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白干皮孔菌发酵液的化学成分研究

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摘 要:首次从白干皮孔菌[*Skeletocutis nivea* (Jungh.) Keller]发酵液提取物的乙酸乙酯层中分离纯化了3个化合物。通过质谱、核磁等方法对其进行结构鉴定,分别为:monomethylsulochrin(**1**)、6-methoxyspirotryprost atin B(**2**)、pseurotin A(**3**)。

关键词:白干皮孔菌;化学成分;结构鉴定

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Chemical Constituents of the Fermentation Broth of *Skeletocutis nivea* (Jungh.) Keller

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Abstract: Three compounds were firstly isolated from the EtOAc extracts of the fermentation broth of *Skeletocutis nivea* (Jungh.) Keller. Based on the spectroscopic data, their structures were determined as: monomethylsulochrin (**1**), 6-methoxyspirotryprost atin B (**2**) and pseurotin A (**3**).

Key words: *Skeletocutis nivea* (Jungh.) Keller; chemical constituents; structural identification

Introduction

Skeletocutis nivea (Jungh.) Keller is classified within the fungi Basidiomycota. They could be predominantly found from broad leaved trees[1]. For the purpose of seeking new natural products or even promising new drug leads, the chemical constituents of the fermentation broth of this higher fungus were studied. In this paper, we reported the isolation and structural identification of monomethylsulochrin (**1**), 6-methoxyspirotryprost atin B (**2**) and pseurotin A (**3**) (Fig. 1).

Materials and Methods

General experimental procedures

NMR spectra were acquired on Bruker AV-600, DRX-500 and AV-400 spectrometers at room temperature, in $CDCl_3$, δ in ppm, and J in Hz. ESI-MS were recorded on a VG Autospec-300 spectrometer. Silica gel (200-300 mesh, Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham BioSciences, Sweden) were used for column chromatography (CC). Precoated silica gel GF254 plates (Qingdao Marina Chemical, Inc., China) were used for thin layer chromatography (TLC). Preparative HPLC was performed on an Agilent 1100 liquid chromatography system equipped with a Zorbax SB-C_{18} column (9.4 mm × 150 mm).

Fungal material and culture

The fungus *Skeletocutis nivea* (Jungh.) Keller was collected from Bawangling National Nature Reserve, Hainan, China, on Nov. 26th 2010, and identified by Prof. DAI Yu-cheng, Beijing Forestry University (BFU). The voucher specimen was deposited in the Institute of Microbiology, BFU. The culture medium consisted of pota-

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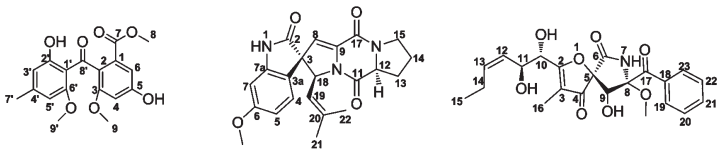
to (peeled, 200 g), glucose (20 g), KH_2PO_4 (3 g), $MgSO_4$ (1.5 g), citric acid (0.1 g) and thiamine hydrochloride (10 mg) per 1 L of deionized H_2O. The pH value was adjusted to 6.5 before autoclaving. Fermentation was carried out on a shaker at room temperature and 150 rpm for 25 days.



1 2 3

Fig. 1 Chemical structures of compounds 1, 2 and 3

Extraction and Isolation

The fermentation broth of *S. nivea* (Jungh.) Keller (20 L) was initially filtered, and the filtrate was extracted 3 times with EtOAc. The organic layer was concentrated under reduced pressure to give a crude extract (6.0 g). The crude extract was subjected to CC over silica gel (200-300 mesh) with a $CHCl_3$-MeOH gradient solvent system to produce fractions A-H. Fraction C (1.5 g) was further repeatedly eluted with a gradient solvent system of petroleum ether-EtOAc to give compounds **4** (6.2 mg), **5** (6.0 mg) and **6** (3.8 mg). Compound **1** (2 mg) was also obtained from Fraction C by preparative HPLC (CH_3CN-H_2O) after repeated silica gel CC. Fraction E (0.9 g) was purified over silica gel CC (petroleum ether-Acetone), then by preparative HPLC (CH_3CN-H_2O) to provide compound **2** (1.2 mg) and **3** (1.0 mg). Compound **7** (5 mg) was gained by reversed-phase C_{18} CC (MeOH-H_2O), Sephadex LH-20 (MeOH) from fraction G (0.8 g), after which was eluted with gradient solvent ($CHCl_3$-MeOH).

Results and Discussion

Structural identification

Compound 1 was obtained as yellowish powder; ESI-MS *m/z* 369 $[M + Na]^+$, *m/z* 385 $[M + K]^+$, *m/z* 715 $[2M + Na]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.59 (1H, d, J = 2.4 Hz, H-4), 7.00 (1H, d, J = 2.4 Hz, H-6), 3.68 (3H, s, H-8), 3.67 (3H, s, H-9), 6.43 (1H, s, H-3′), 6.04 (1H, s, H-5′), 2.27 (3H, s, H-7′) 3.35 (3H, s, H-9′); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 128.6 (C-1), 128.3 (C-2), 156.3 (C-3), 103.1 (C-4), 157.3 (C-5), 108.0 (C-6), 166.3 (C-7), 52.5 (C-8), 56.4 (C-9), 111.2 (C-1′), 164.5 (C-2′), 110.5 (C-3′), 148.3 (C-4′), 103.3 (C-5′), 161.1 (C-6′), 22.7 (C-7′), 199.8 (C-8′), 55.9 (C-9′). Its structure was identified as monomethylsulochrin by comparison of its spectroscopic data with those reported in the literature[2].

Compound 2 was obtained as pale yellow, amorphous powder; ESI-MS *m/z* 416 $[M + Na]^+$, *m/z* 432 $[M + K]^+$, *m/z* 809 $[2M + Na]^+$; ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.51 (1H, s, H-1), 6.93 (1H, d, J = 8.4 Hz, H-4), 6.50 (1H, dd, J = 8.4, 2.4 Hz, H-5), 6.41 (1H, d, J = 2.4 Hz, H-7), 5.76 (1H, s, H-8), 4.32 (1H, dd, J = 10.8, 6.0 Hz, H-12), 2.46 (1H, m, Ha-13), 1.96 (1H, m, Hb-13), 2.10 (1H, m, Ha-14), 1.96 (1H, m, Hb-14), 3.81 (1H, m, Ha-15), 3.55 (1H, m, Hb-15), 5.36 (1H, d, J = 9.0 Hz, H-18), 5.20 (1H, d, J = 9.0Hz, H-19), 1.57 (3H, s, H-21), 1.27 (3H, s, H-22), 3.78 (3H, s, CH_3O-6); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 178.6 (C-2), 61.5 (C-3), 119.0 (C-3a), 128.8 (C-4), 107.3 (C-5), 160.8 (C-6), 97.2 (C-7), 141.6 (C-7a), 116.9 (C-8), 138.2 (C-9), 162.7 (C-11), 61.8 (C-12), 29.5 (C-13), 22.3 (C-14), 45.1 (C-15), 155.3 (C-17), 64.3 (C-18), 120.6 (C-19), 138.6 (C-20), 25.6 (C-21), 18.5 (C-22), 55.7 (OCH_3-6). Its structure was identified as 6-Methoxyspirotryprostatin B by comparison of its spectroscopic data with those reported in the literature[3].

Compound 3 was obtained as amorphous white powder; ESI-MS *m/z* 454 $[M + Na]^+$, *m/z* 470 $[M + K]^+$, *m/z* 885 $[2M + Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.14 (1H, s, H-7), 4.70 (1H, s, H-9),

4.59 (1H, d, J = 4.0 Hz, H-10), 4.75 (1H, dd, J = 4.0, 9.0 Hz, H-11), 5.27 (1H, dd, J = 9.0, 7.6 Hz, H-12), 5.61 (1H, m, H-13), 2.17 (1H, m, Ha-14), 2.10 (1H, m, Hb-14), 0.99 (3H, t, J = 7.5 Hz, CH_3-15), 1.68 (3H, s, CH_3-16), 8.32 (2H, d, J = 7.5 Hz, H-19, H-23), 7.50 (2H, t, J = 7.5 Hz, H-20, H-22), 7.65 (t, J = 7.5 Hz, H-21), 3.44 (3H, s, CH_3O-8); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 185.8 (C-2), 113.3 (C-3), 196.3 (C-4), 92.8 (C-5), 166.6 (C-6), 90.3 (C-8), 70.3 (C-9), 70.6 (C-10), 71.0 (C-11), 126.4 (C-12), 136.5 (C-13), 21.4 (C-14), 14.1 (C-15), 6.1 (C-16), 195.2 (C-17), 132.3 (C-18), 130.7 (C-19, C-23), 128.7 (C-20, C-22), 134.8 (C-21), 51.7 (CH_3O-8). Its structure was identifiedas pseurotin A by comparison of its spectroscopic data with those reported in the literature[4].

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(上接第 189 页)

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