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青霉属真菌液体发酵产物化学成分研究

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摘　要:青霉属(*Penicillic sp.*)真菌的发酵液中提取分离得到 12 个化合物,其结构经波谱鉴定为 Paeonol (**1**), Orsellinic acid(**2**), Dibenz[b,e]oxepin-6,11-dione,1,9-dihydroxy-3-(hydroxymethyl)-10-methoxy-(**3**), Dibenz[b,e]oxepin-6,11-dione,1-hydroxy-3-(hydroxymethyl)-10-methoxy-(**4**), Janthinone(**5**), 对羟基苯甲醇(**6**), 对羟基苯甲醛 (**7**), 1,5-Anhydroxylitol(**8**),Penicillic acid(**9**),β-谷甾醇(**10**),Ergosta-5,7,22-trien-3-ol(**11**),Demethylincisterol A3(**12**)。化合物 **12** 对五株肿瘤细胞(HL-60,A549,SMMC-7721,MCF-7,SW480)具有一定的体外肿瘤生长抑制活性,其 IC_{50} 值分别为 14.29、14.02、13.91、16.45、16.05 μM.

关键词:青霉菌;内酯;青霉酸

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Chemical Studies on the Liquid Fermentinai Products from the *Penicillic* sp.

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Abstract: There were 13 liquid fermentinal products isolated from the *Penicillic sp.* They were identified as Paeonol (**1**), Orsellinic acid (**2**), Dibenz[*b*,*e*]oxepin-6,11-dione,1,9-dihydroxy-3-(hydroxymethyl)-10-methoxy- (**3**), Dibenz[*b*,*e*]oxepin-6,11-dione,1-hydroxy-3-(hydroxymethyl)-10-methoxy-(**4**), Janthinone (**5**), 4-(hydroxymethyl) phenol (**6**), 4-hydroxybenzaldehyde (**7**), 1,5-Anhydroxylitol (**8**), Penicillic acid (**9**), β-sitosterol (**10**), Ergosta-5,7,22-trien-3-ol (**11**), Demethylincisterol A3 (**12**). Compound **12** showed moderate toxicity against five human tumor cell (HL-60, A549, SMMC-7721, MCF-7, SW480) with IC_{50} value of 14.29, 14.02, 13.91, 16.45, 16.05 μM, respectively.

Key words: *Penicillic sp.*; lactones; Penicillic acid

微生物是一类重要的自然资源。微生物资源的开发利用已产生了巨大的社会和经济效益。青霉素的发现带动了其它抗生素及生物活性物质的寻找和发现,促使抗生素工业的形成。我国微生物资源丰富,迄今我们所认识的真菌达 7 万多种,细菌 5,000 多种,放线菌 3,000 多种。此外微生物易培养及大幅提高产率,这是其他植物资源无法比拟的[1]。青霉菌(*Penicillic* sp.)是由云南大学微生物研究所从云南程海中分离得到的一株真菌。本文对其次生代谢产物进行了初步的研究。该菌株经液体摇瓶发酵后,对其发酵液化学成分的分离与鉴定得到 12 个化合物。

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1　仪器与材料

Waters AutoSpec Premier P776 和 Bruker HCT/Esquire 型质谱仪; Bruker AM-400, DRX-500 和 Avance III 600 超导核磁共振仪,TMS(四甲基硅烷)为内标;JASCOD IP-370 和 OA AA-55 型数字旋光仪,浓度 c 单位为 g/mL;薄层色谱和正相柱色谱硅胶(青岛海洋化工);Sephadex LH -20(Pharm acia 公司);ODS(YMC 公司); MCI 树脂(三菱公司)。

青霉菌(*Myrothecium* sp.)菌种由云南大学微生物研究所提供。

2　培养基

2.1　种子培养基

天麻 3 g,H_2O 100 mL,pH 7.5,28 ℃培养 48 h。

2.2 发酵培养基

天麻 3 g,H_2O 100 mL,pH 7.5,28 ℃培养 72 h。

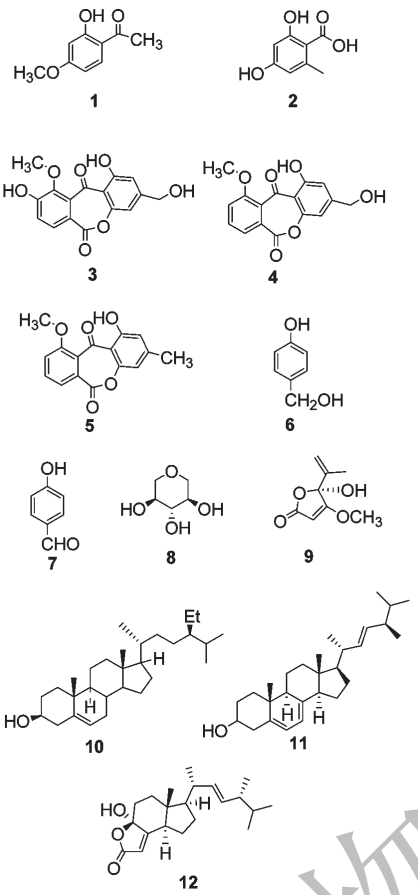

图 1 化合物 1-12 的结构

Fig. 1 The structure of compounds 1-12

3 提取与分离

发酵液 130 L 过大孔树脂,再用甲醇洗脱后,用石油醚和乙酸乙酯萃取,得到乙酸乙酯部位 120 g。用氯仿:甲醇(40:1~1:1)反复梯度洗脱,分为五个部分。对各部分的化合物用 MCI 脱色和粗分后,反复硅胶柱层析和用 Sephadex LH-20 凝胶柱色谱,甲醇冲洗,以及 HPLC 分析得到 13 个化合物。**1** (30 mg),**2** (350 mg),**3** (26 mg),**4** (15 mg),**5** (23 mg),**6** (5 mg),**7** (7 mg),**8** (12 mg),**9** (850 mg),**10** (39 g),**11** (360 mg),**12** (150 mg)。

4 结构鉴定

化合物 1 白色片状结晶(石油醚);mp. 48~50 ℃(lit.);^{1}H NMR ($CDCl_3$) δ:^{1}H NMR ($CDCl_3$) 2.56 (3H, s, -$COCH_3$), 3.82 (3H, s, -OCH_3), 6.47 (1H, d, J = 2.5 Hz, H-3), 6.53 (1H, dd, J = 8.0, 2.5 Hz, H-5), 7.62 (1H, d, J = 8.0 Hz, H-6), 12.64 (1H, s, OH);^{13}C NMR ($CDCl_3$) δ:26.0 (-CO*C*H3), 55.3 (-O*C*H3), 100.6 (C-3), 107.2 (C-5), 113.7 (C-1), 132.1 (C-6), 164.9 (C-2), 165.8 (C-4), 202.4 (-$COCH_3$)。以上数据与文献报道的 Paeonol 基本一致[2]。

化合物 2 无色针状结晶;mp. 188~189 ℃;^{1}H NMR (methanol-d_4) δ:6.25 (1H, d, J = 2.5Hz), 6.20 (1H, d, J = 2.5 Hz), 2.50 (3H, s). ^{13}C NMR (methanol-d_4) δ:24.2 (CH_3), 101.5 (C-3), 105.0 (C-1), 112.1 (C-5), 144.9 (C-6), 163.4 (C-3), 167.1 (C-2), 174.16 (COOH)。以上数据与文献报道的 Orsellinic acid 基本一致[3]。

化合物 3 黄色粉末;^{1}H NMR (DMSO-d_6) δ: 3.84 (3H, s, OCH_3), 4.58 (2H, brs, CH_2), 5.53 (1H, t, OH), 6.73 (1H, d, J = 1.1Hz, H-2), 6.98 (1H, d, J = 1.1Hz, H-4), 7.46 (1H, d, J = 8.0Hz, H-6), 7.62 (1H, d, J = 8.0Hz, H-5), 10.47 (1H, s, OH-7), 12.19 (1H, s, OH-1);^{13}C NMR (DMSO-d_6) δ: 180.1 (C-9), 167.2 (C-8), 160.8 (C-1), 155.8 (C-12), 154.4 (C-3), 151.1 (C-10), 149.2 (C-7), 125.7 (C-6), 120.5 (C-5), 117.4 (C-13, 14), 107.5 (C-2), 106.9 (C-11), 104.2 (C-4), 62.7 (CH_2OH), 52.6 (OCH_3)。以上数据与文献报道的 Dibenz [*b*, *e*] oxepin-6, 11-dione, 1, 9-dihydroxy-3-(hydroxymethyl)-10-methoxy-一致[4]。

化合物 4 黄色粉末;^{1}H NMR (DMSO-d_6) δ: 3.90 (3H, s, OCH_3), 4.59 (2H, d, J = 5.28 Hz, CH_2), 5.59 (1H, t, J = 5.67 Hz, -OH), 6.76 (1H, d, J = 1.1Hz, H-2), 6.99 (1H, d, J = 1.1Hz, H-4), 7.44 (1H, dd, J = 8.3, 1.1 Hz, H-7), 7.74 (1H, d, J = 7.3, 1.1 Hz, H-5), 7.93 (1H, dd, J = 8.3, 7.3 Hz, H-6), 12.05 (1H, s, OH-1);^{13}C NMR (DMSO-d_6) δ: 180.1 (C-9), 168.7 (C-8), 160.5 (C-1), 155.5 (C-12), 155.4 (C-10), 154.4 (C-3), 136.0 (C-6), 133.0 (C-14), 122.9 (C-7), 119.7 (C-5), 116.6 (C-13), 107.7 (C-2), 106.9 (C-11), 104.2 (C-4), 62.7 (CH_2OH), 52.6 (OCH_3)。以上数据与文献报道的 Janthinone 一致[5]。

化合物 5 黄色粉末;^{1}H NMR (DMSO-d_6) δ: 2.43 (3H, s, CH_3), 4.03 (3H, s, OCH_3), 6.63 (1H, d, J = 1.1Hz, H-2), 6.75 (1H, d, J = 1.1Hz, H-4),

7.30 (1H, dd, J = 8.3, 1.1 Hz, H-7), 7.52 (1H, d, J = 7.3, 1.1 Hz, H-5), 7.75 (1H, dd, J = 8.3, 7.3 Hz, H-6), 12.15 (1H, s, OH-1); ^{13}C NMR (DMSO-d_6) δ: 180.4 (C-9), 169.7 (C-8), 161.4 (C-1), 155.9 (C-12), 155.6 (C-10), 149.4 (C-3), 136.0 (C-6), 133.0 (C-14), 122.4 (C-7), 119.4 (C-5), 111.7 (C-13), 107.4 (C-2), 107.4 (C-11), 106.9 (C-4), 53.1 (OCH_3), 22.6 (CH_3)。以上数据与文献报道的 Janthinone 一致[4]。

化合物 6　无色针晶; ^{1}H NMR (DMSO-d_6) δ: 9.21 (1H, s, OH), 7.10 (2H, d, J = 8.1 Hz, H-3, 5), 6.69 (2H, d, J = 8.1 Hz, H-2, 6), 4.92 (1H, s, CH_2OH), 4.35 (2H, s, CH_2OH); ^{13}C NMR (DMSO-d_6) δ: 156.2 (C-1), 132.8 (C-4), 128.1 (C-3, C-5), 114.8 (C-2, C-6), 62.8 (CH_2OH)。以上数据与文献中报道羟基苯甲醇一致。

化合物 7　无色针状结晶; ^{1}H NMR (acetone-d_6) δ: 9.90 (1H, s), 9.40 (1H, s, br), 7.80 (2H, d, J = 8.7), 7.01 (2H, d, J = 8.7); ^{13}C NMR (acetone-d_6) δ: 191.0 (CHO), 163.9 (C-4), 132.8 (C-2, 6), 130.5 (C-1), 116.7 (C-3.5)。以上数据与文献报道的一致,故确定为对羟基苯甲醛[6]。

化合物 8　无色针晶; ^{13}C NMR (DMSO-d_6) δ: 71.43 (C-3), 69.78 (C-2, C-4), 63.95 (C-1, C-5)。以上数据与文献报道的 1,5-Anhydroxylitol 一致[7]。

化合物 9　无色针晶; ^{1}H NMR ($CDCl_3$) δ: 1.75 (3H, s, -CH_3), 3.9 (3H, s, -OCH_3), 5.13 (H, s, =CH-), 5.17, 5.47 (2H, s, =CH_2), 5.74 (H, s, -OH); ^{13}C NMR ($CDCl_3$) δ: 17.54 (C-7), 60.14 (C-8), 89.54 (C-2), 103.56 (C-4), 116.74 (C-6), 139.66 (C-5), 172.08 (C-1), 179.67 (C-3)。以上数据与文献报道的 Penicillic acid 一致[8]。

化合物 10　白色粉末; ^{1}H NMR ($CDCl_3$) δ: 0.66 (3H, s, CH_3-18), 0.82 (3H, d, J = 6.0 Hz, CH_3-27), 0.83 (3H, d, J = 6.0 Hz, CH_3-26), 0.84 (3H, J = 7.0 Hz, CH_3-29), 0.87 (3H, d, J = 7.0 Hz, CH_3-26), 3.52 (1H, m, H-3), 5.35 (1H, m, H-6); ^{13}C NMR ($CDCl_3$) δ: 11.9 (C-18), 12.0 (C-29), 18.8 (C-26), 19.0 (C-21), 19.4 (C-19), 19.8 (C-27), 21.1 (C-11), 23.0 (C-28), 24.3 (C-15), 25.9 (C-25), 28.3 (C-16), 29.4 (C-23), 31.6 (C-1), 31.8 (C-8), 34.0 (C-22), 36.2 (C-20), 36.5 (C-10), 37.2 (C-1), 39.7 (C-12), 42.3 (C-12), 45.9 (C-24), 47.9 (C-13), 50.2 (C-9), 56.0 (C-17), 56.7 (C-14), 71.8 (C-3), 121.7 (C-11), 140.7 (C-9)。以上波谱数据与文献[9]报道的 β-谷甾醇一致。

化合物 11　无色针状晶; ^{13}C NMR ($CDCl_3$) δ: 141.3 (C-8), 140.8 (C-5), 136.1 (C-22), 132.2 (C-23), 119.6 (C-6), 117.2 (C-7), 69.9 (C-3), 56.0 (C-17), 54.8 (C-14), 46.7 (C-9), 43.1 (C-24), 43.1 (C-13), 42.0 (C-4), 40.7 (C-20), 39.4 (C-12), 39.0 (C-1), 37.5 (C-10), 33.4 (C-25), 33.0 (C-2), 28.7 (C-16), 23.4 (C-15), 21.5 (C-11), 21.4 (C-21), 20.2 (C-27), 19.8 (C-26), 17.8 (C-28), 16.6 (C-19), 12.2 (C-18)。EI-MS 显示 396[M]$^+$。以上数据与文献报道的 ergost-5, 7, 22-trien-3-ol 一致[10]。

化合物 12　无色油状物; ^{1}H NMR ($CDCl_3$) 0.61 (3H, s, H-12), 0.83 (3H, d, J = 6.8 Hz, H-19), 0.84 (3H, d, J = 6.8 Hz, H-20), 0.92 (3H, d, J = 6.8 Hz, H-21), 1.04 (3H, d, J = 6.6 Hz, H-14), 2.27 (1H, ddd, J = 14.1, 4.0, 2.4 Hz, H-5eq), 2.64 (1H, ddd, J = 11.9, 6.8, 1.7 Hz, H-8), 5.17 (1H, dd, J = 15.3, 8.3 Hz, H-15), 5.26 (1H, dd, J = 15.3, 7.7 Hz, H-16), 5.63 (1H, d, J = 1.8 Hz, H-2); ^{13}C NMR ($CDCl_3$) 11.7 (C-12), 17.6 (C-18), 19.6 (C-20), 20.0 (C-21), 21.0 (C-14), 21.4 (C-9), 28.8 (C-10), 33.0 (C-19), 35.0 (C-5), 35.3 (C-6), 40.1 (C-13), 42.8 (C-17), 48.8 (C-7), 50.3 (C-8), 55.3 (C-11), 104.7 (C-4), 112.3 (C-2), 132.9 (C-16), 134.6 (C-15), 170.5 (C-3), 170.7 (C-1)。ESI-MS m/z: 355 [M + Na]$^+$。以上数据与文献报道的 Demethylincisterol A3 一致[11]。

5　细胞毒活性

化合物 **12** 按照 MTT 方法,用 HL-60、SMMC-7721、A-549、MCF-7、SW480 五株肿瘤细胞株对化合物进行了 40 uM 浓度的初筛,以在此浓度下对于肿瘤细胞生长抑制率到达 50% 左右的化合物进入梯度浓度复筛。与阳性对照药物顺铂(MW300)及紫杉醇相比,化合物 **12** 具有一定的体外肿瘤生长抑制活性,其 IC_{50} 值分别为 14.29、14.02、13.91、16.45、16.05 μM。

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参考文献

1 Jiang CL(姜成林),Xu LH(徐丽华). Development and utilization of microbial resources(微生物资源开发利用)[M]. Beijing:China Light Industry Press,2001.

2 Takaaki Y,Rinko K,Takahiro N,*et al*. Metabolism of paeonol in rats[J]. *J Nat Prod*,1999,62:1142-1144.

3 M. Iqbal C,Meher A,Atia-tul W,*et al*. New antiglycation and enzyme inhibitors from *Parmotrema cooperi*[J]. *Sci Chi Chem*(中国科学:化学),2011,54:1926-1931.

4 Carvalho MR,Barbosa LCdA,de Queiroz JH,*et al*. Novel lactones from *Aspergillus versicolor*[J]. *Tetrahedron Lett*,2001,42:809-811.

5 Guo ZY,Cheng F,Zou K,*et al*. Secondary metabolites from the mangrove endophytic fungus *Penicillium* sp.(SBE-8)[J]. *Nat Prod Commun*,2009,11:1481-1483.

6 Xiao YQ(肖永庆),Li L(李丽),You XL(游小琳),*et al*. Studies on Chemical Constituents of the Rhizomae of Ligusticum chuanxiong[J]. *China J Chin Mater Med*(中国中医药杂志),2002,27(1):35-36.

7 Elisabetta C,Guido F,Pier LC,*et al*. 1,5-Anhydroxylitol from leaves of *Olea europaea*[J]. *Carbohydr Res*,2004,339:2731-2732.

8 Kang SW,Kim SW. New antifungal activity of penicillic acid against Phytophthora species[J]. *Biotechnology Letters*,2004,26:695-698.

9 Qaisar M,Ahmad,Viqar U,Nisar M,*et al*. Biodirected isolation from Forsskalea tenacissima[J]. *J Chem Soc Pak*,2008,30:854-859.

10 Wang F,Tan JW,Liu JK. Vibratilicin:a novel compound from the basidiomycete cortinarius vibratilis[J]. *HeIv Chim Acta*,2004,87:1912-1915.

11 Francesco DR,Aldo S,Irene I,*et al*. Synthesis of(17R)-17-methylincisterol,a highly degraded marine steroid[J]. *Tetrahedron Lett*,1995,36:4303-4306.

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2 Chen YJ(陈雨洁),Lin QX(林亲熊),Wan DR(万定荣),*et al*. Study on the anti-tumor activities of the different extract fractions and total flavones of the three sedum plant drugs[J]. *J Minzu Univ Chin:Nat Sci*(中央民族大学学报:自科版),2011,20(2):88-92.

3 Qiao JF(乔剑峰),Hu W(胡伟),Li ZG(李祖光),*et al*. Research of active component in Sedum emarginatum Migo[J]. *J Zhejiang Univ Sci & Tech*(浙江科技学院学报),2003,15:26-28.

4 Cheng LM(程琳茗),Li BL(李炳伦),Yan AJ(严爱娟),*et al*. Content determination of total flavonoids in alcohol extract from different parts of sedum emarginatum migo and comparison of their *in vitro* antioxidant activities[J]. *J Mod Med Health*(现代医药卫生),2013,14:2096-2097.

5 Gao L(高莉),Tian H(田华),Lv PJ(吕培军),*et al*. Chemical constituents of sapium sebiferum leaves[J]. *Chin J Chin Mater Med*(中国中药杂志),2015,40:1518-1522.

6 Morikawa T,Zhang Y,Nakamura S,*et al*. Bioactive constituents from chinese natural medicines. XXII. 1) absolute structures of new megastigmane glycosides,sedumosides E1,E2,E3,F1,F2,and G,from sedum sarmentosum(crassulaceae)[J]. *Chem Pharm Bull*,2007,55:435-441.

7 Otsuka H,Zhong XN,Hirata E,*et al*. Myrsinionosides A-E:megastigmane glycosides from the leaves of *Myrsine seguinii* Lev[J]. *Chem Pharm Bull*,2001,49:1093-1097.

8 Ye G(叶冠),Fan MS(范明松),Huang CG(黄成钢). Isolation and identification of flavonoids from *Achnatherum splendens*[J]. *Chin Tradit Herb Drugs*(中草药),2006,37(6):821-823.

9 Li YM(李永梅),Wang TZ(王天志),Wang ZX(王志霄). Studies on chemical constituents in dried buds of *Lonicera similis* Hemsl[J]. *Chin J Chin Mater Med*(中国中药杂志),2001,26(1):45-47.

10 Ma YM(马养民),Jiang SJ(姜少娟),Shi QH(史清华). Extraction and isolation of isorhamnetin and quercetin from marc of sea buckthorn fruit[J]. *J Northwest Forestry Univ*(西北林学院学报),2009,24:121-124.

11 Yang Y(杨茵),Li SG(李硕果),Ye WC(叶文才),*et al*. Studies on chemical constituents of microcos paniculata[J]. *Lishizhen Med Mater Med Res*(时珍国医国药),2010,21:2790-2792.

12 Wu HJ(吴海建),Gong X(龚秀),Yang YT(杨倚天),*et al*. Improvement of carbon tetrachloride drug-induced liver injury model in vitro[J]. *Chin J Chin Mater Med*(中国中药杂志),2012,37:3633-3636.