来源于印度洋深海区的烟曲霉次生代谢产物的研究

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摘要: 为了研究烟曲霉次生代谢产物, 我们采用硅胶柱层析、sephadex LH-20 凝胶柱层析、半制备液相色谱等分离方法对来源于印度洋深海区的烟曲霉 (*Aspergillus fumigatus*)的乙醇提取液进行系统的次生代谢产物分离, 根据所分离得到的化合物的理化性质和波谱数据确定化合物的结构。共分离鉴定了 15 个化合物, 其中 11 个二酮哌嗪类生物碱(diketopiperazine), 4 个喹唑啉类衍生物 (fumiquinazoline), 分别为: fumitremorgin B (**1**) , 13-oxofumitremorgin B (**2**), fumitremorgin C (**3**), 12-hydroxyfumitremorgin C (**4**), cyclotyprostatins B (**5**), cyclotyprostatins C (**6**), cyclotyprostatins E (**7**), verruculogen (**8**), spirotryprostatin C (**9**), spirotryprostatin D (**10**), spirotryprostatin F (**11**), fumiquinazoline C (**12**), fumiquinazoline D (**13**), 3-hydroxyfumiquinazoline A (**14**)和 fumiquinazoline E (**15**)。分离得到的 15 个化合物, 丰富了烟曲霉次生代谢产物的类型, 为烟曲霉的开发利用提供了化学研究基础。

关键词: 烟曲霉(*Aspergillus fumigatus*); 曲霉属(*Aspergillus*); 次生代谢产物; 化学成分; 波谱数据

中图分类号: R914.4　　**文献标识码**: A　　**文章编号**: 1000-3096(2019)08-0043-07

DOI: 10.11759/hykx20190411002

烟曲霉(*Aspergillus fumigatus*)属于丝孢菌纲(Hyphomycetes)、丛梗孢目(Hyphomycetales)、曲霉属(*Aspergillus*)真菌[1], 为曲霉属中最常见的菌种之一。烟曲霉来源很广泛, 如药用植物, 植物共生菌和动物器官等, 其产生的某些代谢成分能够引起机体的严重损害, 导致侵袭性曲霉病感染的形成和扩散, 严重危及生命健康。比如, 烟曲霉的主要次生代谢产物烟曲霉毒素 (Fumitremorgin) 严重危害畜禽健康及食品安全, 是对亚洲地区家禽类和农作物类致病力最强、危害最大的霉菌毒素[2]。

不同于传统的陆生真菌, 海洋真菌因其特有的生存条件, 使得其具有特殊的代谢体系以及耐碱和耐盐的生理特性, 从而能够产生许多结构特异、药理作用优良的次生代谢产物[3]。自 20 世纪 50 年代 Newton[2]等学者从海洋来源的头孢霉菌 (*Cephalosporium acremonium*) 的发酵物中发现抗菌化合物 Cephalosporin C 以来, 海洋微生物的抗菌活性研究日益成为新药研究领域的热门。已经进入治疗非小细胞肺癌 II 期临床实验阶段的 Plinabulin (NPI-2358)[4], 就是从曲霉属海洋真菌 *Aspergillus* sp. CNC-139 中获得的次生代谢产物为模板合成的。鉴于海洋来源的真菌次生代谢产物丰富的化学结构和多样的生物活性, 我们对来源于海拔下 2 434 m 印度洋深海热液区深海沉积物的烟曲霉, 采用大米固体发酵, 对其发酵物的乙醇提取液进行了系统的次生代谢产物化学成分研究, 分离鉴定了 11 个二酮哌嗪类生物碱(diketopiperazine)和 4 个喹唑啉类衍生物 (fumiquinazoline), 分别为: fumitremorgin B (**1**), 13-oxofumitremorgin B (**2**), fumitremorgin C (**3**), 12-hydroxyfumitremorgin C (**4**), cyclotyprostatins B (**5**), cyclotyprostatins C (**6**), cyclotyprostatins E (**7**), verruculogen (**8**), spirotryprostatin C (**9**), spirotryprostatin D (**10**), spirotryprostatin F (**11**), fumiquinazoline C (**12**), fumiquinazoline D (**13**), 3-hydroxyfumiquinazoline A (**14**)和 fumiquinazoline E (**15**)(图 1)。化合物 **13** 和 **15** 为首次从该菌种发现, 丰富了烟曲霉次生代谢产物类型, 为烟曲霉的开发利用提供了化学基础。

1　仪器与试剂

旋光仪: Perkin-Elmer 341 polarimeter; 红外光谱仪: Bruker Vertex 70; 紫外全波长扫描仪: Varian

收稿日期: 2019-04-11; 修回日期: 2019-05-25

基金项目: 国家自然科学基金青年项目(31600266)

[Foundation: Project supported by the National Science Foundation for Young Sciencists of China, No. 31600266]

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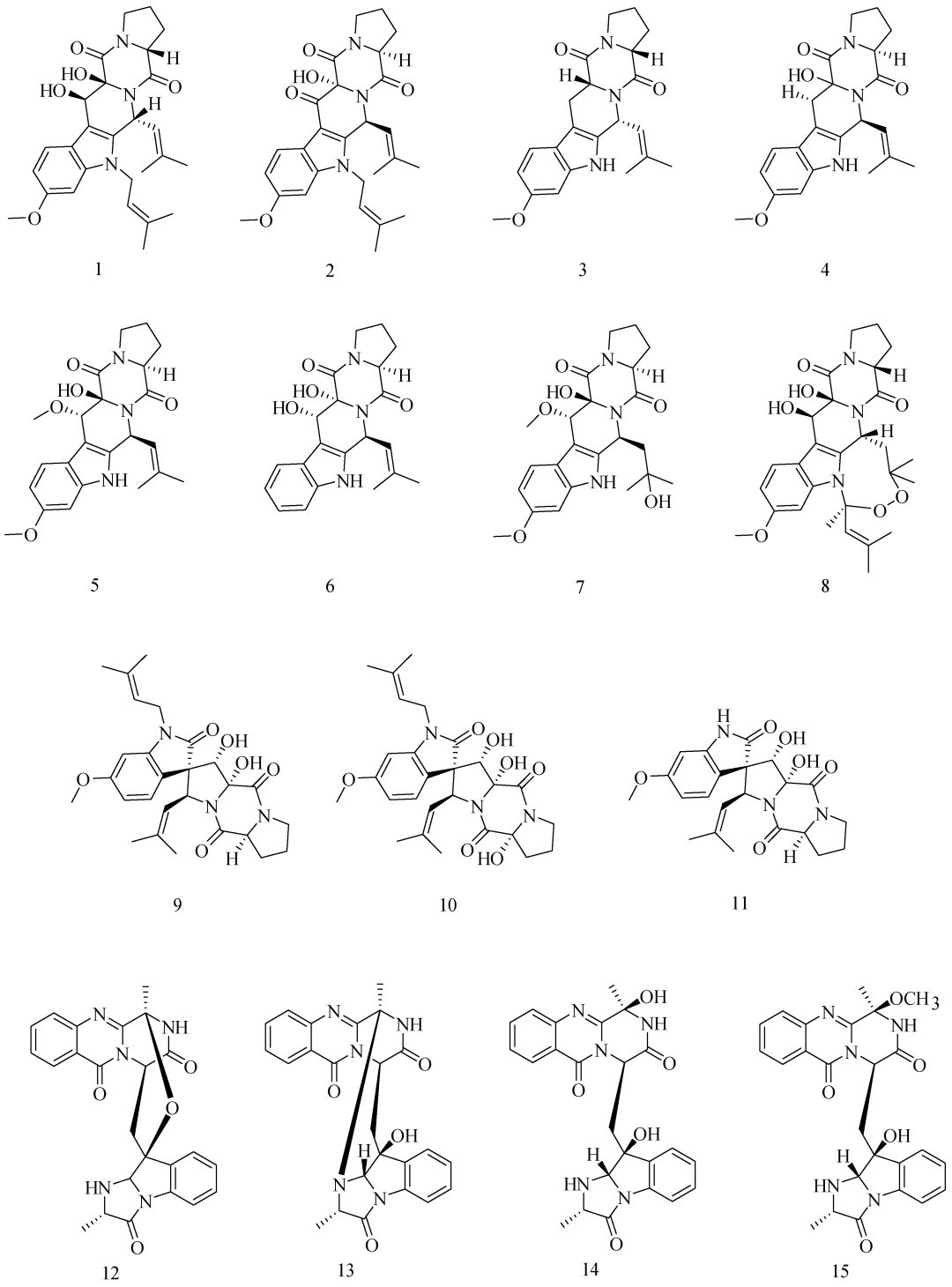

图 1　烟曲霉分离纯化得到的化合物结构

Fig. 1　Chemical structures of compounds 1-15 isolated from *Aspergillus fumigatus*

Cary 50; 高分辨质谱仪: Thermo Fisher LC-LTQ-Orbitrap XL spectrometer; 圆二色光谱: JASCO-810 CD spectrometer; 核磁共振仪: Bruker AM-400; 高效液相色谱仪: 美国 Dionex 公司和美国 Agilent 公司; C_{18}反相填料: 德国 Merck 公司; 凝胶填料: 瑞典 GE Healthcare Bio-Sciences AB 公司; 柱层析、薄层层析硅胶: 青岛海洋化工厂。其他所用试剂均为分析纯或色谱纯。

2 菌株来源

烟曲霉菌株于2014年购买于中国海洋微生物菌种保藏管理中心(Marine Culture Collection of China, MCCC 3A00161), 来源于印度洋深海热液区深海沉积物, 海拔下2 434 m。菌株固体斜面样品保存于华中科技大学同济医学院药学院天然药物化学教研室, 由汪建平副教授通过提取基因组, 基因测序比对鉴定为烟曲霉。

3 菌株发酵及提取分离

3.1 菌株发酵培养

烟曲霉保藏菌株转移接种到PDA培养基上, 放置于28℃恒温箱。该菌生长较快, 约1周后观察菌落表面孢子呈墨绿色块状, 菌落背面呈黄褐色, 可见绒毛状分生孢子。之后将种子培养基在无菌条件下按照10%的接种量转接到灭过菌的混合培养基(0.5%蛋白胨混合固体大米)中, 1 L锥形瓶中含200 g大米和200 mL水, 123℃灭菌0.5 h, 搅拌均匀, 之后静置于28℃培养4周。

3.2 提取分离

室温下, 30 kg烟曲霉大米发酵物加95%乙醇浸泡10 h, 提取6次, 浸提液减压浓缩后得总浸膏(2 kg)。总浸膏用水混悬后, 加等体积的乙酸乙酯溶剂进行萃取, 共得到287 g乙酸乙酯部位萃取物。乙酸乙酯部位进行硅胶柱层析(80~100 目), 二氯甲烷-甲醇(100∶0→0∶100)梯度洗脱, 用TLC检测并合并相同流分, 得到6个组分(A—F)。

组分B经Sephadex LH-20凝胶柱(二氯甲烷∶甲醇, 1∶1)和正相硅胶柱层析(石油醚∶丙酮, 15∶1)划段得到B1和B2两个主要部位; B2经高效液相色谱(乙腈/水40/60, 220 nm)得化合物 **12**(28.23 mg)。B1经反相中压色谱柱(甲醇-水, 20∶80→100∶0)得到3个组分B1-1, B1-2和B1-3, B1-1和B1-2分别经半制备高效液相色谱柱纯化, 得到化合物 **1**(24.12 mg)和 **8**(5.78 mg)。

组分C先用反相中压色谱仪(MPLC)以MeOH-H_2O系统梯度洗脱(30%甲醇→100%甲醇)得到4个极性部分C1—C4。C1再次经MPLC(40/60→80/20甲醇/水)得到C1-1→C1-44个组分, 其中C1-3经正相硅胶色谱及反复的HPLC纯化得到化合物 **3**(16.67 mg), **11**(6.54 mg), **13**(26.45 mg), **14**(6.99 mg)和 **15** (3.40 mg)。C4经Sephadex LH-20凝胶、MPLC、正相硅胶柱、半制备HPLC等色谱分离方法进行分离纯化, 最终得到化合物 **2**(17.0 mg), **4**(6.35 mg)和 **9**(12.3 mg)。

组分E先经Sephadex LH-20凝胶柱(二氯甲烷∶甲醇 1∶1)划段得到3个亚组分E1—E3。YQ3-3-1, YQ3-3-2和YQ3-3-3。E2经MPLC(甲醇/水)反复梯度洗脱、正相硅胶柱层析及半制备HPLC(乙腈/水85/15)纯化得单体化合物 **7**(3.56 mg)和化合物 **10**(6.89 mg)。E3经正相硅胶柱层析(二氯甲烷∶甲醇100∶1→0∶100)划段, 然后经MPLC(MeOH/H_2O 30∶70→100∶0 甲醇)及半制备液相色谱纯化得到化合物 **5**(10.90 mg)和化合物 **6**(10.35 mg)。

4 结构鉴定

化合物 **1**: 白色无定型粉末, $[\alpha]_D^{20}$–13.7 (c = 0.22, $CHCl_3$); ESI-MS: m/z 502.2280 $[M + Na]^+$, 分子式为$C_{27}H_{33}N_3O_5$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 5.87 (1H, d, J=9.97 Hz, H-3), 4.51 (1H, dd, J=4.63, 9.21 Hz, H-9), 5.52 (1H, d, J=1.95 Hz, H-13), 7.67 (1H, d, J=8.66 Hz, H-16), 6.66 (1H, dd, J=2.16, 8.67 Hz, H-17), 6.74 (1H, d, J=2.04 Hz, H-19), 4.48 (2H, d, J=6.1 Hz, H-21), 4.94 (1H, t, J=6.03 Hz, H-22), 1.84 (3H, s, H-24), 1.66 (3H, s, H-25), 4.73 (1H, d, J=10.0 Hz, H-26), 1.58 (3H, s, H-28), 1.91 (3H, s, H-29), 3.73 (3H, s, H-30). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C: 130.5 (C-2), 48.2 (C-3), 170.6 (C-5), 58.6 (C-6), 29.1 (C-7), 22.4 (C-8), 45.2 (C-9), 166.3 (C-11), 83.4 (C-12), 68.3 (C-13), 106.5 (C-14), 120.7 (C-15), 121.3 (C-16), 108.9(C-17), 155.6 (C-18), 93.7 (C-19), 137.6 (C-20), 41.3 (C-21), 120.6 (C-22), 133.9 (C-23), 18.4 (C-24), 25.6 (C-25), 123.8 (C-26), 134.3 (C-27), 18.2 (C-28), 25.6 (C-29), 55.9 (C-30)。通过与文献[5]报道中的数据对比, 确定此化合物为吲哚二酮哌嗪类生物碱fumitremorgin B。

化合物 **2**: 无色固体结晶, $[\alpha]_D^{20}$+21.5 (c = 0.41, $CHCl_3$); δ_H: 6.02 (1H, d, J= 9.78 Hz, H-3), 2.35 (1H, m, H-7a), 2.11 (1H, m, H-7b), 2.09 (1H, m, H-8a), 1.93 (1H, m, H-8b), 3.61 (2H, dd, J=9.02, 16.71 Hz, H-9), 8.11 (1H, d, J=8.63 Hz, H-16), 6.88 (1H, dd, J=8.62, 1.99 Hz, H-17), 6.62 (1H, d, J=1.94 Hz, H-19), 4.47 (2H, m, H-21), 4.95 (1H, t, J=5.56 Hz, H-22), 1.82 (3H, s, H-24), 1.71 (3H, s, H-25), 1.94 (3H, s, H-28), 1.63 (3H, s, H-29), 3.82(3H, s, H-18-OCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C: 147.5 (C-2), 48.7 (C-3), 165.2 (C-5), 60.2 (C-6), 28.6 (C-7), 23.2 (C-8), 45.5 (C-9), 173.1 (C-11), 81.9 (C-12), 181.5 (C-13), 108.2 (C-14), 118.5 (C-15), 123.0 (C-16), 111.7 (C-17), 55.6 (C-18), 94.9 (C-19), 139.0 (C-20), 42.7 (C-21), 118.3 (C-22), 136.4 (C-23), 18.3 (C-24), 25.5 (C-25), 122.1 (C-26),

138.2 (C-27), 18.7 (C-28), 25.9 (C-29), 55.8 (C-18-OCH 3)。以上数据与文献[6]对照，确定该化合物为二酮哌嗪类化合物 13-oxofumitremorgin B。

化合物 **3**: 白色粉末, $[\alpha]_D^{20}$–7.9 (c = 0.13, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 5.85 (1H, d, J = 9.78 Hz, H-3), 4.27 (2H, m, H-6, 12), 2.20 (1H, m, H-7a), 2.01(2H, m, H-8a), 1.85 (2H, m, H-7b, 8b), 3.44 (1H, dd, J=5.86, 7.71 Hz, H-9), 3.31 (1H, dd, J=15.79, 5.21 Hz, H-13a), 2.86 (1H, dd, J=15.82, 10.96 Hz, H-13b), 7.42 (1H, d, J=8.26 Hz, H-16), 6.66(1H, dd, J=8.32, 2.36 Hz, H-17), 6.84 (1H, d, J=2.42 Hz, H-19), 4.84 (1H, d, J=9.24 Hz, H-21), 1.59(3H, s, H-23), 1.94 (3H, s, H-24), 3.81 (3H, s, H-25). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C: 130.8 (C-2), 48.3 (C-3), 167.5 (C-5), 56.8 (C-6), 26.4 (C-7), 21.1 (C-8), 43.2 (C-9), 163.8 (C-11), 54.4 (C-12), 19.3 (C-13), 103.2 (C-14), 116.8 (C-15), 118.7 (C-16), 106.8 (C-17), 153.8(C-18), 93.3 (C-19), 135.2 (C-20), 123.2 (C-21), 131.2 (C-22), 23.8 (C-23), 16.3 (C-24), 53.7 (C-25)。以上数据与文献[7]对照，确定该化合物为二酮哌嗪类化合物 fumitremorgin C。

化合物 **4**: 无色针晶固体, $[\alpha]_D^{20}$+15.8 (c = 0.15, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 6.02 (1H, d, J=9.52 Hz, H-3), 4.38 (2H, dd, J=9.47 Hz, H-6), 3.65 (1H, d, J=15.97 Hz, H-13a), 3.27 (1H, d, J=16.24 Hz, H-13b), 7.53 (1H, d, J=8.68 Hz, H-16), 6.87 (1H, dd, J=2.14, 8.63 Hz, H-17), 6.85 (1H, d, J=2.12 Hz, H-19), 4.76 (2H, d, J=9.37 Hz, H-21), 1.88 (3H, s, H-23), 1.61(3H, s, H-24), 3.78 (3H, s, H-25). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C: 131.8 (C-2), 50.2(C-3), 170.9 (C-5), 59.2 (C-6), 29.2 (C-7), 22.8 (C-8), 45.6 (C-9), 164.3 (C-11), 84.3 (C-12), 30.4 (C-13), 103.5 (C-14), 121.8 (C-15), 119.2 (C-16), 109.9 (C-17), 157.4 (C-18), 95.8 (C-19), 137.6 (C-20), 123.1 (C-21), 134.5 (C-22), 25.8 (C-23), 18.4 (C-24), 55.7 (C-25)。以上数据与文献[8]对照，确定该化合物为二酮哌嗪类化合物烟曲霉毒素 12-hydroxyfumitremorgin C。

化合物 **5**: 淡黄色结晶, $[\alpha]_D^{20}$+19.9 (c = 0.37, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 6.65 (1H, d, J=9.70 Hz, H-3), 4.38 (2H, m, H-6), 2.44 (1H, m, H-7a), 1.96 (1H, m, H-7b), 2.13 (1H, m, H-8a), 1.98 (1H, m, H-8b), 3.72 (2H, m, H-9), 7.45 (1H, d, J= 8.63 Hz, H-16), 6.81 (1H, dd, J=8.62, 2.23 Hz, H-17), 6.88 (1H, d, J=2.12 Hz, H-19), 5.56 (1H, d, J=9.72 Hz, H-21), 1.78 (3H, d, J=0.93 Hz, H-23), 2.04 (3H, d, J=1.08 Hz, H-24), 3.37 (3H, s, H-13-OCH_3), 3.85 (3H, s, H-18-OCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C: 133.7 (C-2), 49.2 (C-3), 167.1 (C-5), 60.1 (C-6), 29.8 (C-7), 22.2 (C-8), 45.9 (C-9), 166.0 (C-11), 84.9 (C-12), 76.7 (C-13), 105.4 (C-14), 122.6 (C-15), 118.5 (C-16), 110.2 (C-17), 156.7 (C-18), 95.4 (C-19), 136.8 (C-20), 123.6 (C-21), 138.0 (C-22), 26.2 (C-23), 18.3 (C-24), 56.8 (C-13-OCH_3), 55.7 (C-18-OCH_3)。以上数据与文献[9]对照，确定该化合物为二酮哌嗪类化合物 cyclotyprostatins B。

化合物 **6**: 白色针晶固体, $[\alpha]_D^{20}$+27.8 (c = 0.22, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 7.93(1H, brs, H-1), 5.94 (1H, d, J=9.70 Hz, H-3), 4.47 (2H, dd, J=1.02, 7.15 Hz, H-6), 2.48 (1H, m, H-7a), 2.08 (1H, m, H-7b), 2.13 (1H, m, H-8a), 1.98 (1H, m, H-8b), 3.62 (2H, m, H-9), 5.77 (1H, d, J=2.88 Hz, H-13), 7.84 (1H, d, J=7.63 Hz, H-16), 7.15 (1H, t, J=7.62 Hz, H-17), 7.19 (1H, t, J=7.82, 1.03 Hz, H-18), 7.33 (1H, d, J=7.78 Hz, H-19), 4.86 (1H, m, J=9.72 Hz, H-21), 1.68 (3H, d, J=0.93 Hz, H-23), 2.04 (3H, d, J=1.02 Hz, H-24). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C: 131.5 (C-2), 49.9 (C-3), 171.0 (C-5), 59.1 (C-6), 29.2 (C-7), 22.5 (C-8), 45.3 (C-9), 166.0 (C-11), 84.9 (C-12), 68.7 (C-13), 105.3 (C-14), 125.9 (C-15), 119.7 (C-16), 120.7 (C-17), 122.5 (C-18), 111.1 (C-19), 136.6 (C-20), 123.8 (C-21), 135.0 (C-22), 21.4 (C-23), 18.2 (C-24)。以上数据与文献[9]对照，确定该化合物为二酮哌嗪类化合物 cyclotyprostatins C。

化合物 **7**: 浅黄色无定型粉末, $[\alpha]_D^{20}$+24.2 (c = 0.22, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H: 5.97 (1H, dd, J=5.96, 6.02 Hz, H-3), 4.29 (1H, dd, J=6.02, 11.03 Hz, H-6), 2.51 (1H, m, H-7a), 1.97 (1H, m, H-7b), 2.13 (1H, m, H-8a), 2.02 (1H, m, H-8b), 2.23 (1H, d, J=6.31 Hz, H-9a), 2.15 (1H, d, J=6.26 Hz, H-9b), 4.83 (1H, s, H-13), 7.48 (1H, d, J=8.96 Hz, H-16), 6.76 (1H, dd, J=2.46, 8.98 Hz, H-17), 6.97 (1H, d, J=2.52 Hz, H-19), 3.69 (2H, m, H-21), 1.37 (1H, s, H-23), 1.43 (3H, s, H-24), 3.37 (3H, s, H-13-OCH_3), 4.83 (3H, s, H-18-OCH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C: 136.3 (C-2), 49.2 (C-3), 1167.8 (C-5), 60.7 (C-6), 31.0 (C-7), 22.7 (C-8), 46.2 (C-9), 169.2 (C-11), 87.4 (C-12), 77.7 (C-13), 105.2 (C-14), 138.2 (C-15), 119.4 (C-16), 110.8 (C-17), 157.4 (C-18), 96.1 (C-19), 123.7 (C-20), 50.6 (C-21), 71.4 (C-22), 31.5 (C-23), 29.1 (C-24), 57.4 (C-13-OCH_3), 56.2 (C-18-OCH_3)。以上数据与文献[10]对照，确定此化合物为二酮哌嗪类化合物 cyclotryprostatin E。

化合物 **8**: 淡黄色晶体, $[\alpha]_D^{20}$+4.7 (c = 0.20, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H: 6.04 (1H, d, J=10.07 Hz, H-3), 4.49 (1H, dd, J=9.45, 7.13 Hz, H-6), 3.64 (2H, dd, J=8.73, 4.33 Hz, H-9), 4.07 (1H, s, H-12-OH), 5.68 (1H, s, H-13), 4.76 (1H, s, H-13-OH), 7.90 (1H, d, J=8.73 Hz, H-16), 6.83 (1H, dd, J=8.73, 2.12 Hz, H-17), 6.59 (1H, d, J=2.02 Hz, H-19), 6.64 (1H, d, J=8.08 Hz, H-21), 5.04 (1H, d, J=8.13 Hz, H-22), 1.74 (3H, s, H-24), 2.00 (3H, s, H-25), 1.02 (3H, s, H-28), 1.72 (3H, s, H-29), 3.83 (3H, s, H-18-OCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C: 131.7 (C-2), 48.9 (C-3), 166.2 (C-5), 58.7 (C-6), 29.0 (C-7), 22.6 (C-8),

51.1 (C-9), 170.7 (C-11), 82.5 (C-12), 68.6 (C-13), 105.5 (C-14), 121.0 (C-15), 121.6 (C-16), 109.3 (C-17), 156.3 (C-18), 93.9 (C-19), 136.2 (C-20), 85.8 (C-21), 118.5 (C-22), 143.1 (C-23), 18.8 (C-24), 24.3 (C-25), 55.4 (C-18-OCH_3)。以上数据与参考文献[11]对照，确定该化合物为二酮哌嗪类化合物 verruculogen。

化合物 **9**: 淡黄色粉末，$[\alpha]_D^{20}$+43.2 (*c* = 0.12, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 7.15 (1H, d, *J*=8.28 Hz, H-4), 6.58 (1H, dd, *J*=2.11, 8.35 Hz, H-5), 6.45 (1H, d, *J*=2.11 Hz, H-7), 4.72 (1H, s, H-8), 2.57 (1H, s, H-8-OH), 5.72 (1H, s, H-9-OH), 4.51 (1H, t, *J*=8.03 Hz, H-12), 2.19 (1H, m, H-14a), 1.88 (1H, m, H-14b), 4.59 (1H, d, *J*=9.05 Hz, H-18), 5.04 (1H, d, *J*=8.95 Hz, H-19), 0.97 (3H, s, H-21), 1.57 (3H, s, H-22), 4.29 (2H, qd, *J*=6.88, 15.46 Hz, H-23), 5.11 (1H, t, *J*=6.22 Hz, H-24), 1.76 (3H, s, H-26), 1.67 (3H, s, H-27), 3.74 (3H, s, H-6-OCH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C : 179.7 (C-2), 61.5 (C-3), 118.3 (C-3a), 127.6 (C-4), 107.2 (C-5), 160.2 (C-6), 96.7 (C-7), 144.7 (C-7a), 74.5 (C-8), 87.0 (C-9), 169.1 (C-11), 60.5 (C-12), 27.9 (C-13), 23.0 (C-14), 44.9 (C-15), 164.7 (C-17), 56.5 (C-18), 123.1 (C-19), 137.0 (C-20), 17.7 (C-21), 25.1 (C-22), 38.1 (C-23), 118.2 (C-24), 136.4 (C-25), 18.3 (C-26), 25.7 (C-27), 55.7 (C-6-OCH_3)。以上数据与文献[12]对照，确定该化合物为具有独特的螺环结构骨架的二酮哌嗪类化合物 spirotryprostatin C。

化合物 **10**: 淡黄色粉末，$[\alpha]_D^{20}$+47.7 (*c* = 0.22, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 7.05 (1H, d, *J*=8.32 Hz, H-4), 6.59 (1H, dd, *J*=2.32, 8.25 Hz, H-5), 6.46 (1H, d, *J*=2.31 Hz, H-7), 4.74 (1H, s, H-8), 2.32 (1H, m, H-13a), 2.41 (1H, m, H-13b), 2.34 (1H, m, H-14a), 2.13 (1H, m, H-14b), 4.62 (1H, d, *J*=8.95 Hz, H-18), 5.14 (1H, d, *J*=9.12 Hz, H-19), 1.09 (3H, s, H-21), 1.59 (3H, s, H-22), 4.31 (2H, dd, *J*=6.78, 15.46 Hz, H-23), 5.14 (1H, t, *J*=6.25 Hz, H-24), 1.74 (3H, s, H-26), 1.65 (3H, s, H-27), 3.82 (3H, s, H-6-OCH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C : 178.9 (C-2), 61.5 (C-3), 118.3 (C-3a), 127.6 (C-4), 107.2 (C-5), 160.2 (C-6), 96.7 (C-7), 144.7 (C-7a), 74.5 (C-8), 87.0 (C-9), 169.3 (C-11), 90.4 (C-12), 33.9 (C-13), 21.5 (C-14), 45.1 (C-15), 164.7 (C-17), 56.5 (C-18), 121.2 (C-19), 139.2 (C-20), 17.9 (C-21), 25.4 (C-22), 38.0 (C-23), 118.1 (C-24), 136.1 (C-25), 18.3 (C-26), 25.4 (C-27), 55.7 (C-6-OCH_3)。以上数据归属情况与参考文献[12]对照，确定此化合物为具有独特螺环结构骨架的二酮哌嗪类化合物 spirotryprostatin D。

化合物 **11**: 淡黄色粉末，$[\alpha]_D^{20}$+35.1 (*c* = 0.25, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 6.96 (1H, d, *J*=8.31 Hz, H-4), 6.54 (1H, dd, *J*=2.21, 8.46 Hz, H-5), 6.42 (1H, d, *J*=2.27 Hz, H-7), 4.77 (1H, s, H-8), 4.56 (1H, t, *J*=7.25, 9.43 Hz, H-12), 2.23 (1H, m, H-13a), 2.03 (1H, m, H-13b), 2.37 (1H, m, H-14a), 1.85 (1H, m, H-14b), 3.69 (2H, m, H-15), 4.69 (1H, m, H-18), 5.34 (1H, d, *J*=8.45 Hz, H-19), 1.97 (3H, s, H-21), 1.27 (3H, s, H-22), 3.76 (3H, s, H-6-OCH_3). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C : 179.6 (C-2), 61.3 (C-3), 114.2 (C-3a), 127.3 (C-4), 107.4 (C-5), 160.4 (C-6), 97.0 (C-7), 142.3 (C-7a), 75.3 (C-8), 87.2 (C-9), 169.5 (C-11), 60.3 (C-12), 27.8 (C-13), 23.2 (C-14), 45.0 (C-15), 165.3 (C-17), 57.5 (C-18), 121.5 (C-19), 139.0 (C-20), 25.3 (C-21), 20.1 (C-22)。以上数据与文献[13]对照，确定该化合物为具有独特的螺环结构骨架的二酮哌嗪类化合物 spirotryprostatin F。

化合物 **12**: 淡黄色粉末，$[\alpha]_D^{20}$–99.7 (*c* = 0.15, $CHCl_3$); ESI-MS: *m/z* 466.1487 $[M+Na]^+$, 分子式 $C_{24}H_{21}N_5O_4$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 7.78 (1H, d, *J*=8.05 Hz, H-7), 7.86 (1H, t, *J*=7.65 Hz, H-8), 7.63 (1H, t, *J*=7.54 Hz, H-9), 8.16 (1H, d, *J*=7.87 Hz, H-10), 5.36 (1H, d, *J*=6.92 Hz, H-14), 2.95 (1H, dd, *J*=7.26, 15.22 Hz, H-15a), 2.04 (1H, d, *J*=15.01 Hz, H-15b), 1.87 (3H, s, H-16), 5.08 (1H, d, *J*=9.18 Hz, H-18), 2.30 (1H, t, *J*=9.42 Hz, H-20), 7.31 (3H, m, H-24, 25, 27), 7.21 (1H, t, *J*=7.45 Hz, H-26). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C : 172.5 (C-1), 85.9 (C-3), 150.7 (C-4), 146.5 (C-6), 128.3 (C-7), 134.8 (C-8), 128.4 (C-9), 126.3 (C-10), 121.5 (C-11), 159.6 (C-12), 51.2 (C-14), 31.5 (C-15), 24.3 (C-16), 87.4 (C-17), 85.9 (C-18), 58.4 (C-20), 170.3 (C-21), 136.4 (C-23), 114.8 (C-24), 130.1 (C-25), 125.8 (C-26), 125.4 (C-27), 138.7 (C-28), 18.1 (C-29)。以上数据与文献[14]对照，确定此化合物确为喹唑啉类衍生物 fumiquinazoline C。

化合物 **13**: 淡黄色粉末，$[\alpha]_D^{20}$–24.7 (*c* = 0.09, $CHCl_3$); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 7.64 (1H, d, *J*=7.89 Hz, H-7), 7.82 (1H, m, H-8), 7.53 (1H, m, H-9), 8.12 (1H, dd, *J*=7.97, 1.16 Hz, H-10), 5.56 (1H, dd, *J*=1.32 Hz, H-14), 3.06 (1H, dd, *J*=10.32, 14.92 Hz, H-15a), 2.08 (1H, dd, *J*=14.96, 2.13 Hz, H-15b), 1.95 (3H, s, H-16), 5.21 (1H, d, *J*=10.02 Hz, H-18), 4.20 (1H, m, H-20), 7.48 (1H, d, *J*=7.42 Hz, H-24), 7.29 (1H, ddd, *J*=7.87, 7.69, 1.12 Hz, H-25), 7015 (1H, ddd, *J*=7.48, 7.46, 1.08 Hz, H-26), 7.35 (1H, d, *J*=7.36 Hz, H-27). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ_C : 172.4 (C-1), 70.7 (C-3), 153.4 (C-4), 146.2 (C-6), 127.6 (C-7), 134.9 (C-8), 127.6 (C-9), 126.5 (C-10), 120.3 (C-11), 160.2 (C-12), 53.2 (C-14), 43.0 (C-15), 18.8 (C-16), 82.8 (C-17), 85.3 (C-18), 58.4 (C-20), 168.7 (C-21), 137.7 (C-23), 114.7 (C-24), 129.4 (C-25), 125.2 (C-26), 124.6 (C-27), 139.2 (C-28), 17.3 (C-29)。以上数据与文献[14]对照，确定该化合物为 fumiquinazoline D。

化合物 **14**: 淡黄色粉末，$[\alpha]_D^{20}$–48.7 (*c* = 0.07, MeOH); ^{1}H-NMR (DMSO-d_6, 400 MHz) δ_H : 7.68 (1H,

d, *J*=8.26 Hz, H-7), 7.84 (1H, t, *J*=7.62 Hz, H-8), 7.41 (1H, d, *J*=7.54 Hz, H-9), 8.06 (1H, dd, *J*=7.87, 1.23 Hz, H-10), 5.65 (1H, m, H-14), 2.53 (1H, m, H-15a), 2.27 (1H, m, H-15b), 1.86 (3H, s, H-16), 5.28 (1H, dd, *J*=5.94, 1.43 Hz, H-18), 4.14 (1H, d, *J*=5.21 Hz, H-20), 7.52 (1H, m, H-24), 7.35 (1H, d, *J*=6.51 Hz, H-25), 7.17 (1H, t, *J*=7.36 Hz, H-26), 7.64 (1H, d, *J*=7.58 Hz, H-27), 1.11 (1H, d, *J*=6.62 Hz, H-29). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ C：171.7 (C-1), 80.3 (C-3), 152.4 (C-4), 146.7 (C-6), 127.6 (C-7), 134.9 (C-8), 127.2 (C-9), 126.5 (C-10), 120.3 (C-11), 160.4 (C-12), 53.0 (C-14), 38.2 (C-15), 26.1 (C-16), 79.9 (C-17), 86.0 (C-18), 58.5 (C-20), 171.3 (C-21), 136.5 (C-23), 114.3 (C-24), 129.1 (C-25), 125.8 (C-26), 124.6 (C-27), 139.6 (C-28), 18.2 (C-29)。以上数据与文献[15]对照，确定该化合物为 3-hydroxyfumiquinazoline A。

化合物 **15**：淡黄色粉末，$[\alpha]_D^{20}$–53.4 (*c* = 0.10, MeOH)；^{1}H-NMR ($CDCl_3$, 400 MHz) δ_H：7.73 (1H, m, H-7), 7.78 (1H, dd, *J*=6.92, 1.41 Hz, H-8), 7.36 (1H, m, H-9), 8.24 (1H, dd, *J*=8.12, 1.15 Hz, H-10), 5.92 (1H, dd, *J*=4.98, 9.01 Hz, H-14), 2.91 (1H, dd, *J*=9.04, 14.43 Hz, H-15a), 2.33 (1H, dd, *J*=4.99, 14.42 Hz, H-15b), 1.97 (3H, s, H-16), 5.48 (1H, d, *J*=4.46 Hz, H-18), 4.17 (1H, m, H-20), 7.56 (1H, m, H-24), 7.33 (1H, m, H-25), 7.16 (1H, ddd, *J*=0.95, 7.56, 7.54 Hz, H-26), 7.58 (1H, d, *J*=8.19 Hz, H-27), 1.33 (1H, d, *J*=6.66 Hz, H-29), 3.33 (3H, s, H-3-OCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C：172.6 (C-1), 85.0 (C-3), 148.2 (C-4), 146.4 (C-6), 128.2 (C-7), 135.1 (C-8), 128.3 (C-9), 127.1 (C-10), 120.7 (C-11), 161.1 (C-12), 53.5 (C-14), 39.0 (C-15), 21.1 (C-16), 80.2 (C-17), 86.4 (C-18), 59.4 (C-20), 171.4 (C-21), 136.9 (C-23), 115.2 (C-24), 129.9 (C-25), 125.3 (C-26), 124.9 (C-27), 138.7 (C-28), 18.1 (C-29), 50.8 (C-3- CH_3)。以上数据与文献[14]对照，确定该化合物为 fumiquinazoline E。

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Study of the secondary metabolites of *Aspergillus fumigatus* from the deepsea zone of the Indian Ocean

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Received: Apr. 11, 2019
Key words: *Aspergillus fumigatus*; *Aspergillus*; the secondary metabolites; chemical constituents; spectral data

Abstract: This study was conducted to analyze the chemical constituents of the secondary metabolites of *Aspergillus fumigatus* for which the chemical constituents of the ethanol extract of *A. fumigatus* from the deep sea zone of the Indian Ocean were separated systematically using silica gel column chromatography, Sephadex LH-20 column chromatography, and RP-HPLC. Physicochemical properties and spectral data were used to determine the structures of the isolated compounds. A total of 15 compounds were isolated that were identified as fumitremorgin B (**1**), 13-oxofumitremorgin B (**2**), fumitremorgin C (**3**), 12-hydroxyfumitremorgin C (**4**), cyclotyprostatins B (**5**), cyclotyprostatins C (**6**), cyclotyprostatins E (**7**), verruculogen (**8**), spirotryprostatin C (**9**), spirotryprostatin D (**10**), spirotryprostatin F (**11**), fumiquinazoline C (**12**), fumiquinazoline D (**13**), 3-hydroxyfumiquinazoline A (**14**), and fumiquinazoline E (**15**). These 15 compounds isolated from *A. fumigatus* enriched the type of the secondary metabolites of the fungus and provided a basis for exploring its development and conducting chemical research.

(本文编辑: 谭雪静)