

云南美登木内生真菌 *Botryosphaeria* sp. MHF 次生代谢产物研究

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[摘要] 目的: 对云南美登木内生真菌 *Botryosphaeria* sp. MHF 的化学成分进行研究。方法: 采用反相、正相等多种柱色谱法进行分离; 应用波谱技术进行结构鉴定。结果: 从云南美登木内生真菌 *B. sp.* MHF 的发酵物中分离得到 8 个化合物: 分别是麦角甾-5-烯-3-醇 (1)、麦角甾-4,6,8,22-四烯-3-酮 (2)、麦角甾-3 β ,5 α ,9 α -三羟基-7,22-二烯-6-酮 (3)、麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇 (4)、麦角甾-5 α ,8 α -环二氧-6,22-二烯-3-醇 (5)、fusaproliferin (6)、脑苷脂 C (7) 和 3,4,5-三羟基-四氢萘酮 (8)。结论: 所有化合物均为首次从以 Murashige-Skoog 培养基培养的该菌株中分离得到。

[关键词] 云南美登木; 内生真菌; *Botryosphaeria* sp. MHF; 化学成分; 麦角甾醇类

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Study on Secondary Metabolites of Endophytic Fungal Strain *Botryosphaeria* sp. MHF of *Maytenus hookeri*

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[Abstract] **Objective:** To study the secondary metabolites of endophytic fungal strain *Botryosphaeria* sp. MHF of *Maytenus hookeri*. **Method:** The chemical constituents were isolated by column chromatography such as normal phase or reverse phase etc. The structures were identified by spectroscopic analysis. **Result:** Eight compounds were obtained and elucidated as 22E, 24R-ergosta-5-en-3 β -ol (1), 22E, 24R-ergosta-4, 6, 8, 22-tetraen-3-one (2), 22E, 24R-3 β , 5 α , 9 α -trihydroxy-ergosta-7, 22-diene-6-one (3), 22E, 24R-ergosta-7, 22-diene-3 β , 5 α , 6 β -triol (4), 22E, 24R-5 α , 8 α -epidioxyergosta-6, 22-dien-3 β -ol (5), fusaproliferin (6), cerebroside C (7) and 3, 4, 5-trihydroxy-1-tetralone (8). **Conclusion:** All these compounds were isolated from this strain cultivated on Murashige-Skoog culture medium for the first time.

[Key words] *Maytenus hookeri*; endophytic fungal strain; *Botryosphaeria* sp. MHF; chemical constituents; ergosterol derivatives

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植物内生菌作为一种新兴的资源已经越来越受到重视。在互利共生的基础上,植物内生菌通常以产生具有生物活性的次生代谢产物来提高宿主植物的生长速度、抗逆性、抗病害及抗动物危害的能力。因此,内生菌成为了发现新的活性化合物和先导化合物的重要来源^[1-2]。国内外研究表明,已经从植物内生菌中分离到数百个化合物,结构类型包括:生物碱、萜类、大环内酯类、醌类、甾醇、黄酮、异香豆素、苯丙素和木质素类、酚和酚酸等。结构类型的多样化也决定了它们具有多种多样的生理活性,如抗肿瘤、抗菌杀虫和调节植物生长等^[3-4]。

云南美登木属于卫矛科美登木属植物,从该植物中发现的美登木素类化合物具有很强的抗肿瘤活性。我们曾对美登木的内生菌进行了系统的研究,并从中分离得到很多新化合物和活性化合物^[5-7]。为了继续从云南美登木内生菌中寻找活性次生代谢产物,我们对云南美登木 *Botryosphaeria* sp. MHF 发酵物的化学成分进行研究。

1 材料

Bruker AM-400 型核磁共振仪 (TMS 为内标), Finnigan LCQ-Advantage 型质谱仪, VG Auto-Spec-3000 型质谱仪。薄层色谱和柱色谱硅胶均为青岛海洋化工厂生产,柱色谱凝胶 Sephadex LH-20 (Amersham Pharmacia 公司),反相硅胶 (Merck 公司)。

真菌 MHF 分离于采自云南西双版纳的美登木属植物云南美登木 *Maytenus hookeri* 的叶片,经 ITS 技术 (ITS1 和 ITS2) 及 5.8S rDNA 序列实验,并比对基因库已知序列,鉴定该菌为 *Botryosphaeria* 属真菌 (accession No. EU523117, GenBank),菌株现存于昆明学院生命科学与技术系。

2 菌株发酵、提取和分离

菌株经琼脂平板发酵,采用 10 L 的 MS (Murashige-Skoog)^[8] 培养基在 28 °C 下培养 10 d。固体发酵培养物连同培养基用结晶刀切成小块,浸泡于乙酸乙酯-甲醇-冰醋酸 (85:15:5) 提取液中冷浸提取,过滤合并 3 次提取液,减压浓缩除去有机溶剂得到浸膏。浸膏以水溶解后经乙酸乙酯萃取,得乙酸乙酯相粗提物 10 g。

粗提物经中压柱色谱,用 RP-18 柱 (145 g) 分别以水,30%,50%,70%,100% 甲醇梯度洗脱 (各梯度洗脱 2 L),TLC 检测合并得 A~E 5 个组分。组分 B 先后经 Sephadex LH-20 (甲醇) 凝胶柱色谱,硅胶柱色谱 (以石油醚-丙酮及氯仿-甲醇梯度洗脱) 得

化合物 **8** (10 mg); 组分 D 用凝胶柱色谱、正相柱色谱 (石油醚-丙酮梯度洗脱) 及反相柱色谱 (甲醇-水梯度洗脱) 得化合物 **6** (5 mg) 和 **7** (4 mg); 组分 E 用 Sephadex LH-20 (氯仿-甲醇) 纯化后,经反复硅胶柱色谱 (石油醚-乙酸乙酯、石油醚-氯仿等多个溶剂系统梯度洗脱) 得化合物 **1** (7 mg), **2** (2 mg), **3** (2 mg), **4** (4 mg) 及 **5** (6 mg)。

3 结构鉴定

化合物 **1** 白色无定形粉末 (氯仿)。分子式 $C_{28}H_{46}O$, EI-MS m/z : 398; ¹H-NMR (400 MHz, CDCl₃) δ : 5.35 (1H, br s, H-6), 5.13-5.22 (2H, m, H-22, 23), 3.55 (1H, m, H-3), 1.01 (3H, d, $J = 6.6$ Hz, H-28), 1.00 (3H, d, $J = 6.5$ Hz, H-21), 0.91 (3H, d, $J = 5.4$ Hz, H-27), 0.84 (3H, d, $J = 5.8$ Hz, H-26), 0.81 (3H, s, H-19), 0.69 (3H, s, H-18); ¹³C-NMR (100 MHz, CDCl₃) δ : 37.3 (C-1), 31.9 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.6 (C-6), 31.7 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.0 (C-11), 39.7 (C-12), 42.2 (C-13), 56.0 (C-14), 24.2 (C-15), 28.5 (C-16), 56.8 (C-17), 11.9 (C-18), 19.4 (C-19), 40.1 (C-20), 20.9 (C-21), 135.8 (C-22), 131.7 (C-23), 42.8 (C-24), 33.1 (C-25), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28)。其波谱数据与文献 [9] 报道基本一致,鉴定为麦角甾-5-烯-3-醇。

化合物 **2** 白色无定形粉末 (氯仿)。分子式 $C_{28}H_{40}O$, EI-MS m/z : 392; ¹H-NMR (400 MHz, CDCl₃) δ : 6.61 (1H, d, $J = 9.4$ Hz, H-7), 6.04 (1H, d, $J = 9.4$ Hz, H-6), 5.73 (1H, s, H-4), 5.16-5.33 (2H, m, H-22, H-23), 1.06 (3H, d, $J = 6.6$ Hz, H-21), 0.99 (3H, s, H-19), 0.96 (3H, s, H-18), 0.93 (3H, d, $J = 6.6$ Hz, H-28), 0.85 (3H, d, $J = 6.8$ Hz, H-27), 0.83 (3H, d, $J = 6.8$ Hz, H-26); ¹³C-NMR (100 MHz, CDCl₃) δ : 34.1 (C-1), 18.9 (C-2), 199.5 (C-3), 122.9 (C-4), 164.4 (C-5), 124.4 (C-6), 134.0 (C-7), 124.4 (C-8), 44.3 (C-9), 36.7 (C-10), 25.3 (C-11), 34.1 (C-12), 43.9 (C-13), 156.0 (C-14), 35.5 (C-15), 27.6 (C-16), 55.6 (C-17), 16.6 (C-18), 18.9 (C-19), 39.2 (C-20), 21.2 (C-21), 134.9 (C-22), 132.5 (C-23), 42.8 (C-24), 33.0 (C-25), 19.6 (C-26), 19.9 (C-27), 17.6 (C-28)。其波谱数据与文献 [10] 报道基本一致,鉴定为麦角甾-4,6,8(14),22-四烯-3-酮。

化合物 **3** 白色无定形粉末 (氯仿)。分子式

$C_{28}H_{44}O_4$, ESI-MS m/z : 445 [M + H]⁺; ¹H-NMR (400 MHz, CDCl₃) δ : 5.57 (1H, br s, H-7), 5.17 ~ 5.26 (2H, m, H-22, H-23), 3.93 (1H, m, H-3), 1.05 (3H, d, $J = 6.6$ Hz, H-21), 0.99 (3H, s, H-19), 0.94 (3H, d, $J = 6.8$ Hz, H-28), 0.86 (3H, d, $J = 6.6$ Hz, H-27), 0.83 (3H, d, $J = 6.8$ Hz, H-26), 0.66 (3H, s, H-18); ¹³C-NMR (100 MHz, CDCl₃) δ : 26.6 (C-1), 30.9 (C-2), 67.8 (C-3), 37.1 (C-4), 80.1 (C-5), 200.1 (C-6), 120.0 (C-7), 165.0 (C-8), 76.1 (C-9), 42.7 (C-10), 29.3 (C-11), 36.1 (C-12), 46.1 (C-13), 52.8 (C-14), 23.3 (C-15), 29.1 (C-16), 57.4 (C-17), 12.5 (C-18), 20.0 (C-19), 41.7 (C-20), 21.5 (C-21), 136.6 (C-22), 133.5 (C-23), 44.3 (C-24), 34.3 (C-25), 20.4 (C-26), 20.5 (C-27), 18.1 (C-28)。其波谱数据与文献[11]报道基本一致, 鉴定为麦角甾-3 β ,5 α ,9 α -三羟基-7,22-二烯-6-酮。

化合物 4 白色无定形粉末(吡啶)。分子式 $C_{28}H_{46}O_3$, ESI-MS m/z : 431 [M + H]⁺; ¹H-NMR (400 MHz, C₅D₅N) δ : 5.74 (1H, br s, H-7), 5.23 (1H, m, H-23), 5.19 (1H, m, H-22), 4.84 (1H, m, H-3), 4.33 (1H, br s, H-6), 1.53 (3H, s, H-19), 1.06 (3H, d, $J = 6.8$, H-21), 0.95 (3H, d, $J = 6.8$, H-28), 0.85 (3H, d, $J = 6.4$, H-27), 0.85 (3H, d, $J = 6.8$, H-26), 0.65 (3H, s, H-18); ¹³C-NMR (100 MHz, C₅D₅N) δ : 32.6 (C-1), 33.8 (C-2), 67.6 (C-3), 42.0 (C-4), 76.1 (C-5), 74.2 (C-6), 120.5 (C-7), 141.5 (C-8), 43.7 (C-9), 38.0 (C-10), 22.4 (C-11), 39.9 (C-12), 43.7 (C-13), 55.2 (C-14), 23.5 (C-15), 28.5 (C-16), 56.0 (C-17), 12.5 (C-18), 18.8 (C-19), 40.9 (C-20), 20.1 (C-21), 136.2 (C-22), 132.0 (C-23), 43.0 (C-24), 33.3 (C-25), 21.4 (C-26), 19.8 (C-27), 17.8 (C-28)。其波谱数据与文献[12]报道基本一致, 鉴定为麦角甾-7,22-二烯-3 β ,5 α ,6 β -三醇。

化合物 5 白色无定形粉末(氯仿)。分子式 $C_{28}H_{44}O_3$, ESI-MS m/z : 429 [M + H]⁺; ¹H-NMR (400 MHz, CDCl₃) δ : 6.50 (1H, d, $J = 8.5$ Hz, H-7), 6.24 (1H, d, $J = 8.5$ Hz, H-6), 5.25 (1H, dd, $J = 15.2, 7.3$ Hz, H-23), 5.12 (1H, dd, $J = 15.2, 7.9$ Hz, H-22), 3.99 (1H, m, H-3), 0.99 (3H, d, $J = 6.6$ Hz, H-21), 0.90 (3H, d, $J = 6.6$ Hz, H-28), 0.88 (3H, s, H-19), 0.82 (3H, d, $J = 6.3$ Hz, H-27), 0.81 (3H, s, H-18), 0.81 (3H, d, $J = 6.3$ Hz, H-26); ¹³C-

NMR (100 MHz, CDCl₃) δ : 34.7 (C-1), 30.2 (C-2), 66.4 (C-3), 39.4 (C-4), 82.1 (C-5), 135.4 (C-6), 130.7 (C-7), 79.4 (C-8), 51.2 (C-9), 37.0 (C-10), 23.4 (C-11), 39.4 (C-12), 44.6 (C-13), 51.7 (C-14), 20.6 (C-15), 28.6 (C-16), 56.3 (C-17), 12.9 (C-18), 18.1 (C-19), 39.6 (C-20), 20.8 (C-21), 135.2 (C-22), 132.4 (C-23), 42.6 (C-24), 32.9 (C-25), 19.8 (C-26), 19.5 (C-27), 17.5 (C-28)。其波谱数据与文献[10]报道基本一致, 鉴定为麦角甾-5 α ,8 α -环二氧-6,22-二烯-3-醇。

化合物 6 无色油状(氯仿)。分子式 $C_{27}H_{40}O_5$, EI-MS m/z 444; ¹H-NMR (400 MHz, CDCl₃) δ : 5.35 (1H, br s, H-3), 5.24 (1H, dd, $J = 9.7, 5.3$ Hz, H-13), 5.11 (1H, br s, H-7), 4.28 (2H, m, H-24), 4.04 (1H, br d, $J = 6.4$ Hz, H-11), 2.75 (1H, m, H-15), 2.63 (1H, m, H-23), 2.00 (3H, s, COCH₃), 1.61 (6H, s, H-20, H-21), 1.54 (3H, s, H-22), 1.29 (3H, d, $J = 7.1$ Hz, H-25), 0.97 (3H, s, H-19); ¹³C-NMR (100 MHz, CDCl₃) δ : 49.5 (C-1), 39.6 (C-2), 121.9 (C-3), 137.0 (C-4), 40.7 (C-5), 24.3 (C-6), 124.7 (C-7), 133.4 (C-8), 35.3 (C-9), 30.3 (C-10), 76.9 (C-11), 138.5 (C-12), 129.3 (C-13), 29.2 (C-14), 50.0 (C-15), 147.7 (C-16), 147.3 (C-17), 208.3 (C-18), 16.6 (C-19), 15.7 (C-20), 16.0 (C-21), 10.8 (C-22), 34.2 (C-23), 66.9 (C-24), 15.0 (C-25), 171.2 (COCH₃), 21.3 (COCH₃)。其波谱数据与文献[13]报道基本一致, 鉴定为 fusaproliferin。

化合物 7 白色无定形粉末(甲醇)。分子式 $C_{43}H_{79}NO_9$, 阴性 FAB-MS m/z : 753 [M]⁻; ¹H-NMR (400 MHz, CD₃OD) δ : 5.86 (1H, dd, $J = 15.3, 6.3$, H-4'), 5.74 (1H, m, H-5), 5.51 (1H, dd, $J = 15.2, 6.1$, H-3'), 5.47 (1H, dd, $J = 15.2, 7.3$, H-4), 5.13 (1H, m, H-8), 4.43 (1H, d, $J = 5.9$, H-2'), 4.27 (1H, d, $J = 7.8$, H-1''), 4.15 (1H, m, H-3), 4.11 (1H, m, H-1b), 3.98 (1H, m, H-2), 3.71 (1H, m, H-1a), 3.21 (1H, dd, $J = 8.9, 7.9$, H-2''), 1.59 (3H, s, H-19), 0.90 (6H, t, $J = 6.7$, H-18, 18'); ¹³C-NMR (100 MHz, CD₃OD) δ : 69.6 (C-1), 54.6 (C-2), 72.8 (C-3), 131.0 (C-4), 134.7 (C-5), 33.8 (C-6), 28.7 (C-7), 124.8 (C-8), 136.7 (C-9), 40.8 (C-10), 29.1 (C-11), 29.3-29.9 (C-12 ~ 15), 33.0 (C-16), 23.7 (C-17), 14.4 (C-18), 16.1 (C-19), 175.4 (C-1'), 74.1 (C-2'), 129.0 (C-3'), 134.5

(C-4'), 33.4 (C-5'), 29.3-29.9 (C-6' ~ 15'), 33.0 (C-16'), 23.7 (C-17'), 14.4 (C-18'), 104.7 (C-1'), 74.9 (C-2''), 77.8 (C-3''), 71.5 (C-4''), 77.9 (C-5''), 62.6 (C-6''). 其波谱数据与文献[14]报道基本一致,鉴定为脑苷脂 C。

化合物 8 白色无定形粉末(甲醇)。分子式 $C_{10}H_{10}O_4$, ESI-MS m/z : 195 [M + H]⁺; ¹H-NMR (400 MHz, CD₃OD) δ : 7.43 (1H, dd, $J = 7.6, 0.7$ Hz, H-8), 7.24 (1H, t, $J = 7.9$ Hz, H-7), 7.06 (1H, dd, $J = 8.0, 1.0$ Hz, H-6), 5.07 (1H, d, $J = 4.2$ Hz, H-4), 4.27 (1H, m, H-3), 3.07 (1H, dd, $J = 16.8, 3.1$ Hz H-2), 2.57 (1H, dd, $J = 16.8, 5.4$ Hz H-2); ¹³C-NMR (100 MHz, CD₃OD) δ : 198.9 (C-1), 42.5 (C-2), 66.9 (C-3), 71.6 (C-4), 129.0 (C-4a), 158.2 (C-5), 118.0 (C-6), 130.0 (C-7), 121.9 (C-8), 133.9 (C-8a)。其波谱数据与文献[15]报道基本一致,鉴定为 3,4,5-三羟基-四氢萘酮。

4 讨论

本文从云南美登木内生真菌 *Botryosphaeria* sp. MHF 中主要分离得到 8 个化合物,结构类型主要包括甾醇类、大环类、脑苷脂类等。与该菌株在马铃薯-葡萄糖-琼脂培养基上生成的次生代谢产物(主要是二萜及四降二萜)完全不同^[16]。该实验结果充分证明了 OSMAC^[2] (one-strain-many compounds, 指在微生物发酵培养的过程中较为系统地改变易变的培养条件,如培养基成分的组成和配比,培养温度,培养时间,加入前体物质和酶抑制剂等,使单一的菌株产生尽可能多的次生代谢产物)是拓宽微生物次生代谢产物资源利用的可选方法之一。

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