



Research Article

CHEMICAL CONSTITUENTS OF THE CULTURES OF THE ENDOPHYTIC FUNGUS *BIPOLARIS MAYDIS*

*Tran Thi Minh Dinh, Nguyen Thi Anh Tuyet, Duong Thuc Huy**

Ho Chi Minh City University of Education, Vietnam

**Corresponding author: Duong Thuc Huy – Email: huydt@hcmue.edu.vn*

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ABSTRACT

*Chemical data of *Bipolaris maydis* are little. This fungus was derived from *Kandelia candel*. Three known compounds, *cis-cyclo(L-Val-L-Phe)* (1), *melithasterol B* (2), and *chrysophanol* (3) were isolated from the cultures of *Bipolaris maydis* using multiple chromatographic methods. Their structures were identified using NMR data analyses and then compared with previous reports. Three compounds were reported for the first time in the *Bipolaris* genus.*

Keywords: *Bipolaris maydis*; *chrysophanol*; *ergosterol*; *Kandelia candel*

1. Introduction

Endophytic fungi isolated from higher plants have produced diverse metabolites. These components possess interesting pharmaceutical properties: antioxidant, anticancer, and antiviral (Aly et al. 2010, Deshmukh et al. 2018). The *Bipolaris* causes many plant pathogens (Manamgoda et al. 2014). Recently, the cultures of *Bipolaris* fungi have attracted the attention of many chemists due to their interesting phytochemical data, for examples *B. oryzae*, *B. eleusines*, and *B. sorokiniana* (Ai et al. 2015, Phuwapraisirisan et al. 2007, Qader et al. 2017, Siriwach et al. 2014). *Bipolaris maydis* was separated from the mangrove plant *Kandelia candel* which was popularly distributed in the South of Vietnam. Little is known about chemical constituents of this fungus and its cultures. In this paper, the isolation of three compounds isolated from cultures of *Bipolaris maydis* was described. The structures of isolated compounds were determined using NMR spectroscopic method, followed by the comparison of this study results with previous studies. Three compounds were elucidated as *cis-cyclo(L-Val-L-Phe)* (1), *melithasterol B* (2), and *chrysophanol* (3).

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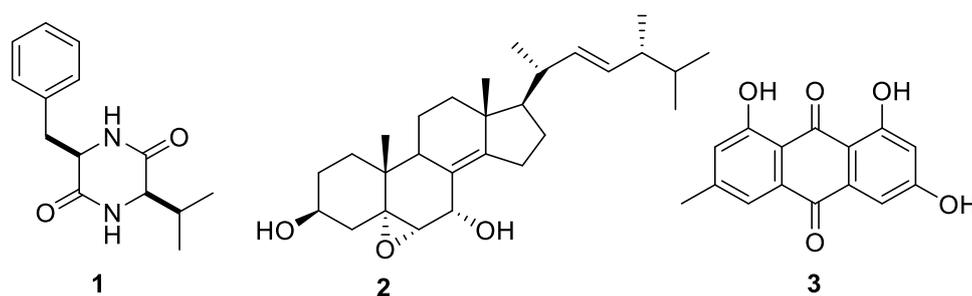


Figure 1. Chemical structures of isolated compounds 1-3

2. Experimental

2.1. General experimental procedures

NMR spectra (1D and 2D) were recorded on Bruker 500 AVANCE spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C) in CDCl_3 , acetone- d_6 , and DMSO- d_6 solutions using tetramethylsilane or residual nondeuterated solvent peak as an internal standard. HRESIMS was recorded on a Bruker MicrOTOF-Q II mass spectrometer. Thin layer chromatography was carried out on precoated Kieselgel 60 F254 or silica gel 60 RP-18 F254S (Merck), and spots were visualized by spraying with 20% H_2SO_4 solution, followed by heating.

2.2. Fungal material

The endophytic fungus was isolated from the leaves of *K. cadell* obtained at Can Gio mangroves, Ho Chi Minh city, Viet Nam. The fungal isolation was carried out applying the method of Ratklao Siriwach (Siriwach et al. 2014). The fungus was identified by sequence analysis of the ITS region using the universal eukaryotic primers of ITS1 and ITS4.

2.3. Fermentation and extraction

Endophytic fungus was grown on potato dextrose agar at 28°C for 5 days. Three pieces ($0.5 \times 0.5 \text{ cm}^2$) of mycelial agar plugs were inoculated into 1000 ml Erlenmeyer flasks containing 200 ml potato dextrose broth and incubated statically at room temperature for 21 days. The mycelia (0.93 kg) were separated by filtration and extracted with equal volumes of EtOAc to provide EtOAc extract, **MEA** (14.47 g).

2.4. Extraction and Isolation

The extract **MEA** (14.47 g) was subjected to normal phase CC to obtain six fractions **A-E**. Fraction **C** (3.21 g) was fractionated by CC, using a gradient of solvent system *n*-hexane-EtOAc-acetone (4:1:1 to 0:1:1, v/v/v) to yield seven subfractions **C1-7**. Fraction **C2** (501.7 mg) was selected for CC, using *n*-hexane-EtOAc (9:1, isocratic) to obtain **2** (7 mg) and **3** (2 mg). Purifying fraction **E** (3.38 g) gave three subfractions **E1-3**. Fraction **E3** (1.1 g) was subjected to reverse phase C18 column chromatography, eluted with acetone-water (5:1, v/v) to provide **1** (14 mg).

- *cis*-Cyclo(L-Val-L-Phe) [(3*S*,6*S*)-3-(1-Methylethyl)-6-(phenylmethyl)-piperazine-2,5-dione] (**1**). Colorless oil; $^1\text{H-NMR}$ (500 MHz, DMSO- d_6) δ_{H} 0.26 (d, 3H, $J = 7.0$ Hz, H-8), 0.64 (d, 3H, $J = 7.0$ Hz, H-9), 1.68 (m, 1H, H-7), 2.87 (dd, 1H, $J = 5.0, 13.5$ Hz, H-10a),

3.14 (dd, 1H, $J = 4.5, 14.0$ Hz, H-10b), 3.52 (m, 1H, H-3), 4.20 (m, 1H, H-6), 7.17 (m, 3H, H-12/16, H-14), 7.23 (m, 2H, H-13/15), 7.89 (s, 1H, NH-1), 8.08 (s, 1H, NH-4). ^{13}C -NMR (125 MHz, DMSO- d_6) δ_{C} 166.6 (C-2), 166.5 (C-5), 136.3 (C-11), 130.3 (C-12/16), 127.9 (C-13/15), 126.4 (C-14), 59.2 (C-3), 55.1 (C-6), 37.9 (C-10), 31.0 (C-7), 18.2 (C-9), 16.2 (C-8) (Stark et al. 2005).

- **Melithasterol B (2)** White amorphous powder; ^1H (CDCl_3 , 500 MHz) and ^{13}C NMR (CDCl_3 , 125 MHz) See Table 1 (Yue et al. 2001).

- **Chrysophanol (3)** Colorless oil; ^1H NMR (Acetone- d_6 , 500 MHz) δ_{H} 12.13 (1H, s, 8-OH), 12.03 (1H, s, 1-OH), 7.83 (1H, t, $J = 8.0$ Hz, H-6), 7.79 (1H, dd, $J = 7.6, 1.6$ Hz, H-5), 7.63 (1H, d, $J = 2.0$ Hz, H-4), 7.37 (1H, dd, $J = 8.4, 1.6$ Hz, H-7), 7.20 (1H, brs, H-2), 2.50 (3H, s, H-11) (Zhang et al. 2012).

3. Results and discussion

The ^1H -NMR spectrum of **2** exhibited the presence of six methyls [δ_{H} 0.88 (*s*, H-18), δ_{H} 0.90 (*s*, H-19), δ_{H} 0.86 (*d*, H-26), δ_{H} 0.86 (*d*, $J = 6.5$ Hz, H-27), δ_{H} 0.94 (*d*, $J = 6.5$ Hz, H-28), δ_{H} 1.03 (*d*, $J = 6.5$ Hz, H-21)], indicative for a sterol skeleton and three oxymethine groups at δ_{H} 3.76 (*m*), 4.33 (*m*) and 2.99 (*d*, $J = 3.5$ Hz). Moreover, the ^1H -NMR spectrum also revealed two olefin protons at δ_{H} 5.25 (*dd*, $J = 15.5$ Hz, 6.5 Hz) and δ_{H} 5.28 ($J = 15.0$ Hz, 6.5 Hz). The analysis of the coupling pattern of these two protons indicated that they were *trans*-coupled and represented for the double bond at C-22 and C-23 of an ergosterol scaffold. The ^{13}C -NMR spectrum in accordance with HSQC spectrum exhibited the presence of 28 carbons: four sp^2 carbons (δ_{C} 127.5, 132.6, 136.4, and 151.0), four oxygenated carbons (δ_{C} 68.6, 67.2, 65.4, and 62.3), seven methylenes, five upfield methines, six methyls, and two quaternary carbons (Table 1).

HMBC correlations of H₃-18 to C-6 (δ_{C} 62.3) and C-9 (δ_{C} 40.0), of H-6 to C-7 (δ_{C} 65.4) and C-8 (δ_{C} 127.5), and of H-7 to C-8 and C-9 indicated the connectivity through C-5-C-6-C-7-C-8-C-9-C-10. In addition, HMBC correlation of H₃-19 to C-14 (δ_{C} 151.0) indicated the presence of the double bond at C-8 and C-14. The NMR comparison of **2** and melithasterol B (Yue et al. 2001) resulted in high similarity, thus **2** was elucidated as melithasterol B.

Table 1. NMR data of **2** and Melithasterol B

N	Melithasterol B (Pyridine- d_5)		2 (CDCl_3)	
	δ_{H} (multi, J)	δ_{C}	δ_{H} (multi, J)	δ_{C}
1		32.3		32.7
2		27.7		28.0
3	4.30 (<i>m</i>)	68.3	3.76 (<i>m</i>)	68.6
4		40.8		40.5

5		66.9		67.2
6	3.37 (d, 3.2)	62.5	2.99 (d, 3.5)	62.3
7	4.67 (d, 3.2)	65.2	4.33 (d, 4.5)	65.4
8		127.3		127.5
9		39.5		40.0
10		36.5		36.8
11		19.6		19.9
12		37.1		37.5
13		43.2		43.6
14		150.5		151.0
15		25.2		25.2
16		33.1		33.2
17		57.3		57.8
18	0.90 (s)	18.4	0.88 (s)	18.4
19	0.91 (s)	16.7	0.90 (s)	16.7
20		40.1		40.2
21	1.03 (d, 6.7)	21.5	1.04 (d, 7.0)	21.6
22	5.19 (m)	136.0	5.25 (dd, 15.5, 6.5)	136.4
23	5.31 (m)	132.3	5.28 (dd, 15.0, 6.5)	132.6
24		43.1		43.8
25		33.4		33.9
26	0.84 (d, 7.0)	19.86	0.86 (d, 6.5)	19.9
27	0.86 (d, 7.0)	20.14	0.86 (d, 6.5)	20.4
28	0.96 (d, 6.8)	17.81	0.94 (d, 7.0)	18.0

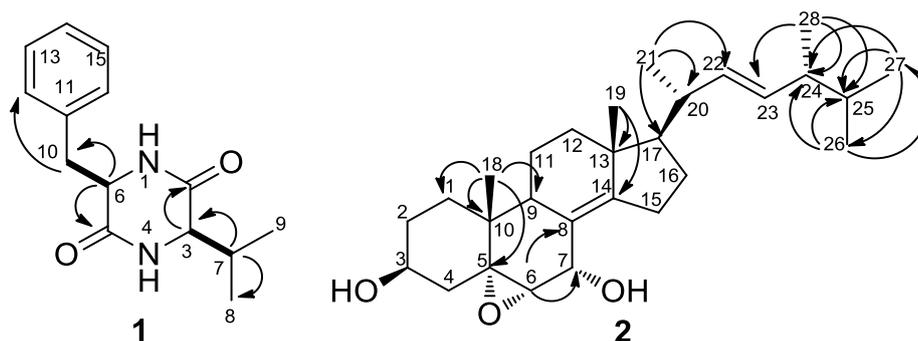


Figure 2. Some key HMBC correlations of **1** and **2**

4. Conclusions

From the cultures of *Bipolaris maydis*, *cis*-cyclo(L-Val-L-Phe) (**1**), melithasterol B (**2**), and chrysophanol (**3**) were isolated and elucidated. To the best of our knowledge, all compounds (**1-3**) were isolated from this species for the first time.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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THÀNH PHẦN HÓA HỌC CỦA SINH KHỐI CỦA LOÀI NẤM *BIPOLARIS MAYDIS***Trần Thị Minh Định, Nguyễn Thị Ánh Tuyết, Dương Thúc Huy****Trường Đại học Sư phạm Thành phố Hồ Chí Minh, Việt Nam***Tác giả liên hệ: Dương Thúc Huy – Email: huydt@hcmue.edu.vn**Ngày nhận bài: 12-10-2020; ngày nhận bài sửa: 14-12-2020; ngày duyệt đăng: 11-3-2021***TÓM TẮT**

*Dữ liệu thành phần hóa học của *Bipolaris maydis* ít được nghiên cứu. Loài nấm này được phân lập từ *Kandelia candel*. Ba hợp chất *cis-cyclo(L-Val-L-Phe)* (1), *melithasterol B* (2) và *chrysophanol* (3) đã được phân lập từ sinh khối của loài nấm *Bipolaris maydis* bằng nhiều phương pháp sắc kí khác nhau. Cấu trúc hóa học của các hợp chất được xác định bằng các phương pháp phổ nghiệm NMR cũng như so sánh với dữ liệu đã công bố. Ba hợp chất trên lần đầu tiên được phân lập từ chi *Bipolaris*.*

Từ khóa: *Bipolaris maydis*; *chrysophanol*; *ergosterol*; *Kandelia candel*