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ACTIVE METABOLITES FROM THE FUNGUS *Pestalotiopsis* sp. YMF1.0474

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The chemical constituents of the fungus Pestalotiopsis *sp. YMF1.0474 were studied. Five compounds, including a new pestalrone C (**1**) and four known compounds, namely, 6-[1-hydroxy-(1*S*)-pentyl]-4-methoxy-(6*S*)-2*H*,5*H*-pyran-2-one (**2**), LL-P880β (**3**), pestalrone A (**4**), and methyl (*E*)-octadec-9-enoate (**5**), were isolated from the culture broth of strain YMF1.0474. Compounds **1–4** showed acetylcholinesterase (AChE) inhibitory activity with IC_{50} 33.90, 81.54, 16.43, and 95.22 μM, respectively.*

Keywords: *Pestalotiopsis* sp., α-pyrone analogue, acetylcholinesterase inhibitory activity.

Fungi of the genus *Pestalotiopsis* are widely distributed in tropical and temperate regions. Some members of *Pestalotiopsis* are rich sources of many interesting compounds [1]. Recently, many active metabolites had been reported from the genus. New salicyloid derivatives vaccinols J–S were isolated from the fungus *Pestalotiopsis vaccinii*, and some of them showed anti-enterovirus 7l (EV71) and cytotoxic activities [2]. Antifungal isocoumarin derivatives were obtained from the endophytic fungus *Pestalotiopsis* sp. [3]. Antiproliferative ambuic acid derivatives were isolated from the Hawaiian endophytic fungus *Pestalotiopsis* sp. FT172 [4]. Polyketide derivatives from a marine-sponge-associated fungus *Pestalotiopsis heterocornis* exhibited cytotoxicity against four human cancer cell lines and also showed antibacterial and antifungal activities [5]. New caryophyllene sesquiterpenoids were isolated from *Pestalotiopsis* sp. and identified as punctaporonin analogues [6]. Drimane sesquiterpenoids and isochromone derivatives were obtained from the endophytic *Pestalotiopsis* sp. [7]. Pestalotiolide A, a new antiviral phthalide derivative, was reported from a soft coral-derived fungus *Pestalotiopsis* sp. [8]. Two oxysporone derivatives, pestalrone A and B, were obtained from the endophytic fungus *Pestalotiopsis karstenii* isolated from *Camellia sasanqua.* Pestalrone B exhibited significant activity agains HeLa, HepG2, and U-251 [9]. In the research on the secondary metabolites of the genus, a strain *Pestalotiopsis* sp. YMF1.0474 was isolated from Gaoligong Mountain, and we had isolated five compounds from it, four of them showing acetylcholinesterase (AChE) inhibitory activity.

Compound **1** was obtained as colorless solid. The HR-ESI-MS data indicated a molecular formula of $C_9H_{14}O_5$ based on the $[M + Na]^+$ ion peak at *m/z* 225.0735 $[M + Na]^+$ (calcd 225.0733). According to the ^1H, ^{13}C, and DEPT NMR (Table 1), compound **1** was an α-pyrone analogue [10]. In comparison with LL-P880α [10], there was one less -CH_2CH_2- unit, and the terminal methyl group was oxidized to CH_2OH. The ^1H–^1H COSY spectrum of compound **1** revealed one fragment by the clear correlations of H-4/H-5/H-6/H-7/H-8. The detail structure was established by HMBC correlations: H-2 (δ 5.15) correlated with the carbons at δ 173.1 (C-3), 166.7 (C-1), and 29.3 (C-4); one proton of methylene at H-4 (δ 2.78) with the carbons at δ 173.1 (C-3), 90.0 (C-2), 78.5 (C-5), and 71.7 (C-6); H-8 (δ 3.88 and 3.93) with the carbon at δ 71.7 (C-6); the oxygenated methyl 3-OCH_3 (δ_H 3.76) with the carbon at δ 173.1 (C-3). The relative configuration of compound **1** was established by comparison of their 1D NMR and specific optical rotation with LL-P880α [10]. Compound **1** and LL-P880α shared the same α-pyrone structural unit, and the relative and absolute configurations of **1** were proposed to be the same as that of LL-P880α. The specific rotation value of **1** $[\alpha]_D^{24}$ –61° (*c* 0.10, $CHCl_3$) was similar to LL-P880α $[\alpha]_D$ –86.2° (*c* 0.14, MeOH). Based on the above data, compound **1** was elucidated to be pestalrone C, which was a new compound.

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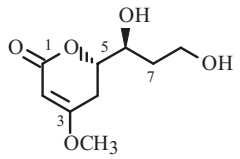
TABLE 1. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR Data of Compound **1** ($CDCl_3$, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC	COSY
1	–	166.7 (C)	–	–
2	5.15 (1H, br.d, J = 1.9)	90.0 (CH)	C-1, C-3, C-4	–
3	–	173.1 (C)	–	–
4	2.32 (1H, ddd, J = 1.5, 13.0, 17.0)	29.3 (CH_2)	–	H-5
	2.78 (1H, dd, J = 4.1, 17.0)		C-2, C-3, C-5, C-6	H-5
5	4.37 (1H, dt, J = 13.0, 4.1)	78.5 (CH)	–	H-4, H-6
6	3.97 (1H, m)	71.7 (CH)	C-7	H-5, H-7
7	1.82 (1H, m)	32.1 (CH_2)	C-6	H-7, H-8
	1.88 (1H, m)		–	H-7, H-8
8	3.88 (1H, m)	60.5 (CH_2)	C-6	H-7
	3.93 (1H, m)		C-6	H-7
3-OCH_3	3.76 (3H, s)	56.2 (CH_3)	C-3	–



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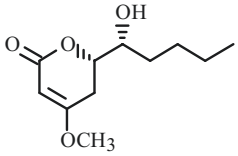

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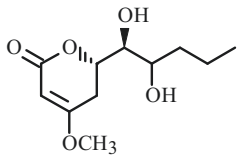

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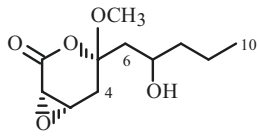

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Compounds **2–5** were identified as 6-[1-hydroxy-(1*S*)-pentyl]-4-methoxy-(6*S*)-2*H*,5*H*-pyran-2-one [11], LL-P880β [12], pestalrone A [9], and (*E*)-octadec-9-enoate [13], respectively, based on reference data.

All compounds obtained from the fungus were assessed for acetylcholinesterase (AChE) inhibition activity. The results showed that compounds **1–4** had AChE inhibition activity with IC_{50} 33.90, 81.54, 16.43, and 95.22 μM, respectively; the IC_{50} of positive control tacrine was 0.234 μM. This is the first report on the AChE inhibition activity of compounds **1–4**.

EXPERIMENTAL

General. UV spectra were measured on a Shimadzu UV-2401PC spectrophotometer (λ_{max} in nm). Optical rotations were measured with a Jasco DIP-370 digital polarimeter. NMR spectra were obtained with a Bruker Avance III-600 NMR spectrometer with TMS as an internal standard. ESI-MS and HR-ESI-MS were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer. Column chromatography (CC) was performed on silica gel G (200–300 mesh, Qingdao Marine Chemical Factory, China) and Sephadex LH-20 (Amersham Pharmacia, Sweden).

Plant Material. *Pestalotiopsis* sp. YMF1.0474 was isolated from Gaoligong Mountain, Yunnan, China. It was stored in State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan University, China.

Pestalrone C (1). Colorless solid; $[\alpha]_D^{24}$–61° (*c* 0.10, $CHCl_3$). UV ($CHCl_3$, λ_{max}, nm) (log ε): 197 (3.50), 233 (3.81). For ^{1}H (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$), see Table 1. ESI-MS *m/z* 225 $[M + Na]^+$; HR-ESI-MS *m/z* 225.0735 $[M + Na]^+$ (calcd 225.0733).

6-[1-Hydroxy-(1*S*)-pentyl]-4-methoxy-(6*S*)-2*H*,5*H*-pyran-2-one (2). Colorless crystals. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.91 (3H, t, J = 7.3, H-10), 1.33 (3H, m, H-9, 8a), 1.50 (1H, m, H-8b), 1.61 (2H, m, H-7), 2.52 (1H, dd, J = 3.7, 17.0, H-4b), 2.91 (1H, dd, J = 1.5, 17.0, H-4a), 3.62 (1H, m, H-6), 3.75 (3H, s, 3-OCH_3), 4.30 (1H, dt, J = 13.0, 4.1, H-5), 5.13 (1H, br.d, J = 1.5, H-2). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 13.9 (C-10), 22.5 (C-9), 27.5 (C-8), 29.5 (C-4), 32.3 (C-7), 56.1 (3-OCH_3), 72.4 (C-6),78.4 (C-5), 90.0 (C-2), 166.6 (C-1), 173.1 (C-3). ESI-MS *m/z* 215 $[M + H]^+$.

LL-P880β (3). Colorless crystals. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.95 (3H, t, J = 7.1, H-10), 1.38–1.53 (4H, m, H-9, 8), 2.33 (1H, dd, J = 3.6, 17.1, H-4a), 2.91 (1H, dd, J = 1.3, 17.1, H-4b), 3.49 (1H, m, H-7), 3.75 (3H, s, 3-OCH_3), 3.79 (1H, br.s, H-6), 4.52 (1H, dt, J = 12.8, 3.9, H-5), 5.13 (1H, br.d, J = 1.3, H-2). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 13.9 (C-10), 18.8 (C-9), 29.3 (C-8), 35.9 (C-4), 70.9 (C-7), 56.2 (3-OCH_3), 73.8 (C-6),78.0 (C-5), 89.7 (C-2), 166.6 (C-1), 173.1 (C-3). ESI-MS *m/z*: 231 $[M + H]^+$, 253 $[M + Na]^+$.

Pestalrone A (4). Colorless crystals. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.82 (1H, br.s, H-3), 3.68 (1H, dd, J = 3.1, 6.6, H-2), 3.64 (1H, m, H-7), 3.38 (3H, s, 5-OCH_3), 2.99, 2.79 (each 1H, d, J = 18.6, H-6), 2.42 (1H, m, H-4a), 1.89 (1H, dd, J = 6.9, 10.0, H-4b), 1.73 (1H, m, H-8a), 1.39 (1H, m, H-8b), 1.55 (2H, m, H-9), 0.96 (3H, t, J = 6.7, H-10). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 14.0 (C-10), 18.6 (C-9), 29.1 (C-4), 32.5 (C-8), 40.7 (C-6), 48.9 (5-OCH_3), 66.3 (C-7), 71.6 (C-3), 76.4 (C-2), 95.7 (C-5), 168.9 (C-1). ESI-MS *m/z* 231 $[M + H]^+$.

Methyl (*E*)-Octadec-9-enoate (5). Pale yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$, δ, ppm): 0.90 (t), 1.26–1.30 (m), 1.42 (m), 1.62 (m), 2.11 (m), 2.31 (t), 3.66 (s), 5.35 (m). ^{13}C NMR (150 MHz, $CDCl_3$, δ, ppm): 14.1, 22.6, 25.2, 26.1, 27.7, 29.1, 29.2, 29.3, 29.4, 31.8, 34.1, 51.5, 130.4, 130.9, 174.3. ESI-MS *m/z* 283 $[M + H]^+$.

AChE Inhibitory Activity. The fungus was grown on PDB medium consisting of glucose (20 g) and potato (200 g, boiled and filtered, per liter of water), and fermented for 20 days at 180 rpm under 28°C. The culture was harvested for further study. The AChE inhibitory activity of the compounds was determined using the modified Ellman spectrophotometric method with DTNB (Dithiobisnitrobenzoic acid) as color developing reagent [14]. Tacrine was used as positive control.

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REFERENCES

1. X. Yu, H. Liu, X. L. Niu, O. Akhberdi, D. S. Wei, D. Wang, and X. D. Zhu, *Microbiol. Res.*, **203**, 29 (2017).
2. J. F. Wang, R. Liang, S. R. Liao, B. Yang, Z. C. Tu, X. P. Lin, B. G. Wang, and Y. H. Liu, *Fitoterapia*, **120**, 164 (2017).
3. R. Y. Song, X. B. Wang, G. P. Yin, R. H. Liu, L. Y. Kong, and M. H. Yang, *Fitoterapia*, **122**, 115 (2017).
4. C. S. Li, B. J. Yang, J. Turkson, and S. Cao, *Phytochemistry*, **140**, 77 (2017).
5. H. Lei, X. Lin, L. Han, J. Ma, K. Dong, X. B. Wang, J. L. Zhong, Y. Mu, Y. H. Liu, and X. S. Huang, *Phytochemistry*, **142**, 51 (2017).
6. I. H. Hwang, D. T. Wicklow, and J. B. Gloer, *Phytochem. Lett.*, **16**, 257 (2016).
7. C. Kuang, S. X. Jing, Y. Liu, S. H. Luo, and S. H. Li, *Nat. Prod. Bioprosp.*, **6** (3), 155 (2016).
8. Y. L. Jia, F. F. Guan, J. Ma, C. Y. Wang, and C. L. Shao, *Nat. Prod. Sci.*, **21** (4), 227 (2015).
9. D. Q. Luo, L. Zhang, B. Z. Shi, and X. M. Song, *Molecules*, **17** (7), 8554 (2012).
10. G. A. Ellestad, W. J. Mcgahren, and M. P. Kunstmann, *J. Org. Chem.*, **37** (12), 2045 (1972).
11. A. S. Kumar, P. Bhaket, and B. V. Rao, *Arkivoc*, **3**, 74 (2005).
12. M. Kirihata, K. Ohta, I. Ichimoto, and H. Ueda, *Agric. Biol. Chem.*, **54** (9), 2401 (1990).
13. K. J. M. S. F. Lie and J. Mustafa, *Lipids*, **32** (10), 1019 (1997).
14. G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, *Biochem. Pharmacol.*, **7** (2), 88 (1961).