·论 文·

一株植物内生真菌漆斑菌次级代谢产物的研究

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摘 要 从一株植物内生漆斑菌属*Myrothecium* sp. 真菌固体发酵物的乙酸乙酯提取物中分离得到12个化合物。采用硅胶、Sephadex LH-20和制备高效液相等柱色谱技术进行分离纯化,通过理化性质研究及波谱学手段对单体化合物进行结构鉴定,分别鉴定为3′-羟基疣孢菌素A(**1**)、疣孢菌素A(**2**)、乙酰基疣孢菌素L(**3**)、疣孢菌素J(**4**)、疣孢菌素K(**5**)、漆斑菌素A(**6**)、漆斑菌素D(**7**)、漆斑菌素H(**8**)、漆斑菌素J(**9**)、verrol 4-acetate (**10**)、(3*S*, 3a*S*, 6α, 6a*R*)-dihydrosporothrioride (**11**)和4,6-二羟基-1(3*H*)-异苯并呋喃(**12**)。其中,化合物**1**、**5**、**9** ~ **12**为首次从漆斑菌属真菌中分离得到,化合物**2**、**3**、**4**、**7**和**8**对白色念珠菌有较强的抑制作用,其最低抑菌浓度MIC_{50}分别是0.318、0.218、0.047、0.569和0.558 μg/mL。

关键词 半夏;内生真菌;漆斑菌属;疣孢菌素;漆斑菌素

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Secondary metabolites of the endophytic fungus *Myrothecium* sp. from *Pinellia ternata*

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Abstract Using a series of purification methods including silica gel, Sephadex LH-20 and preparative high performance liquid chromatography, secondary metabolites of *Myrothecium* sp. were purified from the ethyl acetate extract of the solid fermentation product. Based on structure characterization and investigation on the physical and chemical properties a, twelve monomeric compounds were identified as 3′-hydroxyverrucarin A (**1**), verrucarin A (**2**), verrucarin L acetate (**3**), verrucarin J (**4**), verrucarin K (**5**), roridin A (**6**), roridin D (**7**), roridin H (**8**), roridin J (**9**), verrol 4-acetate (**10**), (3*S*, 3a*S*, 6α, 6a*R*)-dihydrosporothrioride (**11**) and 4,6-dihydroxy-1(3*H*)-isobenzofuranone (**12**). Compounds **1**, **5** and **9**-**12** were isolated from *Myrothecium* sp. for the first time. Compounds **2**, **3**, **4**, **7** and **8** exhibited strong inhibitory effects on *Candida albicans*, with a minimal inhibitory concentration (MIC_{50}) of 0.318, 0.218, 0.047, 0.569 and 0.558 μg/mL, respectively.

Key words *Pinellia ternata*; endophytic fungi; *Myrothecium* sp.; verrucarin; roridin

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植物内生真菌是活性天然化合物的重要来源之一[1]。它们的部分或整个生活史定植在植物中,与宿主共同进化。在这个共同进化的过程中,内生真菌和宿主植物表现出互惠互利的关系。一方

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面内生真菌利用宿主植物的物质完成自身的生长和繁殖,另一方面内生真菌可以产生一些活性代谢产物有助于宿主植物抗病抗虫害,提高宿主的生存能力[2]。目前,从植物内生真菌中已经分离鉴定出丰富的天然产物,如:生物碱、聚酮类化合物、萜类化合物和肽类化合物等,这些化合物具有抗菌、细胞毒、抗结核、抗阿尔茨海默病等广泛的生物活性[3-6]。因此,对植物内生真菌代谢产物的发现与挖掘有利于寻找更多的生物活性化合物,为研究创新药物提供一条有效的途径。

本研究从半夏新鲜的块茎表皮分离得到一株漆斑菌属真菌,对其发酵产物乙酸乙酯提取物进行了抗菌活性初筛,发现其具有较好的抗白色念珠菌活性。通过进一步分离得到12个化合物,分别鉴定为3′-羟基疣孢菌素A(**1**)、疣孢菌素A(**2**)、乙酰基疣孢菌素L(**3**)、疣孢菌素J(**4**)、疣孢菌素K(**5**)、漆斑菌素A(**6**)、漆斑菌素D(**7**)、漆斑菌素H(**8**)、漆斑菌素J(**9**)、verrol 4-acetate(**10**)、(3*S*, 3a*S*, 6α, 6a*R*)-dihydrosporothrioride(**11**)和4,6-二羟基-1(3*H*)-异苯并呋喃(**12**),对分离出的化合物进行抗白色念珠菌活性测试,发现化合物**2**、**3**、**4**、**7**和**8**对白色念珠菌有较强的抑菌作用,MIC_{50}分别为0.318、0.218、0.047、0.569和0.558 μg/mL。

1 实验部分

1.1 材料与仪器

1100系列LC/MSD Trap质谱仪测定(美国Agilient公司);ACF-500 MHz型核磁共振仪(TMS内标);ACF-600 MHz型核磁共振仪(TMS内标)(德国Bruker公司);1260型高效液相色谱仪(美国Agilient公司);LC-8A型制备型高效液相色谱仪(日本岛津公司);100～200、200～300目硅胶,GF_{254}硅胶(青岛海洋化工有限公司);Sephadex LH-20(瑞典Amershan Pharmacia公司);RPMI 1640粉末,氟康唑(美国Gibco公司);分析级、色谱级甲醇(江苏汉邦科技有限公司);乙腈(色谱级,上海凌峰化学试剂有限公司);其他试剂均为市售分析纯。

本实验菌株于2016年从产自甘肃的半夏*Pinellia ternate*块茎中分离获得,通过PCR技术扩增其18S DNA,测序结果与已知真菌漆斑菌属*Myrothecium* sp.(MG 562611.1)有100%的同源性。

1.2 方 法

1.2.1 菌株发酵 将生长在马铃薯葡萄糖琼脂(PDB)斜面培养基上的内生真菌*Myrothecium* sp.接种到马铃薯葡萄糖琼脂(PDA)液体培养基中,28 ℃,160 r/min在摇床中培养4 d作为种子液备用。准备10个2 L的锥形瓶,每瓶装入大米320 g和水450 mL,浸泡过夜后,在121 ℃条件下灭菌20 min。大米培养基凉至室温后,将准备好的种子液接种于培养基中,室温下培养40 d。发酵完成后,内生真菌漆斑菌属固体产物用乙酸乙酯超声提取3次,提取液减压浓缩后得到褐色粗浸膏16 g。

1.2.2 提取与分离 浸膏用100~200目硅胶1∶1进行拌样,再经过200~300目硅胶柱用石油醚-乙酸乙酯(40∶1、20∶1、10∶1、5∶1、2∶1、1∶1、1∶2)梯度洗脱,采用TLC和HPLC检测分析合并得到7个馏份(Fr. A～G)。Fr. B经过Sephadex LH-20柱色谱(二氯甲烷-甲醇,1∶1)得到4个馏分(Fr. B_1～B_4),Fr. B_2经过制备高效液相得到化合物**1**(3 mg)、**3**(7.6 mg)和**12**(6 mg),Fr. B_3经过制备高效液相得到化合物**2**(20 mg)和**4**(6 mg)。Fr. C依次经过硅胶(石油醚-乙酸乙酯20∶1、10∶1、5∶1、2∶1、1∶1)、Sephadex LH-20柱色谱(二氯甲烷-甲醇1∶1),再经制备高效液相得到化合物**5**(4.5 mg)、**6**(5.8 mg)和**7**(2.7 mg),Fr. D经过Sephadex LH-20柱色谱(二氯甲烷-甲醇1∶1)得到化合物**8**(10 mg)和**9**(3 mg),Fr. E经过制备高效液相得到化合物**10**(3.5 mg)和**11**(3.2 mg)。

1.3 结构鉴定

化合物**1** 白色粉末,ESI-MS *m/z*:541[M + Na]$^+$。^{1}H NMR(600 MHz, $CDCl_3$) δ:8.25(1H, dd, J = 15.6 Hz, 12.1 Hz, H-3″),6.73(1H, t, J = 12.1 Hz, H-4″),6.14(1H, d, J = 11.6 Hz, H-5″),6.01(1H, d, J = 15.6 Hz, H-2″),5.75(1H, dd, J = 8.2 Hz, 4.4 Hz, H-4),5.44(1H, d, J = 5.3 Hz, H-10),4.56(1H, d, J = 12.3 Hz, H-15b),4.50(1H, m, H-5′b),4.36(1H, d, J = 12.3 Hz, H-15a),4.21(1H, m, H-5′a),4.10(1H, d, J = 5.9 Hz, H-2′),3.87(1H, d, J = 4.9 Hz, H-2),3.62(1H, d, J = 5.3 Hz, H-11),3.14(1H, d, J = 3.9 Hz, H-13b),2.88(1H, d, J = 3.9 Hz, H-13a),2.49(1H, dd, J = 15.3 Hz, 8.2 Hz, H-3b),2.22(1H, m, H-3a),2.02(1H, m, H-8b),2.00(1H, m, H-4′b),1.97(1H, m, H-7b),

1.85(1H, m, H-7a), 1.78(1H, m, H-4′a), 1.77(1H,m,H-8′a),1.74(3H,s,H-16),1.24(3H,s,H-6′),0.81(3H,s,H-14); ^{13}C NMR(150 MHz,$CDCl_3$) δ:173.2(C-1′),166.4(C-1″),165.2(C-6″),141.4(C-9),139.5(C-4″),139.3(C-3″),126.9(C-5″),125.4(C-2″),117.9(C-10),78.9(C-2),78.1(C-2′),75.7(C-4),73.2(C-3′),67.3(C-11),65.5(C-15),65.0(C-12),60.3(C-5′),49.3(C-5),48.0(C-13),44.1(C-6),35.2(C-3),34.9(C-4′),27.9(C-8),23.3(C-16),23.1(C-6′),20.4(C-7),7.6(C-14)。其波谱数据与文献报道一致[7],故鉴定化合物**1**为3′-羟基疣孢菌素A,其结构见图1。

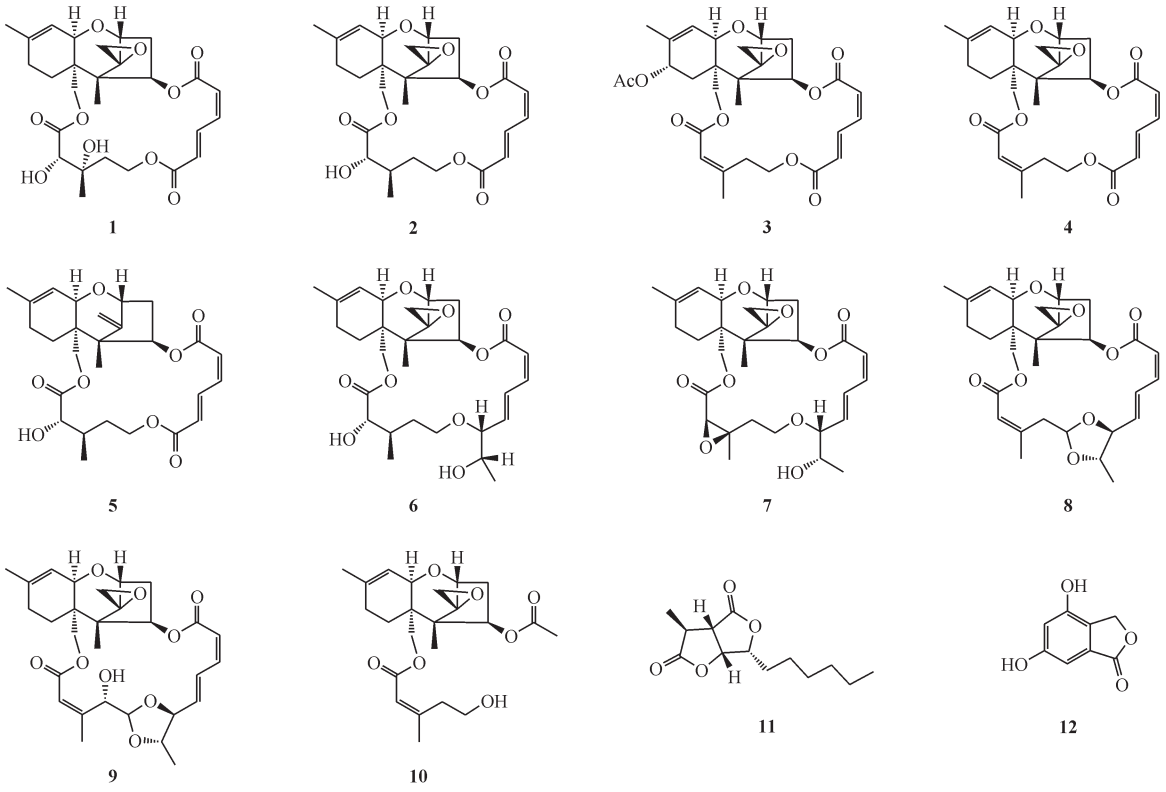

Figure 1　Structures of compounds **1-12**

化合物**2**　白色粉末,ESI-MS *m/z*:525[M + Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) δ:8.03(1H,dd,*J* = 15.6 Hz,11.6 Hz,H-3″),6.69(1H,t,*J* = 11.6 Hz,H-4″),6.16(1H,d,*J* = 11.6 Hz,H-5″),6.06(1H,d,*J* = 15.6 Hz,H-2″),5.82(1H,dd,*J* = 8.2 Hz,4.0 Hz,H-4),5.45(1H,d,*J* = 4.7 Hz,H-10),4.81(1H,d,*J* = 12.1 Hz,H-15b),4.52(1H,m,H-5′b),4.23(1H,d,*J* = 12.1 Hz,H-15a),4.16(1H,s,H-2′),4.00(1H,m,H-5′a),3.88(1H,d,*J* = 5.1 Hz,H-2),3.58(1H,d,*J* = 4.7 Hz,H-11),3.14(1H,d,*J* = 4.0 Hz,H-13b),2.88(1H,d,*J* = 4.0 Hz,H-13a),2.50(1H,m,H-3b),2.37(1H,m,H-3′),2.25(1H,m,H-3a),1.97(1H,m,H-7b),1.96(1H,m,H-4′b),1.80(1H,m,H-4′a),1.77(3H,s,H-16),1.72(1H,m,H-7a),0.89(3H,d,*J* = 6.8 Hz,H-6′),0.86(3H,s,H-14); ^{13}C NMR(150 MHz,$CDCl_3$) δ:174.7(C-1′),166.1(C-1″),165.5(C-6″),141.2(C-9),139.0(C-3″),138.8(C-4″),127.5(C-2″),125.8(C-5″) 117.9(C-10),78.9(C-2),75.5(C-4),74.2(C-2′),66.9(C-11),65.2(C-12),63.5(C-15),61.1(C-5′),49.5(C-5),47.8(C-13),44.2(C-6),34.9(C-3),33.2(C-3′),32.2(C-4′),27.5(C-8),23.3(C-16),20.0(C-7),10.0(C-6′),7.3(C-14)。其波谱数据与文献报道一致[8],故鉴定化合物**2**为疣孢菌素A,其结构见图1。

化合物**3**　白色粉末,ESI-MS *m/z*:565[M + Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) δ:8.01(1H,dd,*J* = 15.7 Hz,11.6 Hz,H-8′),6.62(1H,t,*J* = 11.3 Hz,H-9′),6.08(1H,d,*J* = 11.3 Hz,H-7′),6.01(1H,d,*J* = 15.7 Hz,H-10′),5.93(1H,dd,*J* = 8.2

Hz,4.3 Hz,H-4),5.77(1H,s,H-2′),5.70(1H,*J*=5.4 Hz,H-10),5.19(1H,m,H-8),4.54(1H,d,*J*=12.5 Hz,H-15b),4.49(1H,m,H-5′b),4.21(1H,d,*J*=12.5 Hz,H-15a),4.13(1H,m,H-5′a),3.84(1H,d,*J*=5.1 Hz,H-11),3.80(1H,d,*J*=5.5 Hz,H-2),3.11(1H,d,*J*=3.9 Hz,H-13b),2.84(1H,d,*J*=3.9 Hz,H-13a),2.46~2.57(3H,m,H-3a,4′),2.28(3H,s,H-12′),2.17~2.22(3H,m,H-3b,7),1.95(3H,s,H-18),1.76(3H,s,H-16),0.81(3H,s,H-14);^{13}C NMR(150 MHz,$CDCl_3$) *δ*:170.9(C-13′),165.8(C-11′),165.7(C-6′),165.5(C-1′),157.0(C-3′),139.9(C-8′),138.8(C-9′),136.5(C-9),127.8(C-7′),125.1(C-10′),123.8(C-10),117.7(C-2′),78.9(C-2),74.8(C-4),68.7(C-8),67.0(C-11),65.3(C-12),64.4(C-15),60.4(C-5′),49.0(C-5),47.9(C-13),42.2(C-6),40.2(C-4′),34.9(C-3),26.5(C-7),21.1(C-16),20.5(C-14′),17.1(C-12′),7.0(C-14)。其波谱数据与文献报道一致[9],故鉴定化合物**3**为乙酰基疣孢菌素L,其结构见图1。

化合物**4** 白色粉末,ESI-MS *m/z*:507[M+Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) *δ*:8.08(1H,dd,*J*=15.7 Hz,11.6 Hz,H-3″),6.64(1H,t,*J*=11.6 Hz,H-4″),6.12(1H,d,*J*=11.6 Hz,H-5″),6.02(1H,d,*J*=15.7 Hz,H-2″),6.01(1H,m,H-4),5.85(1H,s,H-2′),5.48(1H,d,*J*=5.2 Hz,H-10),4.48(1H,m,H-5′b),4.44(1H,d,*J*=12.6 Hz,H-15b),4.17(1H,m,H-5′a),3.99(1H,*J*=12.6 Hz,H-15a),3.87(1H,d,*J*=5.1 Hz,H-2),3.77(1H,d,*J*=5.2 Hz,H-11),3.16(1H,d,*J*=4.0 Hz,H-13b),2.85(1H,d,*J*=4.0 Hz,H-13a),2.55(1H,m,H-3b),2.53(2H,m,H-4′),2.30(3H,m,H-6′),2.18(1H,m,H-3a),2.04(2H,m,H-8),1.98(1H,m,H-7b),1.84(1H,m,H-7a),1.73(3H,s,H-16),0.85(3H,s,H-14);^{13}C NMR(150 MHz,$CDCl_3$) *δ*:166.1(C-1′),165.8(C-6″),165.6(C-1″),156.6(C-3′),140.5(C-9),139.5(C-3″),139.1(C-4″),127.4(C-2″),125.5(C-5″),118.6(C-10),79.0(C-2),75.3(C-4),67.3(C-11),65.5(C-12),63.3(C-15),48.8(C-5),48.1(C-13),43.0(C-6),40.2(C-4′),35.1(C-3),27.7(C-8),23.3(C-16),20.8(C-7),17.2(C-6′),7.0(C-14)。其波谱数据与文献报道一致[10],故鉴定化合物**4**为疣孢菌素J,其结构见图1。

化合物**5** 白色粉末,ESI-MS *m/z*:509[M+Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) *δ*:8.04(1H,dd,*J*=15.7 Hz,11.6 Hz,H-3″),6.67(1H,t,*J*=11.6 Hz,H-4″),6.09(1H,d,*J*=11.6 Hz,H-5″),6.06(1H,d,*J*=15.7 Hz,H-2″),5.81(1H,dd,*J*=8.1 Hz,3.8 Hz,H-4),5.40(1H,d,*J*=5.1 Hz,H-10),5.18(1H,s,H-13b),4.77(1H,d,*J*=12.0 Hz,H-15b),4.77(1H,s,H-13a),4.52(1H,m,H-5′b),4.47(1H,d,*J*=5.1 Hz,H-2),4.22(1H,d,*J*=12.0 Hz,H-15a),4.16(1H,d,*J*=5.5 Hz,H-2′),4.00(1H,m,H-5′a),3.58(1H,d,*J*=5.1 Hz,H-11),2.50(1H,m,H-3),2.38(1H,m,H-3′),2.25(1H,m,H-3),1.97(1H,m,H-7b),1.96(1H,m,H-4′b),1.80(1H,m,H-4′a),1.72(3H,s,H-16),1.68(1H,m,H-7a),1.09(3H,s,H-14),0.89(3H,d,*J*=6.8 Hz,H-6′);^{13}C NMR(150 MHz,$CDCl_3$) *δ*:174.7(C-1′),166.0(C-1″),165.5(C-6″),151.6(C-12),140.8(C-9),138.9(C-3″),138.8(C-4″),127.5(C-2″),125.7(C-5″),118.2(C-10),106.5(13),78.7(C-2),75.6(C-4),74.1(C-2′),66.6(C-11),63.7(C-15),61.2(C-5′),52.1(C-5),44.3(C-6),36.0(C-3),33.2(C-3′),32.2(C-4′),27.5(C-8),23.3(C-16),18.8(C-7),12.2(C-14),10.0(C-6′)。其波谱数据与文献报道一致[11],故鉴定化合物**5**为疣孢菌素K,其结构见图1。

化合物**6** 白色粉末,ESI-MS *m/z*:555[M+Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) *δ*:7.65(1H,dd,*J*=15.6 Hz,11.6 Hz,H-8′),6.66(1H,t,*J*=11.6 Hz,H-9′),6.01(1H,d,*J*=15.6 Hz,H-7′),5.79(1H,dd,*J*=11.5 Hz,H-10′),5.78(1H,m,H-4),5.43(1H,d,*J*=4.2 Hz,H-10),4.42(2H,d,*J*=12.3 Hz,H-15),4.09(1H,s,H-2′),3.85(1H,d,*J*=5.0 Hz,H-2),3.68(1H,m,H-6′),3.62(1H,m,H-13),3.60(1H,m,H-11),3.53(2H,m,H-5′),3.11(1H,d,*J*=3.9 Hz,H-13b),2.80(1H,d,*J*=3.9 Hz,H-13a),2.45(1H,d,*J*=15.4,8.3 Hz,H-3b),2.20(1H,m,H-3a),2.03(2H,m,H-8),2.01(1H,m,H-3′),1.90(2H,m,H-7),1.78(2H,m,H-4′),1.73(3H,s,H-16),1.18(1H,d,*J*=6.3 Hz,H-14′),1.08(1H,d,*J*=6.9 Hz,H-12′),0.80(3H,s,

H-14); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 174.8 (C-1′), 166.5 (C-11′), 143.9 (C-9′), 140.9 (C-9), 139.3 (C-7′), 125.9 (C-8′), 118.2 (C-10), 117.4 (C-10′), 83.9 (C-6′), 79.0 (C-2), 75.6 (C-2′), 74.3 (C-4), 70.6 (C-13′), 69.7 (C-5′), 67.1 (C-11), 65.2 (C-12), 64.4 (C-15), 49.3 (C-5), 47.7 (C-13), 43.7 (C-6), 37.0 (C-3′), 34.8 (C-3), 33.0 (C-4′), 27.6 (C-8), 23.3 (C-16), 20.2 (C-7), 18.2 (C-14′), 14.7 (C-12′), 7.5 (C-14)。其波谱数据与文献报道一致[12],故鉴定化合物**6**为漆斑菌素 A,其结构见图1。

化合物**7**　白色粉末,ESI-MS *m/z*: 553 [M + Na]$^+$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.53 (1H, dd, *J* = 15.6 Hz, 11.4 Hz, H-8′), 6.62 (1H, t, *J* = 11.4 Hz, H-9′), 5.98 (1H, dd, *J* = 15.6 Hz, 3.3 Hz, H-7′), 5.85 (1H, m, H-4), 5.83 (1H, m, H-10′), 5.45 (1H, d, *J* = 4.8 Hz, H-10), 4.39 (1H, d, *J* = 12.4 Hz, H-15b), 4.28 (1H, d, *J* = 12.4 Hz, H-15a), 3.87 (1H, d, *J* = 5.1 Hz, H-2), 3.83 (1H, m, H-6′) 3.65-3.70 (2H, m, H-5′), 3.65 (1H, d, *J* = 4.8 Hz, H-11), 3.36 (1H, m, H-13′), 3.32 (1H, s, H-2′), 3.14 (1H, d, *J* = 4.0 Hz, H-13b), 2.83 (1H, d, *J* = 4.0 Hz, H-13a); 2.51 (1H, m, H-3b), 2.48 (1H, m, H-4′b), 2.18 (1H, m, H-4′a), 2.04 (1H, m, H-3a), 1.95 (2H, m, H-8), 1.83 (1H, m, H-7a), 1.74 (3H, s, H-16), 1.63 (3H, s, H-12′), 1.48 (1H, m, H-7b), 1.22 (3H, d, *J* = 6.2 Hz, H-12′), 0.85 (3H, s, H-14); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 168.1 (C-1′), 166.4 (C-11′), 143.1 (C-9′), 140.7 (C-9), 138.3 (C-7′), 126.3 (C-8′), 118.4 (C-10), 118.1 (C-10′), 85.6 (C-6′), 79.0 (C-2), 74.4 (C-4), 70.9 (C-13′), 67.4 (C-5′), 67.0 (C-11), 65.4 (C-12), 64.6 (C-15), 63.3 (C-3′), 58.2 (C-2′), 49.2 (C-5), 47.8 (C-13), 43.2 (C-6), 39.6 (C-4′), 35.1 (C-3), 27.6 (C-8), 23.3 (C-16), 20.6 (C-7), 18.2 (C-14′), 17.4 (C-12′), 7.0 (C-14)。其波谱数据与文献报道一致[13],故鉴定化合物**7**为漆斑菌素 D,其结构见图1。

化合物**8**　白色粉末,ESI-MS *m/z*: 535 [M + Na]$^+$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.71 (1H, m, H-8′), 6.58 (1H, t, *J* = 11.4 Hz, H-9′), 5.98 (1H, dd, *J* = 13.3 Hz, 0.8 Hz, H-7′), 5.96 (1H, m, H-4), 5.82 (1H, d, *J* = 11.1 Hz, H-10′), 5.70 (1H, s, H-2′), 5.55 (1H, dd, *J* = 8.5 Hz, 3.3 Hz, H-5′), 5.46 (1H, d, *J* = 4.2 Hz, H-10), 4.34 (1H, d, *J* = 12.5 Hz, H-15b), 4.08 (1H, m, H-6′), 4.06 (1H, d, *J* = 12.5 Hz, H-15a), 3.86 (1H, d, *J* = 5.1 Hz, H-2), 3.69 (2H, m, H-11, 13′), 3.15 (1H, d, *J* = 4.1 Hz, H-13b), 2.84 (1H, d, *J* = 4.1 Hz, H-13a), 2.66 (1H, dd, *J* = 12.4 Hz, 2.9 Hz, H-4′b), 2.50 (1H, dd, *J* = 15.3, 8, 4 Hz, H-3b), 2.30 (3H, s, H-12′), 2.28 (1H, m, H-3a), 2.19 (1H, m, H-4′a), 2.03 (2H, m, H-8), 1.89 (2H, m, H-7), 1.72 (3H, s, H-16), 1.36 (3H, d, *J* = 6.0 Hz, H-14′), 0.88 (3H, s, H-14); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 166.3 (C-11′), 166.2 (C-1′), 154.9 (C-3′), 142.9 (C-9′), 140.5 (C-9), 134.8 (C-7′), 126.1 (C-8′), 119.0 (C-2′), 118.8 (C-10′), 100.9 (C-2′), 82.0 (C-6′), 79.2 (C-2), 76.7 (C-13′), 73.9 (C-4), 67.7 (C-11), 65.6 (C-12), 63.2 (C-15), 49.0 (C-5), 48.0 (C-4′), 47.6 (C-13), 43.2 (C-6), 34.9 (C-3), 27.6 (C-8), 23.3 (C-16), 20.5 (C-7), 18.4 (C-12′), 16.5 (C-14′), 7.3 (C-14)。其波谱数据与文献报道一致[14],故鉴定化合物**8**为漆斑菌素 H,其结构见图1。

化合物**9**　白色粉末,ESI-MS *m/z*: 551 [M + Na]$^+$。^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.65 (1H, dd, *J* = 15.0 Hz, 11.3 Hz, H-8′), 6.55 (1H, t, *J* = 11.3 Hz, H-9′), 5.92 (1H, m, H-7′), 5.89 (1H, m, H-4), 5.79 (1H, d, *J* = 11.1 Hz, H-10′), 5.76 (1H, s, H-2′), 5.44 (1H, dd, *J* = 3.9 Hz, H-10), 5.25 (1H, d, *J* = 6.9 Hz, H-5′), 4.39 (1H, d, *J* = 12.5 Hz, H-15b), 4.09 (1H, d, *J* = 8.7 Hz, H-6′), 4.02 (1H, d, *J* = 12.5 Hz, H-15a), 3.78-3.79 (2H, m, H-2, 4′), 3.65 (2H, m, H-11, 13′), 3.12 (1H, d, *J* = 3.9 Hz, H-13b), 2.82 (1H, d, *J* = 3.9 Hz, H-13a), 2.47 (1H, dd, *J* = 15.3 Hz, 8.4 Hz, H-3′a), 2.27 (3H, s, H-12′), 2.18 (1H, m, H-3′b), 1.88-2.04 (4H, m, H-7, 8), 1.71 (3H, s, H-16), 1.37 (3H, d, *J* = 6.0 Hz, H-14′), 0.85 (3H, s, H-14); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.2 (C-11′), 165.9 (C-1′), 155.3 (C-3′), 143.1 (C-9′), 140.4 (C-9), 134.5 (C-7′), 126.1 (C-8′), 119.9 (C-2′), 118.8 (C-10′), 118.6 (C-10), 103.3 (C-5′), 82.2 (C-6′), 79.7 (C-4′), 79.2 (C-2), 76.4 (C-13′), 73.8 (C-4), 67.8 (C-11),

65.5(C-12),63.4(C-15),49.2(C-5),48.0(C-13),43.2(C-6),34.7(C-3),27.6(C-8),23.3(C-16),20.4(C-7),15.9(C-12′),13.1(C-14′),7.4(C-14)。其波谱数据与文献报道一致[15],故鉴定化合物**9**为漆斑菌素J,其结构见图1。

化合物**10** 白色粉末,ESI-MS *m/z*:443[M+Na]$^+$。^{1}H NMR(600 MHz,$CDCl_3$) δ:5.95(1H,dd,*J*=7.8 Hz,3.4 Hz,H-4),5.83(1H,d,*J*=1.0 Hz,H-2′),5.48(1H,d,*J*=4.8 Hz,H-10),4.16(1H,dd,*J*=12.5 Hz,H-15),4.13(1H,d,*J*=12.5 Hz,H-15),3.88(1H,m,H-2),3.86(1H,d,*J*=4.8 Hz,H-11),3.84(1H,m,H-5′),3.82(1H,m,H-5′),3.16(1H,d,*J*=4.0 Hz,H-13),2.85(1H,d,*J*=4.0 Hz,H-13),2.31(1H,m,H-3),2.30(1H,m,H-8),2.23(3H,d,*J*=1.0 Hz,H-6′),2.08(3H,s,H-2″),1.97(2H,m,H-4′),1.95(1H,m,H-3),1.88(1H,m,H-8),1.73(3H,s,H-16),1.31(2H,m,H-7),0.88(3H,s,H-14);^{13}C NMR(150 MHz,$CDCl_3$) δ:171.1(C-1″),165.9(C-1′),157.1(C-3′),140.8(C-9),118.4(C-10),117.2(C-2′),79.1(C-2),75.4(C-4),66.7(C-11),65.4(C-12),63.0(C-15),59.8(C-5′),48.6(C-5),48.0(C-13),43.8(C-4′),43.0(C-6),36.7(C-3),27.9(C-15),23.2(C-16),21.5(C-7),21.1(C-2″),18.9(C-6′),6.7(C-14)。其波谱数据与文献报道一致[16],故鉴定化合物**10**为verrol 4-acetate,其结构见图1。

化合物**11** 无色固体,ESI-MS *m/z*:263[M+Na]$^+$。^{1}H NMR(500 MHz,$CDCl_3$) δ:5.16(1H,dd,*J*=6.0,4.0 Hz,H-6a),4.60(1H,ddd,*J*=7.9 Hz,6.5 Hz,3.9 Hz,H-6),3.19(1H,dd,*J*=6.1 Hz,1.0 Hz,H-3a),3.12(1H,dd,*J*=7.6 Hz,1.0 Hz,H-3),1.96(1H,m,H-8a),1.87(1H,m,H-8b),1.55(2H,m,H-9),1.50(3H,d,*J*=7.6 Hz,H-7),1.42(2H,m,H-10),1.36(2H,m,H-11),1.35(2H,m,H-12),0.94(3H,t,*J*=6.8 Hz,H-13);^{13}C NMR(125 MHz,$CDCl_3$) δ:176.8(C-4),174.7(C-2),82.4(C-6),78.3(C-6a),49.0(C-3a),38.4(C-3),31.6(C-11),28.9(C-10),28.8(C-8),25.3(C-9),22.5(C-12),17.1(C-7),14.0(C-13)。其波谱数据与文献报道一致[17],故鉴定化合物**11**为(3*S*,3a*S*,6α,6a*R*)-dihydrosporothrioride,其结构见图1。

化合物**12** 白色固体,ESI-MS *m/z*:189[M+Na]$^+$。^{1}H NMR(600 MHz,DMSO) δ:9.90(1H,s,4-OH),8.49(1H,s,6-OH),6.66(2H,s,H-5,7),5.82(1H,dd,*J*=8.2 Hz,4.0 Hz,H-4),5.45(1H,d,*J*=4.7 Hz,H-10),4.81(1H,d,*J*=12.1 Hz,H-15),5.81(2H,s,H-3);^{13}C NMR(150 MHz,DMSO) δ:170.8(C-1),159.6(C-6),153.0(C-4),127.0(C-4a),124.4(C-7a),108.0(C-7),100.6(C-7),67.9(C-3)。其波谱数据与文献报道一致[18],故鉴定化合物**12**为4,6-二羟基-1(3*H*)-异苯并呋喃,其结构见图1。

1.4 抗白色念珠菌活性研究

对分离鉴定的化合物进行了抗白色念珠菌(*Candida albicans*,ATCC 24433)活性筛选,结果见表1。采用微量肉汤稀释法[19],将白色念珠菌菌悬液浓度用RPMI-1640培养液稀释至每毫升5×10^3 CFU备用,用DMSO溶解化合物**1**~**12**后,再用RPMI 1640培养液进行二倍量稀释,使化合物的质量浓度分别为25,12.5,6.25,3.125,1.562 5,0.781 25,0.390 625 μg/mL(DMSO<1%)。氟康唑为阳性对照,设置其质量浓度为2,1,0.5,0.25,0.125,0.062 5 μg/mL。取配制好的白色念珠菌混悬液和系列质量浓度的化合物**1**~**12**各100 μL于96孔板中作为药物组,取菌液100 μL和RPMI 1640液体培养基100 μL于96孔板中,用作生长对照组,取RPMI 1640液体培养基200 μL于96孔板中作为空白对照组。将96孔板放置于35 ℃培养箱中培养24 h后用酶标仪(540 nm)测试吸收度,计算其MIC_{50}(抑制50%菌生长时所对应的化合物浓度,与生长对照组相比)。试验重复3次。结果(表1)显示,化合物**2**、**3**、**4**、**7**和**8**对白色念珠菌有较强的抑制作用,MIC_{50}分别是0.318、0.218、0.047、0.569和0.558 μg/mL。

2 结 论

本研究从漆斑菌属真菌固体发酵物的乙酸乙酯提取物中分离得到了12个化合物,其中化合物**1**、**5**、**9**~**12**为首次从漆斑菌属真菌中分离得到,化合物**2**、**3**、**4**、**7**和**8**对白色念珠菌有较强的抑制作用。

Table 1　MIC_{50} values of the twelve compounds

Compound	MIC_{50} /(μg/mL)
1	> 25
2	0.318 ± 0.087
3	0.218 ± 0.063
4	0.047 ± 0.015
5	4.387 ± 0.742
6	1.581 ± 0.136
7	0.569 ± 0.259
8	0.558 ± 198
9	2.567 ± 0.652
10	1.275 ± 0.233
11	> 25
12	> 25
Fluconazole	0.25 ± 0.018

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