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西澳粘滑菇的化学成分研究

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摘 要: 利用各种常规色谱分离技术从西澳粘滑菇(*Hebeloma westraliense*)发酵液中分离得到 5个化合物, 经波谱学分析鉴定为 Volemolide(**1**)、过氧化麦角甾醇(**2**)、对羟基苯甲酸(**3**), 对甲氧基苯乙酸(**4**)和对羟基苯乙醇(**5**)。其中化合物 **1**是一个七降麦角甾醇类物质, 系首次从丝膜菌科真菌中分离得到。

关键词: 西澳粘滑菇; 发酵液; Volemolide
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Chemical Constituents of Culture Broth of *Hebeloma westraliense*

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Abstract Volemolide (**1**), a norsterol with a heptanorergostane skeleton was isolated from the culture broth of *Hebeloma westraliense*, together with other four known compounds including ergosterol peroxide (**2**), 4-hydroxybenzoic acid (**3**), 4-methoxyphenylacetic acid (**4**), and 4-hydroxy-benzeneethanol (**5**). The structures of these compounds were elucidated by spectroscopic methods, and the compound **1** was firstly founded in the Cortinariaceae.

Key words *Hebeloma westraliense*; culture broth; volemolide

担子菌亚门丝膜菌科(Cortinariaceae)滑锈伞属(*Hebeloma*)包括多种有毒或非食用性种类, 在北欧洲现已发现 45种[1]。目前仅有 5种该属真菌的化学成分和活性得到研究[2-11]。我们曾对采自云南哀牢山的该属真菌黄滑锈伞(*H. versipelle*)的化学成分进行了研究, 并从中发现了 1个具有中等抗肿瘤活性的新羊毛甾烷型三萜[12]。为了进一步寻找该属真菌中的生理活性物质, 本实验对西澳粘滑菇[13](*H. westraliense*)发酵液的化学成分进行了研究。从中分离并鉴定了 5个化合物, 分别是 Volemolide(**1**)、过氧化麦角甾醇(**2**)、对羟基苯甲酸(**3**)、甲氧基苯乙酸(**4**)和对羟基苯乙醇(**5**)。其中化合物 **1**系首次从丝膜菌科真菌中分离得到, 是一个七降麦角甾醇类化合物。Kenji等[14]首次报道该化合物分离于多汁乳菇(*Lactarius volemus*), 并认为其是红菇科、牛肝菌科等真菌化学分类的关键物质, 其活性有待进一步研究。

1 仪器与材料

XRC-1型显微熔点仪(四川大学科学仪器厂), 温度计未校正; BrukerAM-400核磁共振仪, TMS为内标; VG Auto Spec-3000质谱仪测定; 薄层层析硅胶和柱层析硅胶(青岛海洋化工厂); Sephadex LH-20(Merk)。显色方法为 254、365 nm 荧光、10% 硫酸乙醇溶液和硫酸香草醛处理后加热显色及碘蒸气显色。

西澳粘滑菇(*H. westraliense*)菌株由昆明植物研究所纪大干研究员鉴定并分离。标本菌株存于昆明植物研究所隐花植物标本馆。

2 发酵培养

西澳粘滑菇菌种采用平皿转摇瓶液体培养方法。培养基: 葡萄糖 40 g, 去皮土豆 400 g, $MgSO_4$ 3.0 g, KH_2PO_4 6.0 g, VB_1 20.0 mg, 酵母膏 4 g, 加水混合搅拌至用柠檬酸调 pH 6.5, 温度 25 ℃; 转速: 170 r/min, 暗中培养发酵 25 d, 共得发酵液约 12 L。

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3 提取与分离

西澳粘滑菇发酵液 12 L, 用乙酸乙酯萃取 3次(12 h/次, 室温), 过滤。合并滤液并浓缩, 得浸膏约 1.15 g, 浸膏用甲醇溶解后与 2 g RP-8硅胶拌样, 上 RP-8柱, 用甲醇-水梯度洗脱。甲醇洗脱部分(0.383 g)经反复柱层析得到化合物 **1**(3 mg)、**2**(4 mg)。在甲醇-水(8∶2)部分, 经葡聚糖凝胶层析细分后用薄层制备得到化合物 **4**(4 mg)、**5**(3 mg)。在甲醇-水(4∶6)部分, 经葡聚糖凝胶层析细分, 得到化合物 **3**(26 mg)。

4 结构鉴定

Volemolide (1, 2, 3, 4, 5, 10, 19-heptanorergosta-9α-methoxy-7, 22-dien-6, 9-lactone, 1) 无色油状物; $C_{22}H_{36}O_3$; EI-MS *m/z* (rel. int.): 346 $[M]^+$ (15), 314 (20), 271 (70), 189 (100), 161 (65), 133 (45), 83 (67), 69 (96), 55 (90); ^{1}H NMR (400 MHz, CD_3Cl) δ_H: 5.75 (d, *J* = 1.7 Hz, H-7), 1.77 (1H, m, H-11a), 2.33 (1H, ddd, *J* = 13.3, 3.5, 2.2 Hz, H-11b), 1.57 (1H, m, H-12a), 1.93 (1H, m, H-12b), 2.41 (1H, ddd, *J* = 9.4, 6.0, 1.7 Hz, H-14), 1.49 (1H, m, H-15a), 1.73 (1H, m, H-15b), 1.48 (1H, m, H-16a), 1.90 (1H, m, H-16b), 1.50 (1H, m, H-17), 0.61 (3H, s, H-18), 2.06 (1H, m, H-20), 1.03 (3H, d, *J* = 6.6 Hz, H-21), 5.16 (1H, dd, *J* = 15.3, 7.3 Hz, H-22), 5.26 (1H, dd, *J* = 15.3, 7.3 Hz, H-23), 1.87 (1H, m, H-24), 1.48 (1H, m, H-25), 0.82 (3H, d, *J* = 6.7 Hz, H-26), 0.84 (3H, d, *J* = 6.7 Hz, H-27), 0.92 (3H, d, *J* = 6.7 Hz, H-28), 3.12 (3H, s, OMe); ^{13}C NMR (100 MHz, CD_3Cl) δ_C: 170.7 (s, C-6), 114.2 (d, C-7), 169.1 (s, C-8), 107.8 (s, C-9), 34.5 (t, C-11), 35.1 (t, C-12), 48.9 (s, C-13), 50.6 (d, C-14), 21.3 (t, C-15), 28.9 (t, C-16), 55.5 (d, C-17), 11.9 (q, C-18), 40.1 (d, C-20), 21.0 (q, C-21), 134.6 (d, C-22), 132.9 (d, C-23), 42.8 (d, C-24), 33.0 (d, C-25), 19.7 (q, C-26), 20.0 (q, C-27), 17.6 (q, C-28), 50.1 (s, OMe)。上述数据与文献[14]报道化合物 volemolide数据基本一致。

过氧化麦角甾醇(ergosterol peroxide, **2**) 无色针晶; $C_{28}H_{44}O_3$; mp. 179 ~ 181 °C; EI-MS *m/z* (rel. int.): 428 $[M]^+$ (5), 410 (8), 396 (10), 251 (24), 161 (29), 149 (31), 107 (37), 81 (56), 69 (100), 55 (73); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H: 6.49 (1H, d, *J* = 8.5 Hz), 6.23 (1H, d, *J* = 8.5 Hz), 5.20 (1H, dd, *J* = 7.6, 7.5 Hz), 5.12 (1H, dd, *J* = 7.6, 7.5 Hz), 3.94 (1H, m), 2.08 ~ 1.49 (20H, m), 1.20 (3H, s), 0.98 (3H, d, *J* = 6.6 Hz), 0.88 (3H, d, *J* = 6.8 Hz), 0.86 (3H, s), 0.82 (3H, d, *J* = 3.6 Hz), 0.79 (3H, d, *J* = 3.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 135.4, 135.2, 132.3, 130.7, 82.1, 79.4, 66.5, 56.2, 51.7, 51.1, 44.6, 42.7, 39.7, 39.4, 37.0, 37.0, 34.7, 33.1, 30.1, 28.6, 23.4, 20.9, 20.6, 20.0, 19.6, 18.1, 17.5, 12.9。以上波谱数据与文献[15]一致。

对羟基苯甲酸(**4**-hydroxybenzoic acid, **3**) 无色结晶, $FeCl_3$反应阳性; mp. 214 ~ 216 ℃; EI-MS *m/z* (rel. int.): 138 $[M]^+$ (84), 121 (100), 93 (30), 65 (23)。与标准品点板对照一致, 确定为对羟基苯甲酸。

对甲氧基苯乙酸(**4**-methoxyphenylacetic acid, **4**) 无色固体, mp. 85 ℃; EI-MS *m/z* (rel. int.): 166 $[M]^+$ (33), 149 (27), 107 (100), 57 (14); ^{1}H NMR (400 MHz, CD_3OD) δ_H: 3.56 (2H, s, H-2), 6.76 (2H, dd, *J* = 8.5, 1.5 Hz, H-2′, 6′), 7.13 (2H, dd, *J* = 8.5, 1.5 Hz, H-3′, 5′), 3.70 (3H, s, OMe); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 172.7 (s, C-1), 40.2 (t, C-2), 115.5 (s, C-1′), 130.4 (d, C-2′, 6′), 115.1 (d, C-3′, 5′), 154.8 (s, C-4′), 52.1 (q, OMe)。以上波谱数据与文献[16]一致。

对羟基苯乙醇(**4**-hydroxy-benzeneethanol, **5**) 无色结晶, $FeCl_3$反应阳性; mp. 90 ~ 91 ℃; EI-MS *m/z* (rel. int.): 138 $[M]^+$ (28), 107 (100), 77 (14); ^{1}H NMR (400 MHz, CD_3OD) δ_H: 3.67 (2H, t, *J* = 7.2 Hz, H-1), 2.70 (2H, t, *J* = 7.2 Hz, H-2), 6.69 (2H, d, *J* = 8.3 Hz, H-2′, 6′), 7.02 (2H, d, *J* = 8.3 Hz, H-3′, 5′); ^{13}C NMR (100 MHz, CD_3OD) δ_C: 64.6 (t, C-1), 39.4 (t, C-2), 115.3 (s, C-1′), 130.8 (d, C-2′, 6′), 116.1 (d, C-3′, 5′), 156.7 (s, C-4′)。其核磁数据与文献[17]一致。

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(上接第 750页)

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