A new furan derivative from an endophytic *Aspergillus flavus* of *Cephalotaxus fortunei*

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A new furan derivative named 5-acetoxymethylfuran-3-carboxylic acid (2), together with a known furan compound, 5-hydroxymethylfuran-3-carboxylic acid (1), were isolated from the fermentation of *Aspergillus flavus*, endophytic fungi in *Cephalotaxus fortunei*. The structures of 1 and 2 were elucidated by NMR, IR, UV and MS data, as well as compared with literature data. The compounds 1 and 2 exhibited potent antibacterial activity against *Staphylococcus aureus* with MIC values of 31.3 and 15.6 μg/mL, respectively. The compound 2 showed moderate antioxidant activity.

**Keywords:** furan derivative; 5-acetoxymethylfuran-3-carboxylic acid; *Aspergillus flavus*; *Cephalotaxus fortunei*

1. Introduction

Endophytic fungi that colonise internal tissues of healthy plants represent one of the largest and relatively under-explored resources of biologically active small molecule natural products (Schulz et al. 2002; Strobel 2003; Zhang et al. 2006; Arnold 2007; Rodriguez et al. 2009). Medicinal plants are known to be a promising reservoir of bioactive natural products for drug discovery (Alonso-Castro et al. 2011; Chon 2012). There has been an increasing number of reports of biologically active metabolites from endophytic fungi of medicinal plants (Liu et al. 2011; Luo et al. 2013; Ortega et al. 2013). In the course of our ongoing effort to find valuable compounds from endophytic fungi *Aspergillus* sp. S19 of *Cephalotaxus fortunei* which is a traditional medicinal plant, a new furan derivative 5-acetoxymethylfuran-3-carboxylic acid (2) together with a known compound (1) were found. This article described the isolation and structural elucidation as well as antimicrobial and antioxidant activities evaluation of two compounds.

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2. Results and discussion

2.1. Fungus identification

The strain of S19 was identified as Aspergillus flavus (GenBank accession number KJ473711), based on the internal transcribed spacer (ITS) sequence.

2.2. Structure elucidation

Compound 1 (Figure 1) was obtained as white crystal; m.p. 145.0–147.0°C; IR (KBr) \( \nu_{\text{max}} \) 3266, 3174, 1659, 1610, 1581 and 1283 cm\(^{-1}\). The \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \( \delta_H \): 9.06 (1H, s, –COOH), 8.02 (1H, s, H-2), 6.35 (1H, s, H-4), 5.67 (1H, s, –OH), 4.30 (2H, s, H-7); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \( \delta_C \): 173.87 (C-6), 168.05 (C-5), 145.67 (C-3), 139.23 (C-2), 109.76 (C-4) and 59.38 (C-7). According to literature data, compound 1 was confirmed to be 5-hydroxymethylfuran-3-carboxylic acid (1) (Evidente et al. 2009).

Compound 2 (Figure 1) was obtained as a white crystal. m.p. 123.5–125.5°C. Its molecular formula C\(_8\)H\(_8\)O\(_5\) was determined from the [M – H]\(^-\) at \( m/z \) 183.0302 (calcd. for C\(_8\)H\(_7\)O\(_5\), 183.0293) in HRESI-MS spectrum. The UV spectrum of 2 indicated absorption at 250 and 299 nm. The IR spectrum showed that the structure of 2 contained hydroxyl (3257 cm\(^{-1}\)), carbonyl (1736, 1689 cm\(^{-1}\)), C=O (1630, 1659 cm\(^{-1}\)) and C-O (1254, 1234 cm\(^{-1}\)) functional groups. The IR spectrum of 2 showed extra ester carbonyl group absorption bands at 1736 cm\(^{-1}\) compared with the IR spectrum of 1.

The NMR spectral (Table 1) data of compound 2 were similar to those of compound 1. The differences were in the \(^1\)H NMR of compound 2 in contrast to those of compound 1 disappeared a hydroxyl hydrogen signal at \( \delta_H \) 5.70 and appeared a methyl singlet at \( \delta_H \) 2.11. Meanwhile, the \(^{13}\)C NMR spectrum displayed the addition of one methyl signal (\( \delta_C \) 169.54) and one methyl signal (\( \delta_C \) 20.73) than 1. Furthermore, the methyl group was considered to be bound to carboxyl carbon by HMBC correlation between the methyl proton signal (\( \delta_H \) 2.11) and carboxyl (\( \delta_C \) 169.54). The above evidence suggested that it was an acetyl group. Moreover, the acetyl group was bound to alcoholic hydroxyl group by the HMBC correlation from the \( \delta_H \) 4.94 to \( \delta_C \) 169.54. According to the IR and NMR spectra, compound 2 was identified as acylation of the OH at C-7 in compound 1. Thus, compound 2 was unambiguously confirmed to be 5-acetoxymethylfuran-3-carboxylic acid. The main correlation in the HMBC of compound 2 was indicated in Figure 2. The model of conformation of compounds 1 and 2 is displayed in Figure 3.

2.3. Biological Activities

The screening data of the antibacterial activity showed that compounds 1 and 2 exhibited potent \textit{in vitro} antibacterial activity, especially against Staphylococcus aureus with MIC 31.3 and 15.6 \( \mu \)g/mL, respectively. The compounds exhibited moderate antifungal activity. These results compared with known antibiotics as standards are shown in Table 2. In addition, 5-acetoxymethylfuran-3-carboxylic acid (2) showed moderate activity for the scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals with an IC\(_{50}\) value of 237 \( \mu \)g/mL in Table 2. The antioxidant activity of the compound 1 displayed with IC\(_{50}\) value of 435 \( \mu \)g/ml.

Figure 1. The structures of compounds 1 and 2.
Table 1. $^1\text{H}$ and $^{13}\text{C}$ NMR data of compound 2 (DMSO-$d_6$).

| Position | $\delta_C$ | $\delta_H$ | HMBC               |
|----------|-----------|-----------|--------------------|
| 2        | 140.06    | 8.10 (1H, s) | C-6, C-4, C-5, C-3 |
| 3        | 145.69    |           |                    |
| 4        | 112.47    | 6.54 (1H, s) | C-7, C-3, C-5      |
| 5        | 161.72    |           |                    |
| −COOH    | 173.62    | 9.29 (1H, s) | C-6, C-2           |
| 7        | 61.41     | 4.94 (2H, s) | C-4, C-5, C-8      |
| 8        | 169.54    |           |                    |
| −CH$_3$  | 20.73     | 2.11 (3H, s) | C-8                |

Note: Values in $\delta$ (ppm).

3. Experimental

3.1. General

Melting points were determined on an X-6 micro-melting point apparatus and were uncorrected. HRESI-MS was obtained in the negative ion mode with Bruker maXis 4G UHR-TOF. UV spectra were obtained on a DR 5000. IR spectra were recorded with a Bruker VECTOR-22 FT-IR Spectrometer. 1D and 2D NMR spectra were recorded on Bruker AVANCE III-400 MHz spectrometer with tetramethylsilane as internal standard. Deuterated dimethylsulphoxide was purchased from Beijing Boya Dabei Technological Development (Beijing, China). All the solvents were purchased from Tianjin Hongyan Chemical Reagents Factory (Tianjin, China). The silica gel for column chromatography (200–300 mesh) was purchased from Tsingtao Marine Chemical Factory (Tsingtao, Shandong Province, China). The bacteria ($S$. aureus, Streptococcus lactis, Escherichia coli and Pseudomonas aeruginosa) and the fungi (Botrytis cinerea, Valsa mali, Alternaria alternata and Alternaria brassicae) were provided from the College of Life Science & Engineering, Shaanxi University of Science & Technology (Xi’an, China).

3.2. Material

*C. fortunei* was collected from Taibai Mountains, Shaanxi Province, China, on July 2011. The endopytic fungus (No. S19) isolated from the stem of *C. fortunei* was deposited with the culture collection of the Laboratory of Natural Product Research, College of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology. It was identified according to a molecular biological protocol by DNA amplification and sequencing of the ITS region.

3.3. Solid-state fermentation

S19 was grown on potato dextrose agar at 28°C for 4 days. Five or six pieces (diameter 0.6 cm) of mycelial agar plugs were inoculated into Erlenmeyer flasks (10 × 1000 mL) containing 600 mL Czapek’s medium (sucrose 30 g/L, KH$_2$PO$_4$ 1.0 g/L, MgSO$_4$·7H$_2$O 0.5 g/L, NaNO$_3$ 3.0 g/L, KCl 0.5 g/L and FeSO$_4$ 0.01 g/L). The flasks were incubated for 10 days at 28°C on a rotary shaker.
(120 r/min) to obtain the fungus seed. Then, the seed liquid was added to 350 flasks (500 mL) each containing sterile culture medium consisted of 50 g rice and 60 mL of Czapek’s medium without sugar. Then, the flasks were carried out statically at room temperature for 30 d (Lagemaat & Pyle 2011).

3.4. Extraction and isolation

The air-dried solid culture (3.7 kg) was extracted repeatedly with ethyl acetate (25 L x 3) at room temperature for 24 h. The extract afford a dark brown residue (230 g), which was subjected to silica gel column chromatography eluting gradually with petroleum ether/ethyl acetate gradients (1:0, 1:1, 0:1, V:V) to give three fractions (A–C). The fraction C (125 g) was separated by silica gel column chromatography to yield pure compound 1 (120 g). Fraction B (80 g) was separated by silica gel column chromatography with a gradient eluent of ethyl acetate in petroleum ether to give twelve subfractions (B1–12), B3 (5 g) was separated by repeated silica gel column chromatography and Sephadex LH-20 (methanol) to yield compound 2 (50 mg).

3.5. Antimicrobial assay

The antimicrobial activities were carried out by the continuous dilution method (Bharate et al. 2007; Zhang et al. 2008). Each compound was set at 500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.91, 1.95 and 0.98 μg/mL in DMSO, while the tested strains were incubated in the liquid mediums. For bacteria, the medium was made up of beef extract 3.0 g/L, peptone 10.0 g/L, NaCl 5.0 g/L, pH 7.0–8.0, the culture temperature was 37°C. For fungi, the potato–glucose medium was made up of percolate of 200 g potato under boiling for 30 min, glucose 20 g, and constant volume to be 1 L by water, the culture temperature was 28°C. The in vitro antimicrobial activities were tested against two Gram-positive (G +) bacteria (S. aureus and S. lactis), two Gram-negative (G –) bacteria (E. coli and P. aeruginosa) and four plant pathogenic fungi (B. cinerea, V. mali, A. alternata and A. brassicae). The activities were compared with the same concentrations of known antibiotics including penicillin sodium against G + bacteria, streptomycin sulphate against G- bacteria and ketoconazole against fungi.

3.6. Antioxidation assay

Free radical scavenging activities of compounds were measured by DPPH (Predes et al. 2011) in 96 wells microtitre plates. The activities of the compounds were compared with that of Vc. In brief, 100 μL different concentration solutions (a two-fold dilution from 1.95 μg/mL to 1000 μg/mL in methanol) were mixed with 100 μL of DPPH solution (0.2 mg/mL) and the miscible liquid incubated for 30 min at room temperature. The control was maintained by adding 100 μL of DPPH to 100 μL methanol. The absorbance (A) was measured at 492 nm in triplicate. The antioxidation activities were expressed as the half maximal inhibitory concentration (IC50).
Table 2. Antimicrobial and antioxidant activities of compounds 1 and 2.

|        | E. coli | P. aeruginosa | S. aureus | S. lactis | B. cinerea | V. mali | A. alternata | A. brassicae | DPPH scavenging ability |
|--------|---------|---------------|-----------|-----------|------------|---------|--------------|--------------|-------------------------|
| **1**  | 62.5    | 125           | 31.3      | 31.3      | 125        | 125     | 250          | 125          | 435                     |
| **2**  | 62.5    | 62.5          | 15.6      | 31.3      | 125        | 125     | 125          | 125          | 237                     |
| Streptomycin sulphate | 7.81    | 7.81          | –         | –         | –          | –       | –            | –            | –                       |
| Penicillin sodium     | –       | –             | 7.81      | 7.81      | –          | –       | –            | –            | –                       |
| Ketoconazole          | –       | –             | –         | 62.5      | 31.3       | 31.3    | 31.3         | –            | –                       |
| Vc                  | –       | –             | –         | –         | –          | –       | –            | 4.15         | –                       |

Note: –, the test has not been set.
4. Conclusion

In conclusion, in this study, a new furan derivative together with a known furan compound were isolated and elucidated from *A. flavus*, endophytic fungi in *C. fortunei*. Compounds 1 and 2 exhibited potent antibacterial activity against *S. aureus*. Compound 2 showed moderate antioxidant. Thus, the compound 2 is potential to be a antibacterial drug.

Supplementary material

Supplementary material relating to this article is available online.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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