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A new furan derivative from an endophytic *Aspergillus tubingensis* of *Decaisnea insignis* (Griff.) Hook.f. & Thomson

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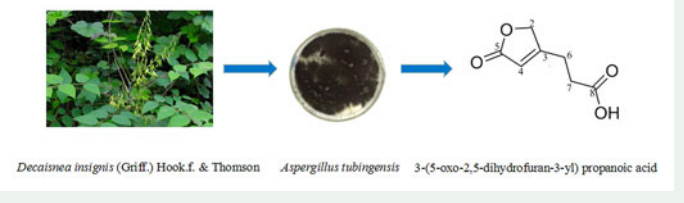
ABSTRACT

A new furan derivative named 3-(5-oxo-2,5-dihydrofuran-3-yl) propanoic acid (**1**) was isolated for the first time. Its structure was elucidated by UV, IR, NMR, HR-ESI-MS and the single-crystal X-ray diffraction spectroscopic data. Meanwhile, the antifungal and antibacterial activities of compound **1** was tested, it exhibited potent antifungal activity against *Fusarium graminearum* with MIC value of 16 μg/mL and medium antibacterial activity against *Streptococcus lactis* with MIC value of 32 μg/mL.



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1. Introduction

Endophytic fungi are considered as one of the most abundant sources of natural products (Xie et al. 2016). Therefore, separated bioactive substances from them secondary metabolites become relative easy (Jiang, 2015). In particular, *Aspergillus* sp. is more likely to produce metabolites with stronger biological activity (Deng et al. 2013; Xiao et al. 2014; Siriwardane et al. 2015). *Decaisnea insignis* (Griff.) Hook.f. & Thomson is widely distributed in the southwest and centure of China (Zhang et al. 2011). It can be used to treat cough, rheumatism and cancer (Bai et al. 2007). So, the *Aspergillus tubingensis* from *Decaisnea insignis* (Griff.) Hook.f. & Thomson is considered as a promising reservoir of biologically active natural products. Our group has conducted a preliminary study of the endophytic fungi of *Decaisnea insignis* (Griff.) Hook.f. & Thomson (Wang et al. 2013). In our going on researching for bioactive

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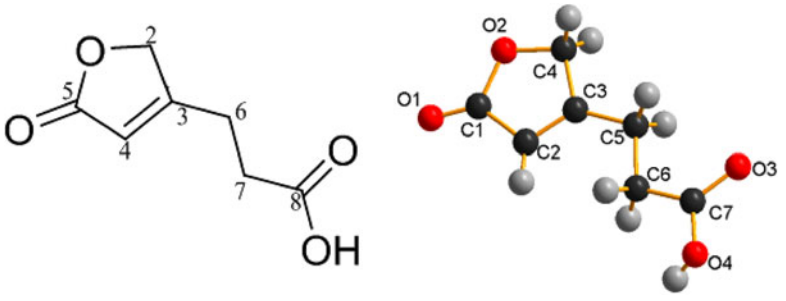

Figure 1. The structure of compound 1.

secondary metabolites from an endophytic *Aspergillus tubingensis* of *Decaisnea insignis* (Griff.) Hook.f. & Thomson, a new furanone (**1**) was obtained. This article described the isolation and structural elucidation as well as antimicrobial activities evaluation of compound **1**.

2. Results and discussion

2.1. Fungus identification

Based on the ITS sequence, the strain of DS37 was identified as *A. tubingensis* (Genbank accession No. MG495096).

2.2. Structure elucidation

Compound **1** was obtained as a colorless flake crystal, exhibited a deprotonated molecular ion peak at m/z 155.0352 $[M\text{-}H]^-$ (calcd. for $C_7H_7O_4$, 155.0344) in its HR-ESI-MS data (supplementary material Figure S2), suggesting the molecular formula $C_7H_8O_4$ (four degrees of unsaturation). The IR spectrum of **1** revealed characteristic absorption bands for a hydroxyl group at 3695 cm^{-1} and a carbonyl group at 1721 cm^{-1}. The UV absorption maximum at 225 nm indicating the presence of a α,β-unsaturated furanone. The ^{1}H-NMR spectrum (supplementary material Figure S5) of **1** showed signals for a hydroxyl proton at δ_H 12.33 (s, 1H) and an ethylene proton at δ_H 5.93 (s, 1H). The signal at δ_H 4.84 (s, 2H) showed the presence of a methylene group attached to the electron withdrawing group, the mutually coupling signals at δ_H 2.61-2.58 (t, 2H, $J = 4.6$ Hz), δ_H 2.57-2.54 (t, 2H, $J = 4.6$ Hz) were attested for two methylene groups. Analysis of the ^{13}C-NMR (including DEPT) (supplementary material Figures S6 and S7) data revealed 7 resonances for two carbonyl carbons (δ_C 173.82, 173.45), three methylene carbons (one O-bearing), one methine carbon and one quaternary carbons. In addition, the ^{1}H-NMR spectra revealed the presence of a methine group and three methylene at δ_H 5.93 (1H, s) and 4.84 (2H, s), 2.60 (2H, t), 2.57 (2H, t), the HSQC spectrum revealed that the C-atoms with signals at 114.08 and 73.11, 30.99, 23.45 were linked with the H-atoms mentioned above, respectively. As supported by HMBC (supplementary material Figure S11) from H-2 to C-3, C-4, and C-5 completed the α,β-unsaturated lactone moiety (Liu et al. 2013). The resonances at δ_H 2.61-2.58 (t, 2H, $J = 4.6$ Hz) and δ_H 2.57-2.54 (t, 2H, $J = 4.6$ Hz) were attributed to an ethylene group. Considering the chemical

Table 1. The preliminary antimicrobial activity result of compound **1**.

	MIC (μg/mL)								
	Bacteria Pathogenic fungi								
	A	B	C	D	E	F	G	H	I
1	64	64	64	32	16	32	64	128	64
Streptomycin sulphate	8	8	–	–	–	–	–	–	–
Penicillin	–	–	8	8	–	–	–	–	–
Carbendazim	–	–	–	–	32	32	32	64	32

Notes: A: E. coli, B: P. aeruginosa, C: S. aureus, D: S. lactis, E: F. graminearum, F: A. alternata, G: S. sclerotiorum, H: B. cinerea, I: P. capsici, –: No set experiment.

shifts of δ_H 2.60 (H-6) and δ_H 2.57 (H-7), they are almost at the diagonal of the COSY spectrum, so it is difficult to observe. Which was attached to C-3 of α,β-unsaturated lactone ring, as supported by NOESY (supplementary material Figure S9) from H-6 to C-2 and C-4, and HMBC (supplementary material Figure S11) correlations of H-6, H-7 with C-3. An oxygen-bearing methylene at δ_H 4.84 (2H, s, H-2) and a methine at δ_H 5. 93 (1H, s, H-4) were assigned at C-2 and C-4 of the α,β-unsaturated lactone ring, which was further confirmed by HMBC correlations of H-2 with C-3 and H-4 with C-3, C-5. The observed HMBC correlations of H-6 and H-7 to C-8 led to the connection of C-8 to C-7. Considering the chemical shift of C-8 (δ_C 173.45), the remaining hydroxyl group should be attached to C-8 to form carboxylic acid unit. It is worth mentioning that crystal of **1** was obtained from methyl alcohol. Consequently, the relative composition of **1** was determined by single-crystal X-ray diffraction (Figure 1). Therefore, the structure of **1** was determined as 3-(5-oxo-2,5-dihydrofuran-3-yl) propanoic acid.

2.3. Antimicrobial activities

The antimicrobial activity result (Table 1) indicated that compound **1** exhibited potent antifungal activity against *Fusarium graminearum* with MIC value of 16 μg/mL and medium antibacterial activity against *Streptococcus lactis* with MIC value of 32 μg/mL.

3. Experimental

3.1. General experimental procedures

IR spectra were recorded with a Bruker VECTOR-22FT-IR; UV spectra were recorded with a Varian Cary 60; MS spectra were obtained in the Bruker Impact HD Q-TOF; Single-crystal X-ray diffraction analyses spectra were recorded with a Bruker APEX-II CCD; 1D and 2D nuclear magnetic resonance (NMR) spectra were recorded on Bruker AVANCEIII-400 MHz spectrometer with tetramethylsilane (TMS) as internal standard; melting points were determined by using an X-6 micro-melting point apparatus were uncorrected. Deuterated dimethylsulfoxide was purchased from Beijing Boya Dabei Technological Development (China), the other 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: *Escherichia coli* (ATCC 11775), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 43300),

Streptococcus lactis (BNCC 336474) and the fungi: *Fusarium graminearum* (BNCC 337560), *Alternaria alternata* (BNCC 341716), *Sclerotinia sclerotiorum* (BNCC 122299), *Botrytis cinerea* (BNCC 338228), *Phytophthora capsici* (BNCC 339721) were provided from the School of Food and Biological Engineering, Shaanxi University of Science & Technology (Xi'an, China).

3.2. Fungal material

Decaisnea insignis (Griff.) Hook.f. & Thomson was collected from Qinling Mountain, Shaanxi Province, China on July 2014. The endophytic fungus (No. DS37) was isolated from the stem of *Decaisnea insignis* (Griff.) Hook.f. & Thomson according to the method described by Tian et al. (Tian et al. 2015), and was deposited with the culture collection of the Laboratory of Natural Product Research, College of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology. The fungus species was initially identified using morphological characteristics, and was further identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region (Tao et al. 2008).The sequenced data derived from the fungus strain have been deposited in the GenBank.

3.3. Solid-state fermentation

DS37 was grown on potato dextrose agar at 28 °C for 3 days. Six or seven pieces (diameter 0.6 cm) of mycelial agar plugs were inoculated into Erlenmeyer flasks (8 × 1000 mL) containing 500 mL Czapek's medium (sucrose 30g/L, KH_2PO_4 1.0 g/L, $NaNO_3$ 3.0 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KCl 0.5 g/L and $FeSO_4$ 0.01 g/L). The flasks were incubated for 7 days at 28 °C on a rotary shaker (180 rpm/min) to obtain the fungus seed. Then, the seed liquid was added to 300 flasks in which contained sterile culture medium, and the sterile culture medium consisted of 75 g rice and 90 mL Czapek's medium without sugar. Finally, the flasks were carried outstatically at room temperature for 35 days.

3.4. Extraction and isolation

The air-dried solid culture (9.2 kg) was extracted repeatedly with petroleum ether, ethyl acetate and methyl alcohol. The extract afforded a brown residue (1500 g), which was subjected to silica gel column chromatography eluting gradually with petroleum ether/ethyl acetate/methyl alcohol gradients (1:0:0, 0:1:0, 0:1:1, 0:0:1 V:V:V) to give four fractions (Fr. A-D). The fraction Fr. B (420 g) was separated by silica gel column chromatography with a petroleum ether/ethyl acetate gradient system (1:0, 5:1, 2:1, 1:1, 1:2, 0:1 V:V) to give six fractions (Fr. B1-B6). The fraction Fr. B6 (25 g) was subjected to silica gel column chromatography eluting gradually with petroleum ether/ethyl acetate gradients (1:0, 10:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 0:1 V:V) to got compound **1** (18.2 mg).

3.4.1. UV, IR, NMR and HR-EI-MS spectra data of 1

Colorless flake crystal; m.p. 146-148.4 °C; IR (KBr) ν_{max} (cm^{-1}): 3695, 3121, 1721, 1632, 1442, 1188, 842; UV (MeOH) λ_{max} (nm): 225; ^{1}H-NMR(400MHz, DMSO-d_6): δ_H 12.33 (1H, s, H-8), 5.93 (1H, s, H-4), 4.84 (2H, s, H-2), 2.61-2.58 (2H, t, $J = 4.6$ Hz, H-6), 2.57-2.54 (2H, t, $J = 4.6$ Hz, H-7); ^{13}C-NMR(100 MHz, DMSO-d_6): δ_C 173.82 (C-5), 173.45 (C-8), 171.55 (C-3), 114.08 (C-4), 73.11 (C-2), 30.99 (C-7), 23.45 (C-6). Its HRESI-MS spectrum exhibited a deprotonated molecular ion peak at m/z 155.0352 $[M-H]^-$ (calcd. for $C_7H_7O_4$, 155.0344), suggesting the molecular formula $C_7H_8O_4$.

3.4.2. X-ray crystallographic analysis of 1

Upon crystallization from MeOH by the vapor-diffusion method, colorless crystals were obtained for **1**. A crystal ($0.120 \times 0.110 \times 0.100$ mm) was separated from the sample and mounted on a glass fiber, then data were collected with a Bruker APEX-II CCD detector with graphite monochromated *Cu Kα* radiation ($\lambda = 0.71073$ Å) at 298 (2) K. The structure was solved using direct methods (SHELXL-2014/7) and refined using full-matrix least squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with the relative isotropic parameters. The final refinement gave $R_f = 0.1648$ and $R_w = 0.3833$ $[I > 2\sigma(I)]$. The following is the basic crystal data: $C_7H_8O_4$ $M = 156.13$, triclinic, space group *P*-1; Unit cell dimensions $a = 5.207(4)$ Å, $b = 7.471(5)$ Å, $c = 10.262(7)$ Å, $\alpha = 105.17(4)°$, $\beta = 99.95(5)°$, $\gamma = 106.51(5)°$, $V = 356.0(4)$ Å^3, $Z = 2$, $D = 1.457$ g/cm^{-3}, F (000) = 164. These messages have been deposited in the Cambridge Crystallographic Data Center with the deposition number CCDC 1814146. A copy of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB21EZ, U.K. (fax, +44 (0) -1233-336033; e-mail, deposit@ccdc.cam.ac.uk).

3.5. Antimicrobial assay

The antimicrobial activity of the compound **1** was determined according to the microdilution method, as described by CLSI guidelines (CLSI, 2017). The minimal inhibitory concentration (MIC) (Bharate et al. 2007) values were determined on four bacteria and five plant pathogenic fungi . Samples (dissolved in DMSO) were serially diluted using 0.9% saline to reach the starting concentration of 256 μg/mL. The inocula were prepared from overnight broth cultures (MH broth for bacteria and PD broth for fungi) and suspensions were adjusted to the required microbial load (1×10^6 CFu/mL). A positive (100 μL of broth medium plus 100 μL inoculum) and a negative control (100 μL of diluted DMSO plus 100 μL inoculum) were considered for each strain. 96-well microtiter plates were incubated 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi (Zheng et al. 2015; Wang et al. 2016). As reference, streptomycin sulphate and penicillin were positive control against bacteria, carbendazim as positive control for the antifungal assay. All the analyses were performed in triplicate.

4. Conclusion

A new furan derivative named 3-(5-oxo-2,5-dihydrofuran-3-yl) propanoic acid was isolated from an endophytic *Aspergillus tubingensis* of *Decaisnea insignis* (Griff.) Hook.f. & Thomson. It indicated strong antibacteria and antifungal activity deserved increasing attention as a source of antimicrobial drug.

Disclosure statement

No conflict of interest was reported by the authors.

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