SHORT REPORT

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Currephila A, a New Chromanol Derivative from the Endophytic Fungus *Curreya pityophila*

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Abstract: A previously undescribed chromanol derivative, named currephila A (**1**), was isolated from the endophytic fungus *Curreya pityophila*, along with two known compounds (**2** and **3**). Their structures were elucidated based on spectroscopic methods, electronic circular dichroism, and spin−spin coupling constants. The cytotoxic activities of compounds **1** and **2** against human breast adenocarcinoma MCF-7 cells were evaluated.

Keywords: *Curreya pityophila*; endophytic fungus; secondary metabolite; chromanol.

1. Fungal Source

The strain *C. pityophila* was isolated from the healthy root tissue of *Fritillaria monatha Migo (*syn.: *Fritillaria hupehensis* P. K. Hsiao & K. C. Hsia) (Liliaceae). The ITS sequence of this strain is almost identical to the strain deposited in Genbank with the accession number MH855249.1 (max identity: 100%, query cover: 100%). The fungal specimen is deposited at South-Central MinZu University, China.

2. Previous Studies

Endophytic fungi inhabit normal tissues and organs of healthy plants without causing discernible manifestation of disease, with rich sources of bioactive natural products [1]. These bioactive natural products provided by endophytic fungi originate from different biosynthetic pathways and belong to diverse structural groups, mainly including terpenoids [2], steroids [3], cytochalasins [4], xanthones [5], quinones [6], phenols [7], and isocoumarins [8]. Many of these compounds possess complex skeletons and have a wide array of pharmacological properties such as anti-inflammatory [9], antitumor [10], antiviral [11], anti-microbial [12], anti-oxidant [12], anti-diabetic [13], anti-allergic [14], neuroprotective [15], and acetylcholinesterase [16] inhibitory activities.

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3. Present Study

In this study, three compounds (**1**-**3**) (Figure 1) were isolated from the EtOAc extract of fermentation broth of *C. pityophila*. Compound **1**, named currephila A, was previously undescribed. Based on NMR spectral data analysis along with a comparison to published data, two known compounds were identified as 2-methyl-5-hydroxybenzoic acid (**2**) [17] curtachalasin H (**3**) [18]. Herein, the isolation, structural elucidation, and biological activities of the new isolates were reported.

The strains were cultured with liquid potato dextrose broth medium in Erlenmeyer flasks for 28 days. Erlenmeyer flasks (500 mL) accommodated 250 mL of potato dextrose broth medium, consisting of 0.5% of yeast extract, 0.05% of KH_2PO_4, 0.05% of $MgSO_4$, 5% of glucose and 0.15% of peptone powder in distilled water. After shaking on a rotary shaker for 28 days (120 rpm at 25 ℃), a total of 25 L supernatant was filtrated to give a mycelium-free sample. The fermentation broth of *C. pityophila* (25 L) was filtered by muslin cloth to separate the supernatant fluid from the mycelia. Afterwards, the fermented material was extracted repeatedly with EtOAc three times and concentrated under a vacuum to obtain the crude extract (100 g). The EtOAc extract was submitted to silica gel CC and eluted with a gradient of CH_2Cl_2-MeOH (100:0-0:100, v/v) to produce five fractions (A-E). Fraction B (24.5 g) was subjected to C18 MPLC using MeOH-H_2O (10:90-100:0, v/v) and yielded eight subfractions (B1-B8). Fraction B5 (2.2 g) was separated into five subfractions B5-1–B5-5 by CC (Sephadex LH-20, MeOH). Fraction B5-2 (12.6 mg) was separated using preparative HPLC with a gradient of MeCN-H_2O from 35:65 to 50:50 in 30 min (4.0 mL/min) to obtain **1** (1.4 mg). Fraction C (4.4 g) was separated into six subfractions C1-C4 by CC (Sephadex LH-20, MeOH). Fraction C2 (14.5 mg) was separated using preparative HPLC with a gradient of MeCN-H_2O from 30:70 to 50:50 in 30 min (4.0 mL/min) to obtain **3** (1.5 mg). Fraction C3 (20.1 mg) was separated using preparative HPLC with a gradient from 45:55 to 60:40 in 30 min (4.0 mL/min) of MeCN-H_2O to obtain **2** (1.3 mg).

Equipment: To obtain 1D and 2D NMR spectra, a Bruker spectrometer (Bruker, Germany, model AM600) was employed. Circular dichroism (CD) spectra were recorded using an Applied Circular Dichroism spectrometer (Chirascan, New Haven, USA). HRESIMS data were collected using a Q Exactive HF mass spectrometer (Thermo Fisher Scientific, USA). Sephadex LH-20 (Pharmacia Fine Chemical Co., Ltd., Sweden), RP-18 gel (Fuji Silysia Chemical Ltd., Japan) and Silica gel (Qingdao Marine Chemical Ltd., Qingdao, China) were used for column chromatography (CC). HPLC studies were performed on an Agilent 1260 HPLC equipped with an Agilent Zorbax SB-C_{18} column (250 × 9.8 mm, 5 μm). To monitor fractions, thin layer chromatography (GF 254) was utilized, and spots were detected by heating silica gel plates covered with vanillin and 10 % H_2SO_4 in ethanol.

Currephila A (**1**): white powder; UV (MeOH) λ_{max} (log ε) = 235 (4.05); $[\alpha]_D^{24}$ + 36 (*c* 0.5, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 5.22 (1H, d, *J* = 15.5 Hz, H-2), 5.34 (1H, d, *J* = 15.5 Hz, H-2), 4.68 (1H, d, *J* = 2.8 Hz, H-9), 2.64 (1H, ddd, *J* = 11.9, 3.5, 2.8 Hz, H-10), 4.29 (1H, dd, *J* = 11.9, 10.7 Hz, H-11), 4.37 (1H, dd, *J* = 10.7, 3.5 Hz, H-11), 4.77 (1H, s, H-12), 5.16 (1H, s, H-12), 1.92 (1H, s, H-14), 2.11 (1H, s, H-15), 4.01 (1H, s, H-16); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) = 169.2 (C, C-1), 67.8 (CH_2, C-2), 148.4 (C, C-3), 112.5 (C, C-4), 159.0 (C, C-5), 120.2 (C, C-6), 157.6 (C, C-7), 109.6 (C, C-8), 60.8 (CH, C-9), 44.5 (CH, C-10), 64.4 (CH_2, C-11), 114.1 (CH_2, C-12), 140.8 (C, C-13), 23.3 (CH_3, C-14), 8.7 (CH_3, C-15), 62.3 (OCH_3, C-16); HRESIMS *m/z* 291.1226 $[M + H]^+$ (calcd for $C_{16}H_{18}O_5$, 291.1227).

Cytotoxicity assay: Breast cancer MCF-7 cell lines were cultured in DMEM medium, with the supplementation of 10% of fetal bovine serum (FBS), 100 U/mL penicillin, and 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA) at 37 °C in humidified environment with 5% of CO_2. Each tumor cell line was exposed to the test compound at concentrations of 0.064, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin as a positive control. The cytotoxicity assay was conducted utilizing the MTS method, and the absorbance at 490 nm was measured using a Spectra Max M5 microplate reader.

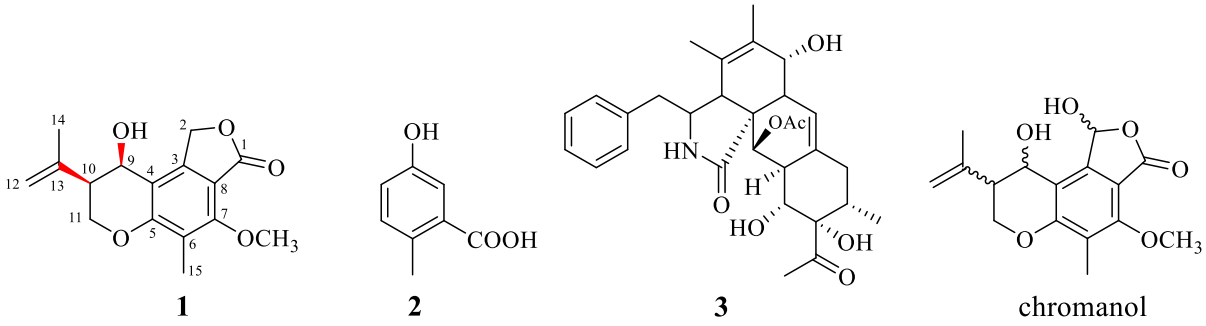

Figure 1. The chemical structures of compounds **1**-**3** and chromanol

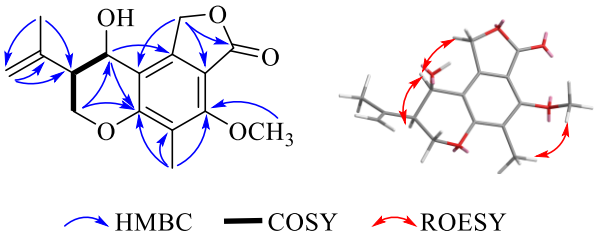

Figure 2. Key HMBC, ^{1}H-^{1}H COSY and ROESY correlations of compound **1**

Compound **1** was isolated as a white amorphous powder, and its molecular formula was established to be $C_{16}H_{18}O_5$ based on HRESIMS that illustrated a protonated molecule at m/z 291.1226 $[M + H]^+$ (calculated for 291.1227) and a sodium adduct at *m/z* 313.1044 $[M + Na]^+$ (calculated for 313.1046), which indicated eight degrees of unsaturation. The ^{13}C NMR and DEPT spectra of compound **1** showed 16 carbon resonances that were classified into two methyl groups (δ_C 8.7, 23.3), one methoxy group (δ_C 62.3), two oxygenated methylene groups (δ_C 64.4, 67.8), two methine group (δ_C 44.5, 60.8), two terminal olefin (δ_C 114.1, 140.8), and seven quaternary groups (δ_C 109.6, 112.5, 120.2, 148.4, 157.6, 159.0, 169.2). The above NMR characteristics of **1** were similar to those of chromanol [19], except that an oxymethine carbon of C-2 (CH, δ_C 96.3) of chromanol was shifted to the methylene group (CH_2, δ_C 67.8) in **1** (Table S1 in Supporting Information), which was further validated by the HMBC correlations (Figure 2) from H-2 (δ_H 5.22) to C-1 (δ_C 169.2), C-4 (δ_C 112.5) and C-8 (δ_C 159.0) of **1**. Therefore, the planar structure of **1** was constructed (Figure 2).

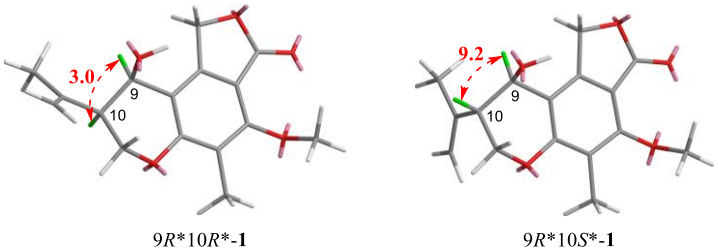

Figure 3. Calculated spin-spin coupling constants of compound **1**

The small ^{1}H-^{1}H coupling constant ($^{3}J_{H\text{-}9,H\text{-}10}$ = 2.8 Hz) allowed for the cis-relationship of H-9/H-10, which was confirmed by comparative analysis of calculated and experimental coupling constants. Compared with the coupling constants between H-9 and H-10 of **1** with the calculated values of two possible configurations, the calculated coupling constant of 9*R**10*R***-1** [3.0 ($^{3}J_{H\text{-}9/H\text{-}10}$)] was nearer to the experimental value [2.8 ($^{3}J_{H\text{-}9/H\text{-}10}$)] than that of 9*R**10*S***-1** [9.2 ($^{3}J_{H\text{-}9/H\text{-}10}$)] (Figure 3). Finally, the 9*R*10*R* configuration for **1** was established by comparing the calculated and experimental ECD spectra. The experimental ECD spectrum of **1** fits well with the calculated spectrum of (9*R*10*R*)-**1** and is opposite that of its enantiomer (9*S*10*S*)-**1** (Figure 4). Therefore, the absolute configuration of **1** was defined as 9*R*10*R*.

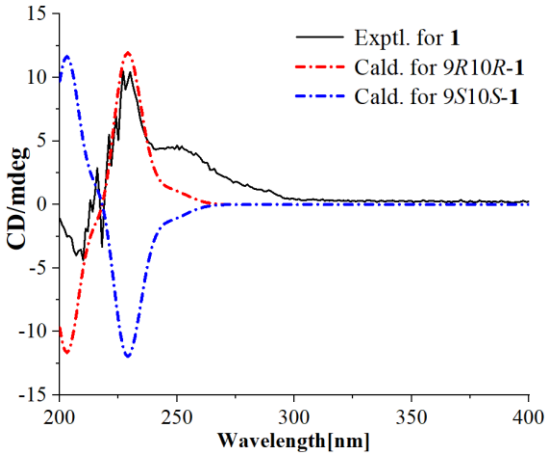

Figure 4. Experimental and calculated ECD spectra of **1** at the M062X/def2svp level in methanol

Compounds **1** and **2** were evaluated for their cytotoxicities against breast cancer MCF-7 cell lines. As a result, none of them exhibited cytotoxicities with $IC_{50} > 40$ 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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References

[1] J. Zhao, T. Shan, Y. Mou and L. Zhou (2011). Plant derived bioactive compounds produced by endophytic fungi, *Mini. Rev. Med. Chem.* **11**, 159-168.

[2] J.J. de Souza, I.J.C. Vieira, E. Rodrigues Filho and R. Braz Filho (2011). Terpenoids from endophytic fungi, *Molecules* **16**, 10604-10618.

[3] H.E. Ortega, D. Torres-Mendoza, E.Z. Caballero and L. Cubilla-Rios (2021). Structurally uncommon secondary metabolites derived from endophytic fungi, *J. Fungi.* **7**, 570. doi: 10.3390/jof7070570.

[4] B.B. Shi, C. Tian, X. Lv, J. Schinnerl, K. Ye, H. Guo, F. Xu, Y. He, H.L. Ai and J.K. Liu (2023). Boerelasins A-D, four unprecedented cytochalasins from the endophytic fungus *Boeremia exigua*, *J. Org. Chem.* **88**, 13926-13933.

[5] X.P. Peng, F.S. Sun, G. Li, C. Wang, Y.H. Zhang, C.Z. Wu, C.Y. Zhang, Y. Sun, S.Y. Wu, Y.X. Zhang, H. Zong, R. Guo and H.X. Lou (2021). New xanthones with antiagricultural fungal pathogen activities from the endophytic fungus *Diaporthe goulteri* L17, *J. Agric. Food Chem.* **69**, 11216-11224.

[6] S.J. Li, X. Zhang, X.H. Wang, and C.Q. Zhao (2018). Novel natural compounds from endophytic fungi with anticancer activity, *Eur. J. Med. Chem.* **156**, 316-343.

[7] H.L. Li, X.M. Li, S.Q. Yang, L.H. Meng, X. Li and B.G. Wang (2019). Prenylated phenol and benzofuran derivatives from *Aspergillus terreus* EN-539, an endophytic fungus derived from marine red alga *Laurencia okamurai*, *Mar. Drugs* **17**, 605.

[8] Y.X. Xu, Z.P. Huang, S.Q. Li, C.Y. Ma and X.M. Zhang (2022). Isocoumarin derivatives from endophytic fungus *Phomopsis sp.* RJ-21. *Biochem. Syst. Ecol.* **105**, 104521.

[9] H.M. Jiang, R.L. Cai, Z.M. Zang, W.C. Yang, B. Wang, G. Zhu, J. Yuan and Z.G. She (2022). Azaphilone derivatives with anti-inflammatory activity from the mangrove endophytic fungus *Penicillium sclerotiorum* ZJHJJ-18, *Bioorg. Chem.* **122**, 105721. doi: 10.1016/j.bioorg.2022.105721.

[10] J. Wei, F. Chen, Y.M. Liu, A. Abudoukerimu, Q. Zheng, X.B. Zhang, Y.P. Sun and D. Yimiti (2020). Comparative metabolomics revealed the potential antitumor characteristics of four endophytic fungi of *Brassica rapa* L., *ACS Omega* **5**, 5939-5950.

[11] I.C.D. Lacerda, J.C. Polonio and H.C. Golias (2022). Endophytic fungi as a source of antiviral compounds - a review, *Chem. Biodivers.* **19**, e202100971.

[12] C.C. Abba, B.O. Umeokoli, P.M. Eze and A.F. Onyegbule (2014). Anti-oxidant and anti-microbial potentials of some metabolites from an endophytic fungi isolated from *Lorantus micranthus* from south-eastern Nigeria, *Planta Med.* **80**, 1398-1398.

[13] Z.L. Shao, Y.Y. Tian, S. Liu, X. Chu and W.J. Mao (2023). Anti-diabetic activity of a novel exopolysaccharide produced by the mangrove endophytic fungus *Penicillium janthinellum* N29, *Mar. Drugs* **21**, 270. doi: 10.3390/md21050270.

[14] M. Elsbaey, A. Sallam, M. El-Metwally, M. Nagata, C. Tanaka, K. Shimizu and T. Miyamoto (2019). Melanogenesis inhibitors from the endophytic fungus *Aspergillus amstelodami*, *Chem. Biodivers.* **16**, e1900237. Doi:10.1002/cbdv.201900237.

[15] R. Vig, F. Bhadra, S.K. Gupta, K. Sairam and M. Vasundhara (2022). Neuroprotective effects of quercetin produced by an endophytic fungus *Nigrospora oryzae* isolated from *Tinospora cordifolia*, *J. Appl. Microbiol.* **132**, 365-380.

[16] Z. Li, N. Ma and P.J. Zhao (2019). Acetylcholinesterase inhibitory active metabolites from the endophytic fungus *Colletotrichum sp.* YMF432, *Nat. Prod. Res.* **33**, 1794-1797.

[17] C. Granchi, G. Bononi, R. Ferrisi, E. Gori, G. Mantini, S. Glasmacher, G. Poli, S. Palazzolo, I. Caligiuri, F. Rizzolio, V. Canzonieri, T. Perin, J. Gertsch, A. Sodi, E. Giovannetti, M. Macchia, F. Minutolo, T. Tuccinardi and A. Chicca (2021). Design, synthesis and biological evaluation of second-generation benzoylpiperidine derivatives as reversible monoacylglycerol lipase (MAGL) inhibitors, *Eur. J. Med. Chem.* **209**, 12857. doi: 10.1016/j.ejmech.2020.112857.

[18] W.X. Wang, G.G. Cheng, Z.H. Li, H.L. Ai, J. He, J. Li, T. Feng and J.K. Liu (2019). Curtachalasins, immunosuppressive agents from the endophytic fungus *Xylaria cf.* curta, *Org. Biomol. Chem.* **17**, 7985-7994.

[19] H. Achenbach, A. Mühlenfeld and G.U. Brillinger (1985). Stoffwechselprodukte von mikroorganismen, XXX. phthalide und phromanole aus *Aspergillus duricaulis*, *Liebigs Ann. Chem.* **8**, 1596-1628.