无花果内生真菌 FL10 中吲哚二酮哌嗪类生物碱的研究

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[摘要] **目的**:研究无花果内生真菌 FL10 的次级代谢产物成分。**方法**:采用反复硅胶柱色谱法、Sephadex LH-20 凝胶色谱法等进行分离纯化,从无花果内生真菌 FL10 的发酵液中分离得到 6 个吲哚二酮哌嗪类生物碱。**结果**:通过理化常数测定和光谱分析,6 个吲哚二酮哌嗪类生物碱鉴定分别为 verruculogen (**1**), cyclotryprostatins B (**2**), fumitremorgin C (**3**), cyclotryprostatin A (**4**), tryprostatin A (**5**), tryprostatin B (**6**)。**结论**:这 6 个吲哚二酮哌嗪类生物碱是首次从无花果内生真菌中分离得到,目前研究表明,无花果内生真菌 *Aspergillus tamarii* 可以作为一种可产生吲哚二酮哌嗪类生物碱的新来源。

[关键词] 内生真菌; 无花果; 吲哚二酮哌嗪; 生物碱
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Indolyl Diketopiperazine Alkaloids Isolated from Endophyic Fungus FL10 of *Ficus carica*

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[**Abstract**] **Objective**: To study chemical constituents from endophytic fungus FL10 of *Ficus carica*. **Method**: The compounds were isolated by column chromatography and identified on the basis of physic-chemical constants and spectral analysis. **Result**: Six indolyl diketopiperazines alkaloid were obtained and elucidated as verruculogen (**1**), cyclotryprostatins B (**2**). fumitremorgin C (**3**), cyclotryprostatin A (**4**), tryprostatin A (**5**), tryprostatin B (**6**). **Conclusion**: Six indolyl diketopiperazine alkaloids were isolated from endophyic fungus of *F. carica* for the first time. The present study revealed that endophytic fungi *Aspergillus tamarii* from *F. carica* are the sources for the production of indolyl diketopiperazine alkaloids.

[**Key words**] endophytic fungus; *Ficus carica*; indolyl diketopiperazine; alkaloid

药用植物中蕴含着大量的内生真菌,这些内生真菌与宿主之间具有紧密的生态关系,产生的次生代谢产物在病虫害的生物防治、医药工业上的用途和范围逐渐增大,成为寻找新的天然活性产物的重要方向[1-4]。吲哚二酮哌嗪是由 2 种氨基酸结合而成的含有吲哚结构单元的特殊生物碱。目前研究发现很多吲哚二酮哌嗪生物碱具有抗菌和抗肿瘤活性。由于其优良的生物活性,此类生物碱合成途径和方式已经成为新药研究领域的热点之一。笔者所在课题组从药用植物内生真菌代谢产物的角度去研究吲哚二酮哌嗪生物碱的来源和合成途径。本文从无花果内生真菌的代谢产物中分离到 6 个吲哚二酮哌嗪类生物碱,为进一步探寻吲哚二酮哌嗪生物碱的新的来源提供依据。

1 材料

JEOL SX102A 型 ESI-MS 质谱仪, Bruker ADVANCE 型核磁共振仪 400 MHz(TMS 作为内标),熔点仪 Fisher-Johns(未校正),柱色谱用硅胶(200 ~ 300 目,青岛海洋化工股份有限公司),凝胶柱层析 Sephadex LH-20(上海安玛西亚公司股份有限公司),其他试剂均为 AR 级。

2 内生真菌的来源和分离

植物样品无花果于 2010 年 9 月采自陕西省周至县秦岭南坡,内生真菌 FL10 分离自该植物的叶部,通过宏观形态学和分子生物学鉴定分析,鉴定这株真菌为 *Aspergillus tamarii*。该内生真菌现被保存在陕西科技大学化学与化工学院天然产物研究室。

3 内生真菌的发酵及代谢产物提取和分离

将内生真菌 FL10 无菌接种于制备好的 PDA 培养平板上,28 ℃,培养 7 d 后,无菌操作从培养平板取 0.6 cm 的内生真菌菌饼,接种至含 100 mL 查氏种子液体培养基 500 mL 锥形瓶中,28 ℃,120 r·min^{-1}摇床上振荡培养 3 d 后,按 10% 的接种量转接到含 400 mL 查氏液体培养基 1 000 mL 锥形瓶中(60 L),28 ℃,130 r·min^{-1}摇床上振荡培养 12 d 后,从摇床中取出培养物,4 层纱布过滤得菌丝和发酵滤液,发酵滤液在 60 ℃浓缩至 5 L 以下,然后用乙酸乙酯提取 5 次(4.5 L),干燥后的菌丝体用甲醇提取 3 次(4 L),减压浓缩提取液,合并乙酸乙酯浓缩液和甲醇浓缩液,阴干后得到褐色粗提取物(37.2 g)。

粗提取物经硅胶柱色谱,以乙酸乙酯和甲醇梯度洗脱(体积比分别为 1∶0,100∶1,20∶1,10∶1,5∶1,2∶1,1∶1,0∶1)得到 8 个部分 Fr. A-H。Fr. A 以石油醚-乙酸乙酯梯度洗脱,得到 5 个部分 sFr. A(1-5)。sFr. A1 再次经硅胶柱色谱洗脱,以石油醚和乙酸乙酯(2∶1)为洗脱剂,得到化合物 **1**,**2**。Fr. B 以乙酸乙酯-石油醚梯度洗脱,得到 5 个部分 sFr. B(1-5)。sFr. B2 再次经硅胶柱色谱洗脱和凝胶柱 Sephadex LH-20(以乙酸乙酯-甲醇 1∶1 为洗脱剂)纯化,得到化合物 **3**,**4**,**5**。同样,sFr. B3 再次经硅胶柱色谱洗脱和凝胶柱 Sephadex LH-20 纯化,得到化合物 **6**。

4 结构鉴定

将分离得到的代谢产物经过光谱表征,这些代谢产物被鉴定为 verruculogen (**1**), cyclotryprostatins B (**2**), fumitremorgin C (**3**), cyclotryprostatin A (**4**), tryprostatin A (**5**), tryprostatin B (**6**)。

化合物 **1** 黄色针状结晶, mp 218 ~ 220 ℃, ESI-MS *m/z*: 534.1 $[M + Na]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$): δ: 7.95 (1H, d, J = 8.0 Hz, H-4), 6.88 (1H, d, J = 8.0 Hz, H-5), 6.58 (1H, s, H-7), 5.66 (1H, s, H-8), 4.45 (1H, t, J = 8.0 Hz, 8.0 Hz, H-12), 2.45 (1H, m, H-13a), 2.10 (1H, m, H-13b), 2.08 (1H, m, H-14a), 1.94 (1H, m, H-14b), 3.63 (2H, d, J = 8.0 Hz, H-15), 6.11 (1H, d, J = 8.0 Hz, H-18), 2.00 (1H, m, H-19a), 1.63 (1H, d, J = 8.0 Hz, H-19b), 1.99 (3H, s, H-21), 1.02 (3H, s, H-22), 6.73 (1H, s, H-23), 5.03 (1H, s, H-24), 1.77 (3H, s, H-26), 1.70 (3H, s, H-27), 3.84 (3H, s, 6-OMe), 4.74 (1H, s, 8-OH), 4.04 (1H, s, 9-OH)。^{13}C-

NMR (100 MHz, $CDCl_3$):δ:131.13 (C-2), 105.50 (C-3), 120.87 (C-3a), 121.64 (C-4), 109.29 (C-5), 156.36 (C-6), 93.82 (C-7), 136.19 (C-7a), 68.96 (C-8), 82.58 (C-9), 166.10 (C-11), 58.76 (C-12), 28.94 (C-13), 22.61 (C-14), 51.11 (C-15), 170.71 (C-17), 49.03 (C-18), 45.29 (C-19), 82.09 (C-20), 25.71 (C-21), 27.00 (C-22), 85.82 (C-23), 118.40 (C-24), 143.11 (C-25), 18.90 (C-26), 24.09 (C-27), 55.72 (6-OMe)。以上数据与文献[5-6]报道的一致,故鉴定该化合物为 verruculogen。

化合物 **2** 白色针状结晶,mp 130 ~ 132 ℃, ESI-MS *m/z*:436.2[M + H]$^+$,^{1}H-NMR (400 MHz, $CDCl_3$)δ:8.10 (1H, s, H-1), 7.43 (1H, d, *J* = 8.0 Hz, H-4), 6.81 (1H, d, *J* = 8.0 Hz, H-5), 6.85(1H, s, H-7), 4.7 (1H, s, H-8), 4.42 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-12), 2.51 (1H, m, H-13a), 2.05(1H, m, H-13b), 2.19(1H, m, H-14a), 1.96 (1H, m, H-14b), 3.75 (1H, m, H-15a), 3.73 (1H, m, H-15b), 6.66 (1H, d, *J* = 8.0 Hz, H-18), 5.62 (1H, d, *J* = 8.0 Hz, H-19), 2.24 (3H, s, H-21), 1.79 (3H, s, H-22), 3.82 (3H, s, 6-OMe), 3.34(3H, s, 8-OMe)。^{13}C-NMR (100 MHz, $CDCl_3$):δ:133.75 (C-2), 105.22 (C-3), 122.60 (C-3a), 118.68 (C-4), 109.99 (C-5), 156.49 (C-6), 95.23 (C-7), 136.69 (C-7a), 76.83 (C-8), 84.77 (C-9), 167.03 (C-11), 59.93 (C-12), 29.69 (C-13), 22.05 (C-14), 45.82 (C-15), 165.73 (C-17), 49.11 (C-18), 123.54 (C-19), 137.92 (C-20), 18.10 (C-21), 26.03 (C-22), 55.77 (6-OMe), 56.57 (8-OMe)。以上数据与文献[6-7]报道的一致,故鉴定该化合物为 cyclotryprostatins B。

化合物 **3** 白色针状结晶,mp 124 ~ 126 ℃, ESI-MS *m/z*:380.2[M + H]$^+$,^{1}H-NMR (400 MHz, $CDCl_3$)δ:7.89 (1H, s, H-1), 7.43 (1H, d, *J* = 8.0 Hz, H-4), 6.82 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-5), 6.86 (1H, d, *J* = 4.0 Hz, H-7), 3.52 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-8a), 3.11(1H, dd, *J* = 12.0 Hz, 12.0 Hz, H-8b), 4.18 (1H, dd, *J* = 4.0 Hz, 4.0 Hz, H-9), 4.11(1H, t, *J* = 8.0 Hz, 8.0 Hz, H-12), 2.41 (1H, m, H-13a), 2.24 (1H, m, H-13b), 2.07 (1H, m, H-14a), 1.95 (1H, m, H-14b), 3.65 (1H, d, *J* = 8.0 Hz, H-15a), 3.63 (1H, d, *J* = 8.0 Hz, H-15b), 5.99 (1H, d, *J* = 12.0 Hz, H-18), 4.91 (1H, d, *J* = 12.0 Hz, H-19), 1.99 (3H, s, H-21), 1.65 (3H, s, H-22), 3.83 (3H, s, 6-OMe)。^{13}C-NMR (100 MHz, $CDCl_3$)δ:132.19 (C-2), 106.09 (C-3), 120.68 (C-3a), 118.84 (C-4), 109.40 (C-5), 156.41 (C-6), 95.26 (C-7), 136.99 (C-7a), 21.90 (C-8), 56.76 (C-9), 169.53 (C-11), 59.21 (C-12), 28.56 (C-13), 23.04 (C-14), 45.41 (C-15), 165.77 (C-17), 50.99 (C-18), 124.09 (C-19), 134.03 (C-20), 18.07 (C-21), 25.72 (C-22), 55.75 (6-OMe)。以上数据与文献[8-9]报道的一致,故鉴定该化合物为 fumitremorgin C。

化合物 **4** 白色针状结晶,mp 110 ~ 112 ℃, ESI-MS *m/z*:380.2[M + H]$^+$,^{1}H-NMR (400 MHz, $CDCl_3$)δ:7.93 (1H, s, H-1), 7.40 (1H, d, *J* = 8.0 Hz, H-4), 6.75 (1H, dd, *J* = 8.0 Hz, 3.0 Hz, H-5), 6.81 (1H, d, *J* = 2.0 Hz, H-7), 5.01 (1H, s, H-8), 4.30 (1H, dd, *J* = 11.0 Hz, 7.0 Hz, H-12), 2.41 (1H, m, H-13a), 2.04 (1H, m, H-13b), 1.95 (1H, m, H-14a), 1.87 (1H, m, H-14b), 3.55 (1H, m, H-15a), 3.68 (1H, m, H-15b), 6.75 (1H, d, *J* = 10.0 Hz, H-18), 5.51 (1H, d, *J* = 10.0 Hz, H-19), 2.09 (3H, s, H-21), 1.78 (3H, s, H-22), 3.80 (3H, s, 6-OMe)。^{13}C-NMR (100 MHz, $CDCl_3$)δ:133.51 (C-2), 107.48 (C-3), 120.81 (C-3a), 118.44 (C-4), 109.70 (C-5), 156.71 (C-6), 95.36 (C-7), 136.82 (C-7a), 69.90 (C-8), 84.99 (C-9), 165.58 (C-11), 59.61 (C-12), 29.65 (C-13), 21.77 (C-14), 45.70 (C-15), 167.04 (C-17), 48.09 (C-18), 123.41 (C-19), 137.93 (C-20), 18.21 (C-21), 25.92 (C-22), 55.74 (6-OMe)。以上数据与文献[7]报道的一致,故鉴定该化合物为 cyclotryprostatin A。

化合物 **5** 淡黄色针状结晶,mp 120 ~ 122 ℃, ESI-MS *m/z*:382.2[M + H]$^+$,^{1}H-NMR (400 MHz, $CDCl_3$)δ:7.98 (1H, s, H-1), 7.40 (1H, d, *J* = 8.0 Hz, H-4), 6.72 (1H, dd, *J* = 8.0 Hz, 3.0 Hz, H-5), 6.86 (1H, d, *J* = 2.0 Hz, H-7), 3.62 (1H, dd, *J* = 15.0 Hz, 4.0 Hz, H-8a), 3.01(1H, dd, *J* = 15.0 Hz, 12.0 Hz, H-8b), 4.30 (1H, dd, *J* = 12.0 Hz, 3.0 Hz, H-9), 5.65 (1H, s, H-10), 4.04 (1H, t, *J* = 8.0 Hz, 8.0 Hz, H-12), 2.31

(1H, m, H-13a), 2.04 (1H, m, H-13b), 2.07 (1H, m, H-14a), 1.95 (1H, m, H-14b), 3.55 (1H, m, H-15a), 3.63 (1H, m, H-15b), 3.79 (2H, m, H-18), 5.11 (1H, d, J = 8.0 Hz, H-19), 1.79 (3H, s, H-21), 1.75 (3H, s, H-22), 3.81 (3H, s, 6-OMe)。^{13}C-NMR (100 MHz, $CDCl_3$) δ: 135.19 (C-2), 104.38 (C-3), 122.61 (C-3a), 118.84 (C-4), 109.40 (C-5), 156.41 (C-6), 94.36 (C-7), 136.39 (C-7a), 25.90 (C-8), 54.76 (C-9), 169.58 (C-11), 59.21 (C-12), 28.46 (C-13), 22.74 (C-14), 45.41 (C-15), 165.77 (C-17), 25.09 (C-18), 120.09 (C-19), 135.03 (C-20), 18.07 (C-21), 25.72 (C-22), 55.85 (6-OMe)。以上数据与文献[9-10]报道的一致,故鉴定该化合物为 tryprostatin A。

化合物 **6** 淡黄色针状结晶,mp 103 ~ 104 ℃, ESI-MS m/z: 352.2 $[M + H]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.12 (1H, s, H-1), 7.45 (1H, d, J = 8.0 Hz, H-4), 7.07 (1H, dd, J = 7.8 Hz, 7.6 Hz, H-5), 7.15 (1H, d, J = 8.0 Hz, H-6), 7.31 (1H, d, J = 8.0 Hz, H-7), 3.62 (1H, dd, J = 15.0 Hz, 4.0 Hz, H-8a), 3.01 (1H, dd, J = 15.0 Hz, 12.0 Hz, H-8b), 4.29 (1H, dd, J = 12.0 Hz, 3.0 Hz, H-9), 5.65 (1H, s, H-10), 4.05 (1H, d, J = 8.0 Hz, 8.0 Hz, H-12), 2.31 (1H, m, H-13a), 2.04 (1H, m, H-13b), 2.04 (1H, m, H-14a), 1.91 (1H, m, H-14b), 3.55 (1H, m, H-15a), 3.68 (1H, m, H-15b), 3.45 (2H, m, H-18), 5.29 (1H, m, H-19), 1.79 (3H, s, H-21), 1.75 (3H, s, H-22)。^{13}C-NMR (100 MHz, $CDCl_3$) δ: 134.99 (C-2), 104.21 (C-3), 127.68 (C-3a), 117.82 (C-4), 119.43 (C-5), 121.55 (C-6), 110.69 (C-7), 136.59 (C-7a), 25.90 (C-8), 55.78 (C-9), 169.51 (C-11), 59.21 (C-12), 28.36 (C-13), 22.05 (C-14), 45.41 (C-15), 165.75 (C-17), 24.99 (C-18), 119.49 (C-19), 135.31 (C-20), 18.01 (C-21), 25.72 (C-22)。以上数据与文献[9, 11]报道的一致,故鉴定该化合物为 tryprostatin B。

这 6 个吲哚二酮哌嗪类生物碱是首次从无花果内生真菌中分离得到,目前的研究表明,无花果内生真菌 *Aspergillus tamarii* 可以作为一种可产生吲哚二酮哌嗪类生物碱的新来源。

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