

红树植物木榄内生真菌 *Pestalotiopsis neglecta* 次级代谢产物的研究

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摘要: 对红树植物木榄内生忽视拟盘多毛孢菌 *Pestalotiopsis neglecta* 的次级代谢产物进行研究。利用硅胶柱层析, Sephadex LH-20 凝胶柱层析, 半制备高效液相色谱仪等分离手段对木榄内生 *P. neglecta* 的提取物进行分离纯化, 并利用各种波谱方法, 结合文献对照, 确定化合物结构。从提取物中分离得到 7 个化合物, 经鉴定为麦角甾-6, 22-二烯-5 α , 8 α -环二氧-3-醇(1), 麦角甾-4, 6, 8(14), 22-四烯-3-酮(2), 24 β -乙基胆甾-4-烯-3 β -醇(3), 24-降胆甾-22-烯-3 β -醇(4), 麦角甾-5, 7, 22-三烯醇(5), 胆甾醇(6), 24-甲基胆甾-5-烯-3 β , 7 α -二醇(7)。这 7 个化合物均为首次从木榄内生真菌 *P. neglecta* 中分离得到。

关键词: 红树植物; 木榄; 内生真菌; 忽视拟盘多毛孢菌; *Pestalotiopsis neglecta*

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Studies on the Secondary Metabolites of Endophytic Fungus *Pestalotiopsis neglecta* Isolated from Mangrove Plant *Bruguiera gymnorrhiza*

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Abstract: To investigate the secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* which was isolated from the leaves of mangrove plant *Bruguiera gymnorrhiza*. A variety of chromatographic purification methods like CC, PTLC, HPLC, and Sephadex LH-20 were applied and seven compounds were isolated. Their structures were identified according to their physicochemical properties and spectral data, they are as follows: 1, 6, 8-trihydroxy-3-(hydroxymethyl) anthraquinone (1), ergosta-4, 6, 8(14), 22-tetraen-3-one (2), 24 β -ethylcholest-4-en-3 β -ol (3), 24-norcholesta-22-en-3 β -ol (4), ergosta-5, 7, 22-trienol (5), cholesterol (6), 24-methylcholest-5-en-3 β , 7 α -diol (7). These compounds are isolated from endophytic fungus *P. neglecta* for the first time.

Key words: mangrove plant; *Bruguiera gymnorrhiza*; endophytic fungus; *Pestalotiopsis neglecta*

由于自身特殊的生存环境, 海洋微生物具有独特的代谢途径, 产生了诸多结构新颖、生物活性显著的次级代谢产物, 使得海洋微生物成为又一个天然活性物质资源的巨大宝库^[1]。红树林作为自然生长在热带和亚热带河口潮间带的一种特殊的木本植物群落, 一直处于高盐、频繁的潮汐、强风、高温、强紫外辐射和缺氧污泥的特殊环境^[2], 为寄生在植物内部的微生物提供独特的生活环境, 使得其次级代谢

产物结构类型丰富多样且具有不同的生物活性, 因而引起人们的广泛关注。如具有抗菌及抗肿瘤活性的 divergolides A-D^[3], 抗真菌活性的 viburspiran^[4] 和 oxepinamides^[5] 等。目前已分离鉴定的红树林真菌超过 200 种, 成为海洋真菌的第二大类群。因此, 红树林内生真菌成为新药研发的重要资源。为了开发利用我国的海洋微生物资源, 从中寻找具有生物活性及药用前景的海洋天然产物, 我们对采自我国南海海域红树植物木榄叶子中分离得到的一株忽视拟盘多毛孢菌 *Pestalotiopsis neglecta* 的次级代谢产物进行了研究, 从中分离得到 7 个化合物, 均为首次从该种真菌中得到。

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1 材料和方法

1.1 实验材料

该菌株分离自我国南海海域红树植物木榄的叶子,经鉴定为 *P. neglecta*。该菌株现存放于中国科学院南海海洋研究所,广东海洋药物重点实验室。

1.2 仪器和试剂

NMR 谱用 Bruker AV-500 核磁共振仪测定;质谱图采用 API2000 型 ESI 质谱仪测定;中压制备柱色谱(Buchi 公司产品 C615/605);ZYJ-S 超净工作台(苏州净化设备公司);MCO-15AC 型 CO₂ 培养箱(SANYO);高效液相色谱仪(岛津公司);葡聚糖凝胶 Sephadex LH-20(Pharmacia 公司);薄层色谱硅胶和柱色谱硅胶,购自青岛海洋化工厂;化学试剂均为分析纯(天津市富宇精细化工有限公司和广州化学试剂厂)。

1.3 菌株发酵

将保藏于 4 °C 的菌株接种到麦芽提取物固体培养基(麦芽提取物 1.5 g,琼脂粉 1.5 g,蒸馏水 100 mL,PH 7.4 ~ 7.8)平板上,并将接种后的平板置于 25 °C 培养 7 d。将活化后的菌种转接到装有 10 mL 的麦芽提取物液体培养基(麦芽提取物 1.5 g,蒸馏水 100 mL,PH 7.4 ~ 7.8)的 100 mL 三角瓶中,28 °C 下 180 rpm 摇床培养 2 d。将 10 mL 种子培养液转接于装有 大米固体培养基(大米 200 g,自来水 200 mL)的 1 L 三角瓶中,自然光照下,于 25 °C 静止培养 35 d,共发酵 30 瓶。

1.4 化合物的提取分离和结构鉴定

将固体发酵培养物切成小块,加入丙酮(2:1,V:V),充分搅拌后,超声提取 15 分钟,再浸泡过夜,抽滤,收集上清液。残渣再以乙酸乙酯(1:1,V:V)浸提 3 次,每次充分搅拌后,超声提取 15 min,再浸泡过夜,抽滤,收集上清液。将 4 次浸提得到的上清液合并后进行减压蒸馏,得粗浸膏(约 150 g)。将粗浸膏经硅胶柱层析,以石油醚-丙酮系统(100:0~0:100)梯度洗脱,经 TLC 薄层检测合并后,得到 16 个馏分 Frs. 1-16。Frs. 3 经 Sephadex LH-20 凝胶柱(氯仿-甲醇,1:1)分离,得到 4 个馏分 Frs. 17-21,其中 Frs. 17 经硅胶柱分离,以石油醚-乙酸乙酯梯度洗脱并重结晶得化合物 1(8.1 mg);Frs. 18-19 经硅胶柱分离,以石油醚-乙酸乙酯梯度洗脱并重结晶得化合物 2(5.5 mg)和化合物 3(6.1 mg),Frs. 4 经正相硅胶柱分离,石油醚-乙酸乙酯(50:1~5:1)梯度

洗脱得到化合物 4(7.8 mg);Frs. 5 经 Sephadex LH-20 凝胶柱(氯仿-甲醇,1:1)分离,得到 5 个馏分 Frs. 22-Frs. 26,其中 Frs. 23 经硅胶柱分离,石油醚-丙酮(20:1~2:1)梯度洗脱,得到化合物 5(3.5 mg);Frs. 25 经硅胶柱分离,氯仿-丙酮(50:1~2:1)梯度洗脱,得到化合物 6(5.8 mg);其中 Frs. 26 经硅胶柱分离,氯仿-甲醇(20:1~2:1)梯度洗脱,得到化合物 7(5.3 mg)。

2 结果

化合物 1 白色针状结晶;¹H NMR (CDCl₃, 500 MHz) δ:6.51 (1H,d,*J* = 8.5 Hz,H-7),6.25 (1H,d,*J* = 8.5 Hz,H-6),5.22 (1H,m,H-23),5.14 (1H,m,H-22),3.98 (1H,m,H-3),1.06 (3H,s,H-19),1.02 (3H,d,*J* = 6.5 Hz,H-26),0.93 (3H,d,*J* = 6.5 Hz,H-27),0.86 (3H,d,*J* = 6.5 Hz,H-28),0.83 (3H,d,*J* = 6.5 Hz,H-21);¹³C NMR (CDCl₃, 125 MHz) δ:135.8 (d,C-22),132.3 (d,C-23),131.0 (d,C-7),135.3 (d,C-6),82.5 (s,C-5),80.1 (s,C-8),66.5 (d,C-3),56.1 (d,C-17),52.0 (d,C-14),51.9 (d,C-9),44.8 (s,C-13),40.6 (t,C-12),43.0 (d,C-24),40.1 (d,C-20),37.1 (s,C-10),36.9 (t,C-4),34.8 (t,C-1),33.5 (d,C-25),30.2 (t,C-2),29.9 (t,C-16),23.7 (t,C-11),21.4 (q,C-21),21.0 (t,C-15),20.6 (q,C-27),19.6 (q,C-26),18.5 (q,C-28),18.0 (q,C-19),13.0 (q,C-18)。以上数据与文献^[6]对照基本一致,确定化合物 1 为麦角甾-6,22-二烯-5 α ,8 α -环二氧-3-醇。

化合物 2 白色针状结晶;¹H NMR (CDCl₃, 500 MHz) δ:6.59 (1H,d,*J* = 9.4 Hz,H-7),6.01 (1H,d,*J* = 9.4 Hz,H-6),5.23 (1H,dd,*J* = 7.6,15.2 Hz,H-23),5.17 (1H,dd,*J* = 7.6,15.2 Hz,H-22),1.03 (3H,d,*J* = 6.7 Hz,H-21),1.01 (3H,s,H-19),0.93 (3H,s,H-18),0.91 (3H,d,*J* = 6.7 Hz,H-28),0.83 (3H,d,*J* = 6.7 Hz,H-27),0.80 (3H,d,*J* = 6.7 Hz,H-26);¹³C NMR (CDCl₃, 125 MHz) δ:199.4 (s,C-3),164.3 (s,C-5),156.0 (s,C-14),135.0 (d,C-22),133.9 (d,C-7),132.5 (d,C-23),124.5 (s,C-8),124.4 (d,C-6),123.0 (d,C-4),55.7 (d,C-17),44.8 (d,C-9),44.0 (s,C-13),42.9 (d,C-25),39.2 (d,C-20),36.7 (s,C-10),35.6 (t,C-12),34.2 (t,C-2),34.1 (t,C-1),33.1

(d, C-24), 25.3 (t, C-15), 21.2 (q, C-21), 19.9 (q, C-27), 19.6 (t, C-26), 20.6 (q, C-27), 18.9 (t, C-11), 18.9 (q, C-18), 17.6 (q, C-28), 16.6 (q, C-19)。以上数据与文献^[7]对照基本一致, 故鉴定化合物 **2** 为麦角甾-4,6,8(14),22-四烯-3-酮。

化合物 3 白色针状结晶; ¹H NMR (CDCl₃, 500 MHz) δ : 5.35 (1H, d, $J = 4.8$ Hz, H-4), 3.55 (1H, m, H-3), 1.01 (3H, s, H-19), 0.93 (3H, s, H-21), 0.86 (3H, d, $J = 6.6$ Hz, H-26), 0.83 (3H, d, $J = 7.3$ Hz, H-29), 0.81 (3H, d, $J = 6.6$ Hz, H-27), 0.68 (3H, s, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ : 140.8 (s, C-5), 121.8 (d, C-4), 71.8 (d, C-3), 56.7 (d, C-14), 56.1 (d, C-17), 50.3 (d, C-9), 46.0 (d, C-24), 42.3 (t, C-12), 42.3 (s, C-13), 39.4 (t, C-22), 36.5 (s, C-10), 36.3 (d, C-20), 34.0 (t, C-7), 32.0 (d, C-8), 31.7 (t, C-2), 31.2 (t, C-1), 29.2 (d, C-25), 28.3 (t, C-16), 26.1 (t, C-23), 24.4 (t, C-6), 24.3 (t, C-15), 23.0 (t, C-28), 21.1 (t, C-11), 19.8 (q, C-19), 19.5 (q, C-26), 19.1 (q, C-27), 18.7 (q, C-21), 12.0 (q, C-18), 11.9 (q, C-29)。以上数据与文献^[8]对照基本一致, 故鉴定化合物 **3** 为 24 β -乙基胆甾-4-烯-3 β -醇。

化合物 4 白色粉末; ¹H NMR (CDCl₃, 500 MHz) δ : 5.26 (1H, m, H-22), 5.17 (1H, m, H-22), 4.01 (1H, m, H-3), 1.02 (3H, s, H-19), 0.94 (3H, d, $J = 6.5$ Hz, H-21), 0.91 (3H, d, $J = 6.6$ Hz, H-26), 0.86 (3H, d, $J = 6.6$ Hz, H-27), 0.66 (3H, s, H-26); ¹³C NMR (CDCl₃, 125 MHz) δ : 135.2 (d, C-22), 132.5 (t, C-23), 71.4 (d, C-3), 56.7 (d, C-14), 56.0 (d, C-17), 54.1 (d, C-9), 42.9 (d, C-24), 42.3 (s, C-13), 40.8 (t, C-12), 39.9 (s, C-5), 39.7 (d, C-20), 37.1 (t, C-4), 35.9 (d, C-8), 35.5 (t, C-10), 35.3 (t, C-1), 33.1 (d, C-25), 31.9 (t, C-7), 30.7 (t, C-2), 28.9 (t, C-6), 28.2 (t, C-16), 24.3 (t, C-15), 20.8 (q, C-26), 20.7 (q, C-21), 21.0 (t, C-11), 21.0 (q, C-27), 19.4 (q, C-19), 19.2 (q, C-28), 12.7 (q, C-18)。以上数据与文献^[9]对照基本一致, 故鉴定化合物 **4** 为 24-降胆甾-22-烯-3 β -醇。

化合物 5 白色针状结晶; ¹H NMR (CDCl₃, 500 MHz) δ : 5.57 (1H, m, H-6), 5.38 (1H, m, H-7), 5.20 (1H, m, H-22), 5.20 (1H, m, H-23), 3.65 (1H, m, H-3), 1.01 (3H, d, $J = 6.5$ Hz, H-21),

0.88 (3H, d, $J = 6.7$ Hz, H-28), 0.86 (3H, s, H-19), 0.81 (3H, d, $J = 6.7$ Hz, H-26), 0.80 (3H, d, $J = 6.7$ Hz, H-27), 0.62 (3H, s, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ : 141.1 (s, C-8), 139.1 (s, C-5), 135.0 (d, C-22), 131.3 (d, C-23), 119.6 (d, C-6), 116.0 (d, C-7), 73.3 (d, C-3), 56.1 (d, C-17), 53.6 (d, C-14), 46.2 (d, C-9), 42.7 (s, C-13), 42.5 (d, C-24), 40.1 (d, C-20), 39.3 (t, C-12), 37.1 (t, C-4), 36.5 (t, C-1), 33.5 (d, C-25), 30.2 (t, C-2), 30.1 (s, C-10), 28.1 (t, C-16), 22.1 (t, C-15), 21.5 (t, C-11), 21.5 (q, C-26), 20.6 (q, C-21), 20.6 (q, C-27), 18.5 (q, C-28), 16.3 (q, C-19), 12.0 (q, C-18)。以上数据与文献^[10]对照基本一致, 故鉴定化合物 **5** 为麦角甾-5,7,22-三烯醇。

化合物 6 白色晶体; ¹H NMR (CDCl₃, 500 MHz) δ : 0.66 (3H, s, H-18), 0.86 (6H, d, $J = 6.5$ Hz, H-27), 0.89 (3H, d, $J = 6.5$ Hz, H-26), 0.95 (3H, d, $J = 6.8$ Hz, H-21), 1.16 (3H, s), 3.78 (1H, m, H-3), 5.23 (1H, m, H-6); ¹³C NMR (CDCl₃, 125 MHz) δ : 135.8 (s, C-5), 117.5 (d, C-6), 71.8 (d, C-3), 56.7 (d, C-14), 56.1 (d, C-17), 50.3 (d, C-9), 42.3 (t, C-12), 42.3 (s, C-13), 39.4 (t, C-22), 38.5 (t, C-24), 36.5 (s, C-10), 36.3 (d, C-20), 34.0 (t, C-7), 32.0 (d, C-8), 31.7 (t, C-2), 31.2 (t, C-1), 29.5 (t, C-4), 29.2 (d, C-25), 28.3 (t, C-16), 26.1 (t, C-23), 24.3 (t, C-15), 23.0 (t, C-28), 21.1 (t, C-11), 19.8 (q, C-19), 19.5 (q, C-26), 19.1 (q, C-27), 18.7 (q, C-21), 12.0 (q, C-18), 11.9 (q, C-29)。以上数据与文献^[11]对照基本一致, 故鉴定化合物 **6** 为胆甾醇。

化合物 7 无色针状结晶; ¹H NMR (CDCl₃, 500 MHz) δ : 5.61 (1H, d, $J = 5.0$ Hz, H-6), 3.86 (1H, brs, H-7 β), 3.58 (1H, m, H-3 α), 2.34 (1H, dd, $J = 13.0, 11.0$ Hz, H-4 α), 2.38 (1H, ddd, $J = 13.0, 5.5, 2.0$ Hz, H-4 β), 1.00 (3H, s, H-19), 0.93 (3H, d, $J = 6.5$ Hz, H-21), 0.85 (3H, d, $J = 7.0$ Hz), 0.78 (3H, d, $J = 7.0$ Hz), 0.78 (3H, d, $J = 7.0$ Hz), 0.68 (3H, s, H-18); ¹³C NMR (CDCl₃, 125 MHz) δ : 146.2 (s, C-5), 123.9 (d, C-6), 71.4 (d, C-3), 65.3 (d, C-7), 55.6 (d, C-17), 49.4 (d, C-14), 39.1 (t, C-12), 39.0 (d, C-24), 37.5 (d, C-8), 37.4 (s, C-10), 36.9 (t, C-1), 35.4 (d, C-20), 33.7 (t, C-22), 31.5 (d, C-26), 31.4 (t, C-2), 30.4

(t, C-23), 28.2 (t, C-16), 24.3 (t, C-15), 21.2 (t, C-11), 20.7 (q, C-21), 18.9 (q, C-27), 18.2 (q, C-28), 17.6 (q, C-19), 15.5 (q, C-25), 11.6 (q, C-18)。以上数据与文献^[12]对照基本一致,故鉴定化合物 7 为 24-甲基胆甾-5-烯-3 β ,7 α -二醇。

3 结论

从红树植物木榄内生真菌忽视拟盘多毛孢菌 *P. neglecta* 的大米发酵物中共分离得到 7 个化合物,这些化合物均为首次从该种真菌中分离得到。

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