NOTE

Pyrenocine I, a new pyrenocine analog produced by *Paecilomyces* sp. FKI-3573

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Human African trypanosomiasis, also known as sleeping sickness, is caused by two subspecies of the parasitic protozoan, *Trypanosoma brucei*. Unique to sub-Saharan Africa, human African trypanosomiasis has, in past epidemics, caused significant and widespread mortality and morbidity. The World Health Organization estimated that human African trypanosomiasis caused 52 000 deaths in 2004, projecting a continuing fall to a total of 36 000 in 2015 (<http://www.ndi.org/>).¹ There are significant problems with the four parenteral drugs currently used for treatment (suramin, pentamidine, eflornithine and melarsoprol) as they are highly toxic, expensive, difficult to administer, and parasite resistance to them is increasing. Although a new combination therapy using drugs was introduced in 2009, only one molecule is currently in clinical development for treatment of human African trypanosomiasis (<http://www.ndi.org/>).² Therefore, there is an urgent need for new antitrypanosomal drugs that are more effective, safer, affordable easier to use and which, ideally, have a novel mode of action.

Our research group has focused on the screening of antitrypanosomal agents from microbial metabolites.^{3–5} Our ongoing studies have led to the discovery of a novel pyrenocine, pyrenocine I (**1**), which was isolated from the culture broth of *Paecilomyces* sp. FKI-3573, along with the known pyrenocines A (**2**)⁶ and B (**3**)⁶ and citreoviridin (**4**) (Figure 1).⁷ All of the compounds exhibit *in vitro* antitrypanosomal activity. In this article, we report their fermentation, isolation, structure elucidation and antitrypanosomal activity.

Fungal strain FKI-3573 was isolated from a soil sample collected in Hilo, HI, USA. The ITS rDNA sequence of FKI-3573 was a 95.6% match to *Paecilomyces lilacinus* ATCC 10114 (GenBank accession number AY213665). From this information, combined with morphological characteristics, FKI-3573 was identified to be a member of the *Paecilomyces* genus.⁸ A stock culture of strain FKI-3573 was inoculated into 100 ml of seed medium, consisting of glucose (2.0%), Polypepton (0.5%; Nihon Pharmaceutical, Tokyo, Japan), yeast extract (0.2%), KH₂PO₄ (0.1%), MgSO₄·7H₂O (0.05%) and agar (0.1%), adjusted to

pH 5.7 before sterilization. The mixture was subsequently incubated on a rotary shaker at 27 °C for 3 days. Seed culture (1 ml) was transferred into each of 10 Erlenmeyer flasks (500 ml) together with 100 ml of production medium containing potato dextrose broth (2.4%), malt extract (0.5%), Mg₃(PO₄)₂·8H₂O (0.5%) and agar (0.1%). The mixture was adjusted to pH 6.0 before sterilization. Fermentation was carried out on a rotary shaker at 27 °C for 3 days, followed by stationary culture for 10 days.

The culture broth (1.0 l) was added 1.0 l of EtOH. It was concentrated under reduced pressure after removing the mycelia by centrifugation and filtration. The resulting aqueous solution was extracted with EtOAc and the organic layer was concentrated to dryness *in vacuo* to afford a yellow solid (488 mg). It was applied to ODS column chromatography (eluted with 20, 40 and 60% aqueous CH₃CN). The concentrated 20% CH₃CN eluate (42.8 mg) was purified by HPLC (column, Pegasil ODS (20 φ×250 mm, Senshu Scientific, Tokyo, Japan); mobile phase, 30% aqueous CH₃CN; flow rate, 5.0 ml min^{−1}; detection, UV at 210 nm). Pyrenocine B (**3**, 16.2 mg) and **1** (3.5 mg) were obtained at 16 and 18 min, respectively. The 40% CH₃CN eluate (65 mg) was purified by HPLC (column, Pegasil ODS (20 φ×250 mm); mobile phase, 40% aqueous CH₃CN containing 0.1% TFA; flow rate, 5.0 ml min^{−1}; detection, UV at 210 nm) to yield 15 mg of pyrenocine A (**2**). The 60% CH₃CN eluate (70 mg) was also purified by HPLC (column, XBridge Prep C8 (10 φ×250 mm, Waters, Milford, MA, USA); mobile phase, 32% aqueous CH₃CN; flow rate, 3.0 ml min^{−1}; detection, UV at 210 nm) to produce 9.9 mg of citreoviridin (**4**), which was identified by comparison of spectral data with published values.⁹

Compound **1** was obtained as a light yellow amorphous solid ([α]_D²³ = −1.0; c = 0.1 in MeOH; UV (MeOH) λ_{max} (ε): 229 (16 000), 289 nm (4700)). The molecular formula was elucidated to be C₁₁H₁₄O₄ by HR-FAB-MS (found, 209.0805 [M-H][−]; calculated, 209.0819 for C₁₁H₁₃O₄). The IR spectrum showed characteristic absorptions at 3431, 1697, 1633 and 1558 cm^{−1}, suggesting the

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Table 1 ^1H and ^{13}C NMR data of pyrenocines I, A and B (in CDCl_3 ; ^1H , 300 MHz; ^{13}C , 75 MHz)

Position	δ_{H} (int, J in Hz)			δ_{C}		
	Pyrenocine I (1)	Pyrenocine A (2)	Pyrenocine B (3)	1	2	3
2				163.8	163.1	163.9
3	5.47 s (1H)	5.47 s (1H)	5.48 s (1H)	87.8	87.7	87.8
4				170.1	168.7	168.2
4-OCH ₃	3.82 s (3H)	3.79 s (3H)	3.86 s (3H)	56.1	56.3	56.5
5				110.0	113.9	115.4
6				159.2	161.4	162.4
6-CH ₃	2.32 s (3H)	2.17 s (3H)	2.27 s (3H)	18.5	18.5	18.6
1'	6.23 d (1H, 16.0)			118.4	190.6	201.1
2'	5.97 dd (1H, 16.0, 6.0)	6.30 dq (1H, 15.5, 1.5)	Ha: 2.81 dd (1H, 17.5, 8.5) Hb: 2.91 dd (1H, 17.5, 3.5)	139.8	133.1	52.8
3'	4.45 qd (1H, 6.5, 6.0)	6.80 dq (1H, 15.5, 7.0)	4.30 dqd (1H, 8.5, 6.5, 3.5)	69.0	147.4	64.3
4'	1.36 d (3H, 6.5)	1.96 dd (3H, 7.0, 1.5)	1.24 d (3H, 6.5)	23.5	18.2	22.7

Figure 1 Structures of pyrenocines I, A and B (1–3) and citreoviridin (4). **Figure 2** ^1H – ^1H COSY and HMBC correlations of pyrenocine I (1).**Table 2** *In vitro* antitrypanosomal activity and cytotoxicity of pyrenocines I, A and B and citreoviridin

Compound	IC_{50} ($\mu\text{g ml}^{-1}$)		
	Antitrypanosomal activity <i>T. b. b.</i> GUTat 3.1	Cytotoxicity MRC-5	Selectivity index MRC-5/GUTat 3.1
Pyrenocine I (1)	1.8	8.8	4.9
Pyrenocine A (2)	0.12	0.38	3.2
Pyrenocine B (3)	0.76	0.98	1.3
Citreoviridin (4)	0.48	45	94
Suramin	1.6	> 100	> 63

presence of an hydroxy group and an α -pyrone ring. These physico-chemical properties and ^1H and ^{13}C NMR spectra of **1** were similar to those of pyrenocines⁶ (Table 1). Compound **1** had the same 4-methoxy-6-methyl- α -pyrone as **2** and **3**, as shown by comparison of the NMR spectra (Table 1). However, the signal of 1'-ketone of **2**

and **3** was not observed in the ^{13}C NMR spectrum of **1**. Two olefinic protons (δ 5.97 and 6.23) were observed and their J value (16.0 Hz) suggested an *E*-configuration. Correlations of COSY from 1'-H (δ 6.23) through 2'-H (δ 5.97) and 3'-H (δ 4.45) to 4'-H (δ 1.36) revealed that its side chain is a 3-hydroxy-1-butenyl group. HMBC study established the connection of C-1' of this side chain to C-5 of the α -pyrone ring (Figure 2). Thus, the planar structure of the new pyrenocine analog, pyrenocine I (**1**), was deduced to be 5-[(1*E*)-3-hydroxy-1-butenyl]-4-methoxy-6-methyl-2*H*-pyran-2-one.

In vitro antitrypanosomal activity of **1**–**4** was evaluated using the GUTat 3.1 strain of *Trypanosoma brucei brucei*, by the method reported earlier.^{3–5} Pyrenocine A (**2**) showed the most potent antitrypanosomal activity, with an IC_{50} value of $0.12 \mu\text{g ml}^{-1}$ (Table 2). Pyrenocine I (**1**) had a similar antitrypanosomal activity ($\text{IC}_{50}=1.8 \mu\text{g ml}^{-1}$) to the commonly used drug suramin, although it was 10-fold less active than **2**. However, among **1**–**3**, **1** was the least cytotoxic against a human embryonic lung fibroblast cells, MRC-5 (Table 2). Citreoviridin (**4**) exhibited antitrypanosomal activity of the same order as **2** and **3** ($\text{IC}_{50}=0.48 \mu\text{g ml}^{-1}$) but was less cytotoxic than both.

Although pyrenocines show several biological activities, reportedly being phytotoxic, antifungal, antibacterial, cytotoxic and antimalarial,^{10–13} there is hitherto no report of their antitrypanosomal activities. In the past, it has been reported that citreoviridin inhibited the mitochondrial F_1 -ATPase of *Trypanosoma cruzi*.¹⁴ The structural similarity of citreoviridin and pyrenocines suggests that pyrenocines may effect their antitrypanosomal activity through a similar mode of action.

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