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Two undescribed pairs of isoprenyl hydroxybenzoic acid derivatives from coculture of *Pestalotiopsis* sp. and *Penicillium bialowiezense*: enantioseparation and their absolute configurations

Fengli Li,^{sa} Zhangyan Huang,^{sa} Shuyuan Mo,^{sa} Saisai Gu,^b Sitian Zhang,^a Beiye Yang,^a Jianping Wang,^a

Zhengxi Hu,*a and Yonghui Zhang*a

^a Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy,

Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

^b Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and

Technology, Wuhan 430030, China

^{*}Corresponding author.

Tel.: 86-027-83692892.

E-mail addresses: <u>zhangyh@mails.tjmu.edu.cn</u> (Y. Zhang); <u>hzx616@126.com</u> (Z. Hu).

[§]These authors contributed equally to this work.

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1. General experimental procedures

Optical rotations were collected from a Perkin-Elmer 341 spectropolarimeter (Waltham, MA, USA) in MeOH. UV spectra were measured on a Varian Cary 50 instrument (Santa Clara, CA, USA) in MeOH. IR spectra were collected from a Vertex 70 FT-IR spectrophotometer (Billerica, MA, USA) with KBr pellets. CD data were recorded on a JASCO-810 spectrometer (Oklahoma City, OK, USA) in MeOH. HRESIMS data were tested on a Bruker MicrOTOF II spectrometer using electrospray ionization (Bruker, Germany). NMR spectra were tested on a Bruker AM-400 spectrometer. The chemical shifts (δ) were expressed in ppm with reference to the solvent signals for methanol- d_4 (δ_H 3.31 and δ_C 49.0). Semi-preparative HPLC was performed on HITACHI L-2400 HPLC system with a reversed-phase (RP) C₁₈ column (5 μ m, 10 × 250 mm, Welch Ultimate XB-C₁₈). Column chromatography (CC) was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden). Fractions were monitored based on TLC and spots were visualized by heating silica gel plates sprayed with H₂SO₄–EtOH (10:90, v/v).

2. Fungal material

The fungal samples were stored in the culture collection center of Tongji Medical College, Huazhong University of Science and Technology. The fungus *Pestalotiopsis* sp. was isolated from the leaves of tea tree, collected from the Enshi Autonomous Prefecture of Hubei Province in March 2016.¹ The fungus *Penicillium bialowiezense* was isolated from the fresh soft coral *Sarcophyton subviride*, collected from the Xisha Island in the South China Sea in October 2016.² The strains were identified based on their morphology analysis and ITS (Internal Transcribed Spacer) sequencing data of the rDNA. The ITS sequences data have been deposited at the GenBank (accession number: MH845232 for *Pestalotiopsis* sp. and MH443003 for *Penicillium bialowiezense*).

- [1] B. Yang, Q. Tong, S. Lin, J. Guo, J. Zhang, J. Liu, J. Wang, H. Zhu, Z. Hu and Y. Zhang, *Phytochem. Lett.*, 2019, 29, 186–189.
- [2] Q. Zhang, B. Yang, F. Li, M. Liu, S. Lin, J. Wang, Y. Xue, H. Zhu, W. Sun, Z. Hu and Y. Zhang, *Mar. Drugs*, 2018, **16**, 230–239.

3. Extraction and isolation

To prepare the seed cultures, each fungus was cultured on potato dextrose agar (PDA) plates at 28 °C for 7 days, which was used to inoculate Erlenmeyer flasks (100 × 1 L), containing 250 g of rice and 250 mL of distilled water. After incubating for 45 days at room temperature. At the time of harvest, each flask

was soaked in 300 mL of 95% EtOH for 48 h and the fermented materials of coculture were extracted with EtOAc for six times. The solvent was removed under reduced pressure to obtain a crude extract (220 g).

The crude extract was fractionated by silica gel CC with a gradient elution of petroleum ether (PE)– ethyl acetate (EtOAc) system ($30:1\rightarrow0:1$) to obtain six main fractions (A–F) based on the TLC analysis. Fraction C (5 g) was purified by Sephadex LH-20 (MeOH), silica gel CC (200-300 mesh) eluted with PE/EtOAc ($20:1\rightarrow1:1$), and RP-HPLC to afford **1** (12.1 mg, CH₃CN–H₂O, 34:66, v/v, 2.5 mL/min, $t_R = 40$ min) and **2** (4.6 mg; CH₃CN–H₂O, 45:55, v/v, 2.0 mL/min, $t_R = 34$ min). Compound **1** (12.1 mg) was separated by chiralpak IC column eluted with CH₃OH–H₂O (59:41, v/v; 1.0 mL/min) to give **1a** (8.2 mg, $t_R = 9$ min) and **1b** (3.1 mg, $t_R = 10.5$ min). Compound **2** (4.6 mg) was separated by chiralpak IA column eluted with CH₃CN– H₂O (35:65, v/v; 1.0 mL/min) to give **2a** (2 mg, $t_R = 20$ min) and **2b** (1.5 mg, $t_R = 22$ min).

Glyceryl 4-hydroxy-3-prenyl-benzoate (**1**): Acicular crystals; UV (MeOH) λ_{max} (log ε): 210 (4.30), 260 (4.12) nm; IR (KBr) v_{max} : 3417, 2934, 1694, 1605, 1280, 1119, 1031, 770 cm⁻¹; HRESIMS *m/z* [M + Na]⁺ 303.1220 (calcd for C₁₅H₂₀O₅Na⁺, 303.1203); For ¹H and ¹³C NMR data, see Table 1.

(1*a*): [α]25 D: +15.0 (*c* 1, MeOH); ECD (MeOH) λ_{max} (Δε): 211 (+4.30) nm.

(**1b**): $[\alpha]$ 25 D: -15.7 (*c* 1, MeOH); ECD (MeOH) λ_{max} ($\Delta \varepsilon$): 208 (-10.02) nm.

Anofinic glyceride (**2**): Colorless oil; UV (MeOH) λ_{max} (log ε): 239 (4.55) nm; IR (KBr) v_{max} : 3419, 2937, 1710, 1608, 1368, 1275, 1193, 1125, 1097, 1031, 766 cm⁻¹; HRESIMS m/z [M + Na]⁺ 301.1070 (calcd for C₁₅H₁₈O₅Na⁺, 301.1046); For ¹H and ¹³C NMR data, see Table 1.

(**2a**): [α]25 D: –4.5 (*c* 1, MeOH); ECD (MeOH) λ_{max} (Δε): 234 (–3.87) nm.

(**2b**): [α]25 D: +4.1 (*c* 1, MeOH); ECD (MeOH) λ_{max} (Δε): 243 (+2.36) nm.

4. X-ray crystal structure analysis

A suitable crystal of compound **1** was obtained in mixed MeOH–H₂O (20:1). Intensity data were collected at 296 K on a Bruker APEX DUO diffractometer equipped with an APEX-II CCD using Cu K α radiation. Bruker SAINT was used for cell refinement and data reduction. The structures were solved by direct methods with SHELXL-2014/7.¹⁻² The crystallographic data (CCDC 2055732 for **1**) have been deposited in the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. + 44 (0) (1223) 336 033; e-mail: <u>deposi@ccdc.cam.ac.uk</u>].

Crystallographic data for **1**: $C_{15}H_{20}O_5$, M = 280.31, a = 4.9574(5) Å, b = 11.4656(7) Å, c = 13.0020(8) Å, $\alpha = 107.096(6)^\circ$, $\beta = 100.136(7)^\circ$, $\gamma = 93.672(6)^\circ$, V = 690.06(9) Å³, T = 100(10) K, space group P-1, Z = 2, μ (Cu K α) = 0.835 mm⁻¹, 12040 reflections collected, 2344 independent reflections ($R_{int} = 0.0703$). The final R_1

values were 0.0652 ($l > 2\sigma(l)$). The final wR_2 values were 0.1827 ($l > 2\sigma(l)$). The final R_1 values were 0.0754 (all data). The final wR_2 values were 0.1918 (all data). The goodness of fit on F^2 was 1.054.

[1] G. M. Sheldrick, Acta Cryst., 1990, A46, 467–473.

[2] C. B. Hubschle, G. M. Sheldrick and B. Dittrich, J. Appl. Crystallogr., 2011, 44, 1281–1284.

5. Dimolybdenum tetraacetate induced reaction

According to the published method,¹⁻² to a stock solution of about 0.6 mg/mL of commercial $Mo_2(AcO)_4$ in commercial DMSO (analysis grade), a quantity of the chiral sample (compound **1a** and **2a**) was added so that the ligand to metal ratio is approximately 1.0/1.2. The first ICD spectrum is recorded immediately after the mixing, and its time evolution is controlled with a rate of about one spectrum every 5 minutes, until a stationary ICD is reached (40 min after the mixing).

M. Górecki, E. Jabłońska, A. Kruszewska, A. Suszczyńska, Z. Urbańczyk-Lipkowska, M. Gerards, J. W. Morzycki, W. J. Szczepek and J. Frelek, *J. Org. Chem.*, 2007, **72**, 2906–2916.
L. Di Bari, G. Pescitelli, C. Pratelli, D. Pini and P. Salvadori, *J. Org. Chem.*, 2001, **66**, 4819–4825.

6. In vitro β-glucuronidase activity assay

The *in vitro* inhibitory activity against bacterial β -glucuronidase (GUS) was carried out in a 96-well plate. Each reaction was prepared in the assay buffer (PBS, pH 7.4), including 20 μ L of tested compound, 40 μ L of GUS (Sigma-Aldrich, 20 ng), and 20 μ L of 5 mM pNPG (4-nitrophenyl β -D-glucuronide). The GUS was treated with each tested compound at 37 °C for 15 min and sequentially incubated with pNPG for 30 min. The GUS activity was measured by detecting the presence of pNP with OD at the wavelength of 405 nm. IC₅₀ values were calculated based on inhibition percentages of series dilutions by GraphPad Prism 7.0 software. DSA was used as positive control.

7. In vitro BChE activity assay

The *in vitro* inhibitory activity against BChE was tested based on the modified Ellman's method.¹ BChE, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and acetylthiocholine iodide (ATCI) were purchased from Sigma-Aldrich. Briefly, 40 μ L of different concentrations of the tested compounds in PBS buffer (pH 7.4) and 20 μ L BChE (final concentration: 0.02 U/mL) were added and pre-incubated for 15 min at room temperature. The reaction mixture was then incubated for 10 min at 37 °C. After that, 20 μ L DTNB (5 mM) and 20 μ L ATCI (10 mM) were added. The activity was determined by measuring absorbance at 405 nm. Each concentration was repeated three times, and IC₅₀ values were calculated as the concentration of

tested compounds with 50% inhibitory activity. Neostigmine was used as positive control.

[1] W. Ma, J. Bi, C. Zhao, Y. Gao and G. Zhang, *Carbohydr. Res.*, 2020, **491**, 107977.

Figure S1. ¹H NMR spectrum of compound **1** (Recorded in methanol- d_4)



Figure S2. ¹³C NMR and DEPT spectra of compound **1** (Recorded in methanol- d_4)









Figure S4. $^{1}H-^{1}H$ COSY spectrum of compound **1** (Recorded in methanol- d_{4})



Figure S5. HMBC spectrum of compound **1** (Recorded in methanol- d_4)



Figure S6. NOESY spectrum of compound **1** (Recorded in methanol- d_4)

Figure S7. HRESIMS spectrum of compound 1



Figure S8. IR spectrum of compound 1





Figure S10. ¹H NMR spectrum of compound **2** (Recorded in methanol- d_4)



Figure S11. ¹³C NMR and DEPT spectra of compound **2** (Recorded in methanol- d_4)





Figure S12. HSQC spectrum of compound 2 (Recorded in methanol- d_4)







Figure S14. HMBC spectrum of compound **2** (Recorded in methanol- d_4)



Figure S15. NOESY spectrum of compound **2** (Recorded in methanol- d_4)

Figure S16. HRESIMS spectrum of compound 2



Figure S17. IR spectrum of compound 2





Figure S19. Chiral HPLC of 1a and 1b



Figure S20. Chiral HPLC of 2a and 2b



