3 (3.2 g) by normal phase silica gel column chromatography with the gradient of petroleum ether-EtOAc (20:1 - 0:1, v/v), which resulted in the separation of eight additional fractions (D-3-3-1 – D-3-3-8). Fraction D-3-3-3 (70.3 mg), Which was purified consecutively with preparative HPLC with Agilent C_{18} column to afford compound **1** (1.2 mg, t_R = 10.8 min) and compound **2** (3.5 mg, t_R = 13.6 min).Fraction D-3-3-4 (83.4 mg), Which was purified consecutively with preparative HPLC with Agilent C_{18} column to afford compound **3** (1.6 mg, t_R = 14.5 min) and compound **4** (2.0 mg, t_R = 18.8 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). The optical circular dichroism (OCD) was measured by a Horiba SEPA-300 polarimeter, and the circular dichroism spectrum was recorded through a Chirascan circular dichroism spectrometer (USA, New Haven).

Curtaterpene A (**1**): white amorphous powder; mp. 180–183 ℃; UV (MeOH) λ_{max} (log ε) = 235 (3.36) nm; $[\alpha]_D^{18}$ -28 (*c* 0.4, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 6.05 (1H, dd, *J* = 9.9, 2.2 Hz, H-6), 5.95 (1H, dd, *J* = 10.2, 3.0 Hz, H-7), 5.32 (1H, s, H-14), 3.70 (2H, t, H-16, *J* = 7.3 Hz), 2.21 (1H, d, H-1a, *J* = 13.5 Hz), 2.15 (1H, s, H-5), 1.94 (1H, m, H-9), 1.84 (1H, dt, *J* = 13.9, 3.7 Hz, H-2b), 1.74 (1H, d, *J* = 7.3 Hz, H-1b), 1.63 (1H, m, H-11a), 1.60 (2H, m, H-15), 1.53 (1H, m, H-2a), 1.48 (2H, d, *J* = 2.8 Hz, H-12), 1.36 (1H, m, H-11b), 1.31 (3H, s, H-18), 1.07 (2H, dd, *J* = 13.6, 4.8 Hz, H-3), 0.98 (3H, s, H-17), 0.62 (3H, s, H-20); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 37.5 (CH_2, C-1), 19.6

(CH_2, C-2), 37.5 (CH_2, C-3), 43.4 (C, C-4), 55.4 (CH, C-5), 127.7 (CH, C-6), 128.2 (CH, C-7), 134.9 (C, C-8), 49.2 (CH, C-9), 37.7 (C, C-10), 19.0 (CH_2, C-11), 34.0 (CH_2, C-12), 34.4 (C, C-13), 134.2 (CH, C-14), 46.2 (CH_2, C-15), 60.0 (CH_2, C-16), 28.1 (CH_3, C-17), 28.3 (CH_3, C-18), 182.1 (C, C-19), 11.9 (CH_3, C-20); HRESIMS: *m/z* 319.22675 $[M + H]^+$ (calcd for $C_{20}H_{31}O_3$, 319.22677).

Curtaterpene B (**2**): white amorphous powder; mp. 185–188 ℃; UV (MeOH) λ_{max} (log ε) = 240 (2.83); $[\alpha]_D^{18}$ -19 (*c* 0.4, MeOH); ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 1.94 (1H, m, H-1b), 1.36 (1H, m, H-1a), 2.01 (1H, m, H-2a), 1.54 (1H, m, H-2b), 2.14 (1H, m, H-3a), 1.04 (1H, m, H-3b), 1.47 (1H, m, H-5), 2.72 (1H, m, H-6b), 2.33 (1H, m, H-6a), 5.48 (1H, m, H-7), 5.44 (1H, m, H-11), 2.04 (1H, m, H-12a), 1.90 (1H, m, H-12b), 4.03 (2H, m, H-14), 1.54 (1H, m, H-15a), 1.46 (1H, m, H-15b), 3.61 (2H, m, H-16), 0.90 (3H, s, H-17), 1.19 (3H, s, H-18), 0.90 (3H, s, H-20); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 38.8 (CH_2, C-1), 21.1 (CH_2, C-2), 39.4 (CH_2, C-3), 45.0 (C, C-4), 51.7 (CH, C-5), 25.9 (CH_2, C-6), 124.5 (CH, C-7), 139.1 (C, C-8), 146.7 (C, C-9), 38.4 (C, C-10), 118.0 (CH, C-11), 40.4 (CH_2, C-12), 32.6 (C, C-13), 45.2 (CH_2, C-14), 42.5 (CH_2, C-15), 59.5 (CH_2, C-16), 26.6 (CH_3, C-17), 29.4 (CH_3, C-18), 181.5 (C, C-19), 20.8 (CH_3, C-20); HRESIMS: *m/z* 317.21222 $[M - H]^+$ (calcd for $C_{20}H_{29}O_3$, 317.21338).

4-methoxy-6-pentanoyl-2H-pyran-2-one (**3**): Yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 6.76 (1H, d, *J* = 2.4 Hz, H-5), 5.70 (1H, d, *J* = 2.4 Hz, H-3), 3.85 (3H, s, H-12), 2.89 (2H, t, *J* = 7.4 Hz, H-8), 1.64 (2H, m, H-9), 1.37 (2H, m, H-10), 0.93 (3H, t, *J* = 7.3 Hz, H-11); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 194.1 (C, C-7), 170.0 (C, C-4), 162.6 (C, C-2), 154.8 (C, C-6), 103.9 (CH, C-5), 93.6 (CH, C-3), 56.5 (CH_3, C-12), 38.1 (CH_2, C-8), 25.3 (CH_2, C-9), 22.3 (CH_2, C-10), 14.0 (CH_3, C-11).The above data was consistent with literature data [14].

6-pentyl-4- methoxy-pyran-2-one (**4**): Yellow oil; ^{1}H NMR (600 MHz, CD_3OD): δ (ppm) = 5.99 (1H, d, *J* = 2.2 Hz, H-5), 5.51 (1H, d, *J* = 2.2 Hz, H-3), 3.83 (3H, s, H-12), 2.47 (2H, t, *J* = 7.6 Hz, H-7), 1.63 (2H, m, H-8), 1.33 (4H, m, H-9, 10), 0.90 (3H, t, *J* = 7.0 Hz, H-11); ^{13}C NMR (150 MHz, CD_3OD): δ (ppm) = 174.0 (C, C-4), 167.8 (C, C-2), 167.5 (C, C-6), 101.3 (CH, C-5), 88.1 (CH, C-3), 56.9 (CH_3, C-12), 34.3 (CH_2, C-7), 32.2 (CH_2, C-9), 27.6 (CH_2, C-8), 23.4 (CH_2, C-10), 14.3 (CH_3, C-11).The above data is consistent with literature data [15].

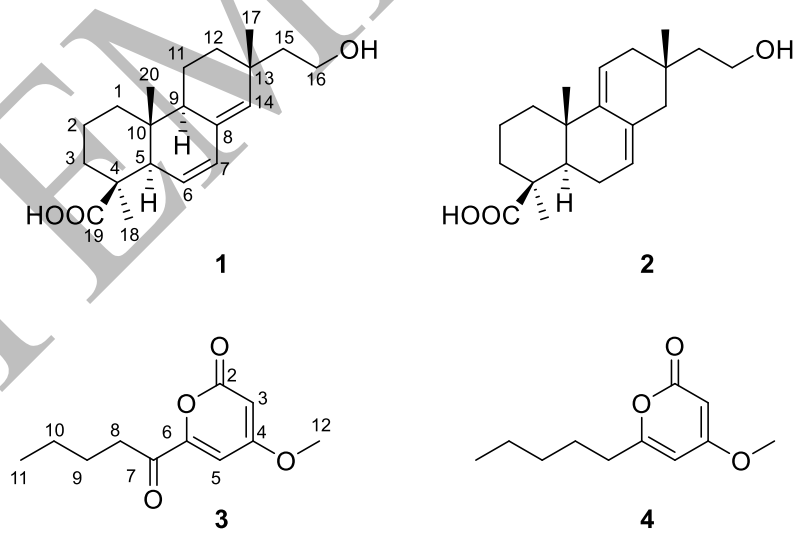

Figure 1. The chemical structures of compounds **1**–**4**.

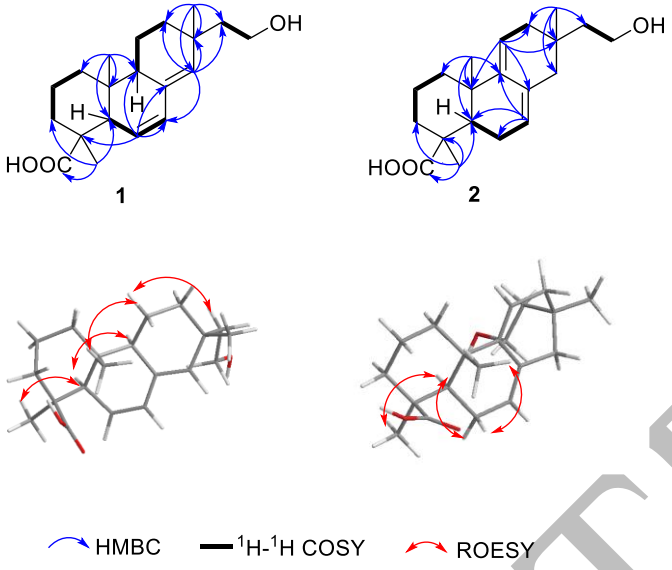

Figure 2. Key HMBC, ^{1}H-^{1}H COSY and ROESY correlations of compounds **1**–**2**.

Curtaterpene A (**1**) was obtained as a white amorphous powder. The molecular formula was determined to be $C_{20}H_{30}O_3$ by the positive HR-ESI-MS (*m/z* 319.22675 $[M + H]^+$, calculated for $C_{20}H_{31}O_3$, 319.22677), indicating a molecular unsaturation index of six. By comparing the 1D NMR spectroscopic data, the data of compound **1** was similar to that of hymatoxin D [16], a known isopinacrine diterpene compound, suggesting that the parental skeleton of compound **1** was consistent with that of hymatoxin D. Further analysis of HMBC and ^{1}H-^{1}H COSY correlation data determined the planar structure of compound **1**. To begin with, according to the ^{1}H-^{1}H COSY correlation between H_2-15/H_2-16, H_2-12/H_2-11/H-9 and H_2-1/H_2-2/H_2-3, the linkage shown in the figure 2 was identified. Then the coupling interactions of H_3-18 with C-3/C-4/C-5/C-19 and H_3-20 with C-1/C-5/C-9/C-10 in the HMBC spectrum were observed, confirming the presence of two six-membered rings. Furthermore, the existence of the C ring was established through HMBC correlations between H_3-17 and C-12/C-13/C-14/C-15. Meanwhile, ROESY spectrum showed the correlation of H_3-20/H-11β/H3-17/H-2β and H_3-18/H-5/H-9 as well as the non-correlation of H_3-20/H-9, which determined the relative configuration of the compound. Finally, the absolute configuration of the compound was determined through ECD calculation (Fig 3).

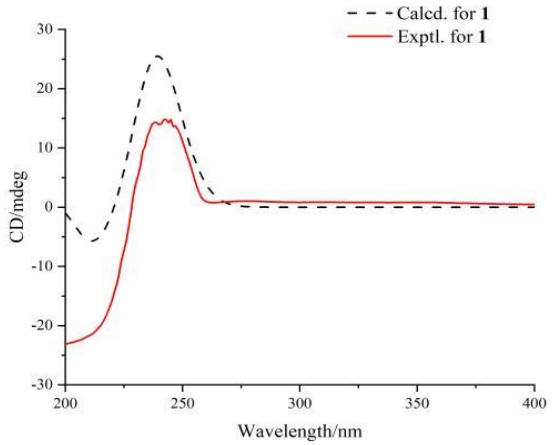

Figure 3. Experimental and calculated ECD curves of compounds **1.**

Curtaterpene B (**2**), a white amorphous powder, presented the HR-ESI-MS $[M - H]^-$ ion at m/z 317.21222 (calculated for $C_{20}H_{29}O_3$, 317.21338) in the HRESI data, indicating a molecular unsaturation index of six. ^{13}C NMR and DEPT revealed 20 carbons, which included three methyl

signals [δ_C 20.8 (C-20), 26.6 (C-17), 28.4 (C-18), eight methylene signals [δ_C 21.1 (C-2), 25.9 (C-6), 38.8 (C-1), 39.4 (C-3), 40.0 (C-12), 42.5 (C-14), 45.2 (C-14), 59.5 (C-16), one hypomethyl signal [δ_C 51.7 (C-5), three quaternary carbon signals [δ_C 32.6 (C-13), 38.4 (C-10), 40.5 (C-4), four olefinic carbon signals [δ_C 118.0 (C-11), 124.5 (C-7), 131.9 (C-8), 146.7 (C-9), and one carbonyl carbon signal [δ_C 181.5 (C-19)]. The ^{1}D NMR spectroscopic data of **2** were similar to those of compound **1** except for the position of the double bonds. Regarding the identification of double bond positions, firstly, HMBC spectrum showed the correlation of H_3-20 with C-1/C-5/C-9/C-10, H_3-17 with C-12/C-13/C-14/C-15 and H_2-12 with C-5/C-10/C-7/C-8. Secondly, the ^{1}H-^{1}H COSY spectrum showed H-11/H_2-12 correlation, determining two double bonds of **2** at C-7/C-8 and C-9/C-11. ROESY spectrum showed the correlations of H_3-20/H_2-6β/, H_2-6β/H_2-1β and H_2-6α/H_3-18/, H_3-18/H-5, which determined the relative configuration of compound **2**. Finally, the absolute configuration of the compound **2** was determined through ECD calculation (Fig 4).

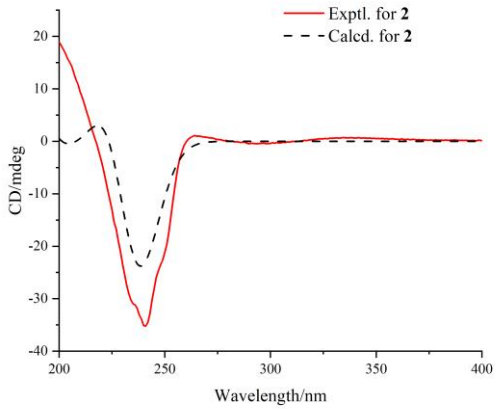

Figure 4. Experimental and calculated ECD curves of compounds **2.**

In the activity testing section, curtaterpenes A (**1**) and B (**2**) were tested for cytotoxic activity and anti-inflammatory activity. Both the compounds did not show significant activity in cytotoxicity and anti-inflammatory tests at a concentration of 40 μM.

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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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