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海南大风子活性成分研究

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摘要:用色谱技术对海南大风子(*Hydnocarpus hainanensis* (Merr.) Sleum.)枝条的乙醇提取物进行分离纯化,从中分离得到了6个化合物,经波谱数据分析与文献数据对照,分别鉴定为coniferaldehyde (**1**)、mulberroside C (**2**)、mulberrofuran G (**3**)、mulberrofuran K (**4**)、morusin (**5**)和胡萝卜苷(**6**),以上化合物均为首次从海南大风子中分离得到。细胞毒测试结果表明,化合物**1**对人肝癌细胞(SMMC-7721)和人胃癌细胞(SGC-7901)有弱活性,化合物**3**和**5**对SGC-7901有明显的抑制作用。

关键词:海南大风子;枝条;化学成分;细胞毒活性
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Bioactive Constituents from *Hydnocarpus hainanensis* (Merr.) Sleum.

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Abstract: The ethanol extract from the stems of *Hydnocarpus hainanensis* (Merr.) Sleum. was separated and purified by means of chromatographic technology, and six compounds were isolated. On the basis of spectral data, they were identified as coniferaldehyde (**1**), mulberroside C (**2**), mulberrofuran G (**3**), mulberrofuran K (**4**), morusin (**5**) and daucosterol (**6**). All the compounds were isolated from *H. hainanensis* for the first time. Compound **1** showed inhibitory activities towards human hepatoma cell line (SMMC-7721) and human gastric carcinoma (SGC-7901) cell line, and compounds **3** and **5** showed obvious inhibitory activities towards SGC-7901.

Key words: *Hydnocarpus hainanensis*; Stem; Chemical constituent; Cytotoxicity

海南大风子(*Hydnocarpus hainainanensis* (Merr.) Sleum.)为大风子科(Flacourtiaceae)大风子属植物[1],别名龙角、高根和麻风子。大风子科植物全世界约有86属850种,主要分布于热带和亚热带地区,极少数延至温带地区,在我国分布有6族12属45种6变种。大风子科植物的生物活性主要有抗癌和抗病毒活性,水提物和醇提物也表现出较好的降血糖、降血脂及中和神经毒的作用[2]。海南大风子在我国主要分布于海南及广西部分地区,具有祛风湿、攻毒杀虫之功效,临床多用于麻风、疹癣、杨梅疮等症[3]。为揭示海南大风子的活性物质基础,我们在活性筛选的指导下对海南大风子的乙醇提取物作了较为系统的植物化学成分分析,现予以报道。

1 材料和方法

1.1 材料

海南大风子(*H. hainanensis*)枝条于2008年5月采自海南省文昌市,经中国热带农业科学院热带作

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物品种资源研究所王祝年副研究员鉴定，凭证标本(AN200805)存放于中国热带农业科学院热带生物技术研究所。柱层析硅胶(200～300目)和薄层层析硅胶均为青岛海洋化工厂产品，Sephadex LH-20为Merck公司产品。

1.2 仪器

熔点采用北京泰克X-5型显微熔点仪测定(温度未校正)；MS谱用Autospec-3000质谱仪测定；NMR用Brucker AV-400型超导核磁仪测定，以TMS为内标。

1.3 提取和分离

海南大风子枝条(21.0 kg)晒干后加工成粉末，用95%乙醇热提3次。滤液经真空减压浓缩至无醇味后加水分散成混悬液，依次用石油醚、乙酸乙酯、正丁醇萃取，萃取液分别减压浓缩至干，得到石油醚萃取物(79.7 g)，乙酸乙酯萃取物(43.0 g)和正丁醇萃取物(92.0 g)。乙酸乙酯萃取物(43.0 g)经减压硅胶柱层析，以氯仿-甲醇(100∶1～0∶1)梯度洗脱，得到9个组分(Fr. 1～9)。Fr. 4部分(9.0 g)依次经Sephadex LH-20凝胶层析(95%乙醇)和硅胶柱层析，以氯仿-甲醇(25∶1～5∶1)梯度洗脱得到化合物**1**(4.6 mg, 0.00002%)、化合物**2**(7.6 mg, 0.00004%)、化合物**3**(8.0 mg, 0.00004%)和化合物**5**(13.0 mg, 0.00006%)。Fr. 8部分(12.0 g)依次经Sephadex LH-20凝胶(95%乙醇)和硅胶柱层析以氯仿-甲醇(5∶1～1∶1)梯度洗脱得到化合物**4**(2.8 mg, 0.00001%)和化合物**6**(112.2 mg, 0.00005%)。

1.4 细胞毒活性筛选方法

采用MTT法[4]。实验设阴性对照组(DMSO溶剂)、阳性对照组(丝裂霉素C)和6个不同浓度(0.2, 0.6, 1.8, 5.4, 16.2和48.6 μg mL^{-1})的待测样品，每个浓度设3个平行。

选取对数生长期细胞，用RPMI 1640完全培养基制成单细胞悬浮液，血球计数板计数，按50000个 mL^{-1}接种90 μL于96孔平底细胞培养板，置于5% CO_2、湿度90%以上、37℃温箱中培养。将SMMC-7721和SGC-7901培养24 h后加入待测样品10 μL，继续培养72 h后取出，置于显微镜下观察每孔细胞形态。然后每孔加入5 mg mL^{-1}的MTT溶液(溶于平衡盐溶液PBS) 15 μL，37℃反应4 h后，吸弃上清液，再向各孔加入100 μL DMSO，充分溶解，将细胞培养板置于酶标仪上，用490 nm波长测量各孔的吸光度(A)，求生长抑制率：生长抑制率(%) = (1 －用药组平均A值/阴性对照组平均A值)×100%

以样品浓度为横坐标，以抑制率为纵坐标，根据浓度梯度利用Origin软件拟合出抑制率的曲线图，求出抑制率为50%时样品的浓度(IC_{50})，样品活性结果即以IC_{50}表示。

1.5 结构鉴定

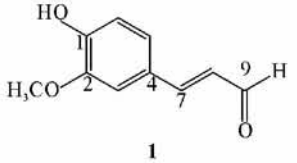
Coniferaldehyde（**1**） 黄色油状物，EI-MS *m/z*: 178 [M]$^+$; ^{1}H NMR (Acetone-d_6, 400 MHz): δ 9.63 (1H, d, *J* = 8.0 Hz, H-9), 7.56 (1H, d, *J* = 16.0 Hz, H-7), 7.38 (1H, d, *J* = 2.0 Hz, H-3), 7.21 (1H, dd, *J* = 8.0, 2.0 Hz, H-5), 6.90 (1H, d, *J* = 8.0 Hz, H-6), 6.65 (1H, dd, *J* = 16.0, 8.0 Hz, H-8), 3.92 (3H, s, OCH_3); ^{13}C NMR (Acetone-d_6, 100 MHz): δ 194.8 (C-9), 154.9 (C-7), 151.9 (C-1), 149.8 (C-2), 129.3 (C-4), 128.0 (C-8), 125.7 (C-5), 117.2 (C-3), 112.7 (C-6)。上述波谱数据与文献[5]报道的Coniferaldehyde基本一致。

Mulberroside C（**2**） 白色晶体(甲醇)，mp 206℃～208℃; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.23 (1H, s, H-4), 6.98 (1H, t-like, H-6′), 6.93 (1H, s, H-7), 6.92 (1H, t-like, H-2′), 6.85 (1H, s, H-3), 6.48 (1H, t-like, H-4′), 3.78 (1H, dd, *J* = 7.2, 5.2 Hz, H-2″), 3.12 (1H, dd, *J* = 16.4, 5.6 Hz, H-1″a), 2.83 (1H, dd, *J* = 16.4, 7.2 Hz, H-1″b), 1.36 (3H, s, H-5″), 1.26 (3H, s, H-4″); xyl: 5.00 (1H, d, *J* = 6.8 Hz, H-1‴), 3.95 (1H, m, H-5‴a), 3.58－3.42 (4H, m, H-2‴, 3‴, 4‴, 5‴b); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.4 (C-5′), 160.1 (C-3′), 156.2 (C-2), 156.0 (C-7a), 152.7 (C-6), 133.7 (C-1′), 124.1 (C-3a), 121.9 (C-4), 117.9 (C-5), 106.7 (C-4′), 105.4 (C-6′), 105.2 (C-2′), 103.0 (C-3), 99.7 (C-7), 78.3 (C-3″), 70.4 (C-2″), 32.4 (C-1″), 26.0 (C-4″), 21.1 (C-5″); xyl: 102.3 (C-1‴), 77.7 (C-3‴), 74.7 (C-2‴), 71.0 (C-4‴), 66.9 (C-5‴)。上述波谱数据与文献[6]报道的Mulberroside C数据基本一致。

Mulberrofuran G（**3**） 黄色针晶(甲醇)，mp 179℃～181℃, FAB-MS *m/z*: 562 [M + H]$^+$; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.29 (1H, d *J* = 8.4 Hz, H-4), 7.08 (1H, d, *J* = 8.0 Hz, H-14″), 7.04 (1H, d, *J* = 8.8 Hz, H-20″), 6.87 (1H, s, H-3), 6.85

(2H, d, J=1.6 Hz, H-7, 2′), 6.76 (1H, d, J=1.6 Hz, H-6′), 6.68 (1H, dd, J=8.4, 2.0 Hz, H-5), 6.40 (1H, dd, J=8.8, 2.0 Hz, H-13″), 6.35 (1H, br s, H-2″), 6.29 (1H, d, J=2.4 Hz, H-11″), 6.27 (1H, d, J=2.4 Hz, H-17″), 6.10 (1H, dd, J=8.8, 2.0 Hz, H-19″), 3.26 (1H, m, H-3″), 2.90 (1H, m, H-4″), 2.72 (1H, dd, J=17.2, 5.2 Hz, H-6″b), 1.98 (1H, m, H-6″a), 1.73 (3H, s, H-7″); ^{13}C NMR (CD_3OD, 100 MHz): δ 160.0 (C-10″), 158.4 (C-18″), 157.9 (C-12″), 157.7 (C-6), 157.2 (C-5′), 156.7 (C-2), 155.7 (C-3′), 154.9 (C-16″), 153.6 (C-7a), 133.9 (C-1″), 131.5 (C-1′), 130.5 (C-14″), 128.0 (C-20″), 123.3 (C-2″), 123.1 (C-3a), 122.0 (C-4), 118.3 (C-4′), 117.4 (C-15″), 113.9 (C-9″), 113.2 (C-5), 110.0 (C-19″), 107.0 (C-13″), 105.5 (C-6′), 105.0 (C-2′), 104.6 (C-17″), 104.2 (C-11″), 103.1 (C-8″), 102.1 (C-3), 98.5 (C-7), 37.6 (C-3″), 36.7 (C-5″), 35.4 (C-4″), 28.8 (C-6″), 23.9 (C-7″)。上述波谱数据与文献[7]报道的 Mulberrofuran G 基本一致。

Mulberrofuran K（4）　黄色针晶(甲醇), mp 174℃～175℃, FAB-MS m/z: 628 $[M+H]^+$; ^{1}H NMR (CD_3OD, 400 MHz): δ 7.41 (1H, d, J=2.4 Hz, H-7), 7.34 (1H, d, J=8.4 Hz, H-4), 7.11 (1H, d, J=8.4 Hz, H-20″), 6.97 (1H, d, J=8.6 Hz, H-14″), 6.93 (1H, s, H-3), 6.90 (1H, d, J=1.5 Hz, H-2′), 6.82 (1H, d, J=1.6 Hz, H-6′), 6.73 (1H, dd, J=8.4, 2.2 Hz, H-5), 6.64 (1H, d, J=10.0 Hz, H-21″), 6.46 (1H, dd, J=8.4, 2.5 Hz, H-19″), 6.39 (1H, br d, J=4.2 Hz, H-2″), 6.32 (1H, d, J=2.4 Hz, H-17″), 6.16 (1H, d, J=8.6 Hz, H-13″), 5.61 (1H, d, J=10.0 Hz, H-22″), 3.38 (1H, m, H-4″), 3.35 (1H, m, H-3″), 2.94 (1H, m, H-5″), 2.67 (1H, dd, J=17.2, 6.4 Hz, H-6b″), 2.02 (1H, m, H-6a″), 1.79 (3H, s, H-7″), 1.32 (1H, s, H-24″), 1.30 (1H, s, H-25″); ^{13}C NMR (CD_3OD, 100 MHz): δ 158.5 (C-10″), 157.8 (C-18″), 157.3 (C-12″), 156.8 (C-6), 155.7 (C-5′), 155.1 (C-2), 155.0 (C-3′), 153.5 (C-16″), 153.0 (C-7a), 133.9 (C-1″), 131.7 (C-1′), 129.5 (C-14″), 129.2 (C-22″), 128.0 (C-20″), 123.2 (C-2″), 123.1 (C-3a), 122.0 (C-4), 119.1 (C-15″), 118.3 (C-4′), 118.3 (C-21″), 113.7 (C-9″), 113.3 (C-5), 111.6 (C-11″), 111.0 (C-19″), 107.7 (C-13″), 105.5 (C-6′), 104.8 (C-2′), 104.1 (C-17″), 102.6 (C-8″), 102.2 (C-3), 98.5 (C-7), 77.0 (C-23″), 38.2 (C-3″), 37.0 (C-5″), 35.1 (C-4″), 28.7 (C-6″), 27.7 (C-24″), 27.7 (C-25″), 23.9 (C-7″)。上述波谱数据与文献[8]报道的 Mulberrofuran K 基本一致。



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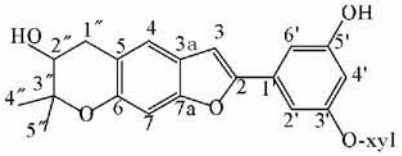

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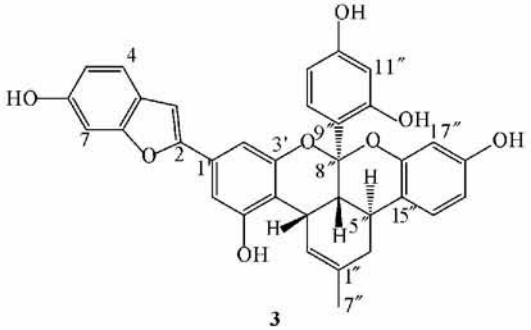

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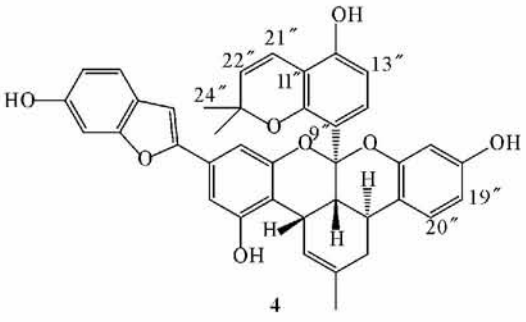

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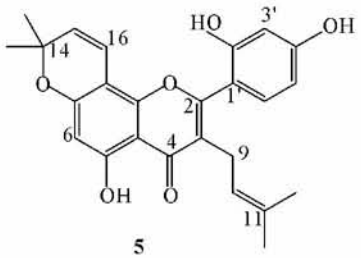

5

Morusin (5) 黄色晶体(氯仿),EI-MS *m/z*: 420 [M]$^+$;^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.15 (1H, d, *J*=0.7 Hz, H-6), 3.13 (1H, br d, *J*=6.8 Hz, H-9), 1.57 (3H, d, *J*=1.2 Hz, H-12), 1.45 (3H, s, H-13), 6.59 (1H, dd, *J*=10.0, 0.7 Hz, H-14), 5.64 (1H, d, *J*=10.0 Hz, H-15), 5.16 (1H, dt, *J*=6.8, 1.4 Hz, H-10), 1.44 (6H, s, H-17, 18), 6.57 (1H, d, *J*=2.2 Hz, H-3′), 6.51 (1H, dd, *J*=8.4, 2.3 Hz, H-5′), 7.24 (1H, d, *J*=8.4 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.5 (C-2), 122.8 (C-3), 184.3 (C-4), 106.6 (C-4a), 163.8 (C-5), 100.7 (C-6), 161.0 (C-7), 102.6 (C-8), 154.3 (C-8a), 24.1 (C-9), 123.5 (C-10), 133.3 (C-11), 25.5 (C-12), 15.3 (C-13), 79.8 (C-14), 129.0 (C-15), 113.7 (C-16), 18.6 (C-17), 26.8 (C-18), 133.3 (C-1′), 158.4 (C-2′), 105.0 (C-3′), 162.6 (C-4′), 109.2 (C-5′), 116.4 (C-6′)。上述波谱数据与文献[9]报道的 Morusin 数据基本一致。

胡萝卜苷(Daucosteral, 6) 白色粉末,mp 295℃~297℃。薄层色谱的 Rf 值与胡萝卜苷对照品相同,两者混合物的熔点不降,因此鉴定为胡萝卜苷。

2 结果和讨论

从海南大风子枝条乙酸乙酯萃取物经正相硅胶、Sephadex LH-20 进行分离、纯化,得到 6 个化合物,经波谱数据分析及文献对照,分别鉴定为: coniferaldehyde (**1**)、mulberroside C (**2**)、mulberrofuran G (**3**)、mulberrofuran K (**4**)、morusin (**5**)和胡萝卜苷 (**6**),以上化合物均为首次从该植物中分离得到。本文采用 MTT 法测定了化合物 **1**~**5** 的细胞毒活性,结果表明,化合物 **1** 对 SMMC-7721 和 SGC-7901 有弱活性,其 IC_{50}分别为 62.5 μg mL^{-1}和 25.0 μg mL^{-1},化合物 **3** 和 **5** 对 SGC-7901 有明显的抑制作用,其 IC_{50}分别为 1.6 μg mL^{-1}和 2.0 μg mL^{-1},而阳性对照丝裂霉素 C 对 SMMC-7721 和 SGC-7901 的 IC_{50}分别为 2.20 μg mL^{-1}和 8.80 μg mL^{-1}。

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