

大叶山棟根化学成分与细胞毒活性的研究

黄森^{1,2}, 梅文莉², 蔡彩虹², 王辉², 董文化², 曲有乐^{1*}, 戴好富^{2*}

(1. 佳木斯大学药学院, 黑龙江 佳木斯 154007; 2. 中国热带农业科学院热带生物技术研究所, 农业部热带作物生物学与遗传资源利用重点实验室, 海口 571101)

摘要: 为了解大叶山棟(*Aphanamixis grandifolia* Bl.)根中的抗肿瘤活性成分, 利用各种色谱技术从其95%乙醇提取物中分离得到9个化合物, 经波谱分析分别鉴定为: 7-hydroxycadalene (**1**)、dregeana-1 (**2**)、4-oxopinoresinol (**3**)、4-ketopinoresinol (**4**)、6-deoxyjacareubin (**5**)、schleicheol 1 (**6**)、豆甾醇 (**7**)、 β -谷甾醇 (**8**)和胡萝卜昔 (**9**)。其中化合物**1~6**为首次从山棟属植物中分离得到, 并首次报道了化合物**1**的碳谱数据。生物活性测试结果表明, 化合物**1**和**5**对慢性髓原白血病细胞K562有生长抑制活性, 化合物**1**、**5**和**6**对人胃癌细胞SGC-7901有生长抑制活性。

关键词: 棣科; 大叶山棟; 化学成分; 抗肿瘤活性

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Studies on Chemical Compositions and Cytotoxicities from Roots of *Aphanamixis grandifolia* Bl.

HUANG Miao^{1,2}, MEI Wen-li², CAI Cai-hong², WANG Hui², DONG Wen-hua², QU You-le^{1*}, DAI Hao-fu^{2*}

(1. School of Pharmacological Sciences, Jiamusi University, Jiamusi 154007, China; 2. Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China)

Abstract: In order to understand the antitumor active ingredients of *Aphanamixis grandifolia* Bl., nine compounds were isolated from 95% ethanol extract of its roots. They were identified as 7-hydroxycadalene (**1**), dregeana-1 (**2**), 4-oxopinoresinol (**3**), 4-ketopinoresinol (**4**), 6-deoxyjacareubin (**5**), schleicheol 1 (**6**), stigmasterol (**7**), β -sitosterol (**8**), and daucosterol (**9**) on the basis of spectral data. Compounds **1~6** were isolated from the genus *Aphanamixis* for the first time, and the ^{13}C NMR spectral data of compound **1** was reported for the first time. Compounds **1** and **5** showed inhibition of the proliferation of K562 cell line, and compounds **1**, **5** and **6** possessed inhibitory activity against SGC-7901 cell line.

Key words: Meliaceae; *Aphanamixis grandifolia* Bl.; Chemical constituent; Antitumor activity

大叶山棟(*Aphanamixis grandifolia* Bl.)为棣科(Meliaceae)山棟属植物。山棟属植物约有25种, 我国产4种, 分布于广东、广西、海南、云南及台湾等地^[1]。《新华本草纲要》记载大叶山棟的根及叶具有祛风除湿、舒筋活络及通痹等功效, 为祛风止痛药。

前人对大叶山棟的枝叶、茎皮、果实和种子做了大量研究, 化学成分主要有三萜、二萜等结构类型, 具有抗肿瘤、抗疟及抗菌等活性^[2~11], 目前对大叶山棟根的化学成分研究较少, 为了寻找其中的生物活性成分, 本文对大叶山棟根的95%乙醇提取物的乙

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作者简介: 黄森(1988~), 女, 硕士研究生, 研究方向为天然药物化学。E-mail: huangmiaoitbb@163.com

*通信作者 Corresponding author. E-mail: daihaofu@itbb.org.cn; Youle1960@163.com

酸乙酯萃取部分进行了分离纯化,根据波谱数据从中鉴定了9个化合物。

1 材料和方法

1.1 材料

大叶山棟(*Aphanamixis grandifolia* Bl.)样品于2013年9月采自海南省海口市石山镇,经中国热带农业科学院热带生物技术研究所刘寿柏博士鉴定,凭证标本(No. AG 20130920)存放于中国热带农业科学院热带生物技术研究所。

人胃癌细胞SGC-7901和慢性髓原白血病细胞K562均购于中国科学院上海细胞库。

1.2 仪器和试剂

NMR采用Bruker AV-500型超导核磁仪(TMS内标,瑞士Bruker公司);MS采用Autospec-3000质谱仪;薄层色谱硅胶板,柱色谱硅胶(200~300目,60~80目)均为青岛海洋化工厂产品;Sephadex LH-20填料柱为Merck公司产品;反相材料RP-18为Merck公司产品;GALAXY型CO₂培养箱为英国RSBiotech公司产品;UNIVERSL32R型台式高速冷冻离心机为德国HETTICH公司产品;ELX-800型酶标仪为美国宝特公司产品;小牛血清为HyClone公司产品;四甲基偶氮唑盐(MTT)为Sigma公司产品;平衡盐溶液PBS为北京欣经科公司产品;紫杉醇为江苏红豆杉药业有限公司产品。

1.3 提取和分离

大叶山棟根(8.5 kg)自然晾干后加工成粉末,用95%乙醇回流提取3次,每次3 h。提取液减压浓缩,得到浸膏305.6 g,将其分散于水中成悬浊液,依次用石油醚、乙酸乙酯、正丁醇各萃取3次,得石油醚萃取物、乙酸乙酯萃取物、正丁醇萃取物、水溶液4部分,减压回收各部分得到石油醚萃取物(12.6 g)、乙酸乙酯萃取物(104.0 g)、正丁醇萃取物(10.4 g)。

乙酸乙酯萃取物(104.0 g)经减压硅胶柱色谱,以氯仿-甲醇(1:0~0:1)梯度洗脱得到10个部分(Fr.1~Fr.10)。Fr.2(2.8 g)经Sephadex LH-20凝胶柱色谱(以氯仿-甲醇1:1为洗脱系统)和反复硅胶柱色谱(以石油醚-乙酸乙酯,石油醚-丙酮为洗脱系统)分离得到化合物**1**(5.3 mg)、**5**(5.3 mg)和**8**(30.0 mg)。Fr.4(5.1 g)经MCI柱色谱、反相RP-18

柱色谱、Sephadex LH-20凝胶柱色谱(以氯仿-甲醇1:1,甲醇为洗脱系统)和反复硅胶柱色谱(以石油醚-乙酸乙酯,石油醚-丙酮,石油醚-氯仿为洗脱系统)分离得到化合物**3**(20.0 mg)、**4**(8.0 mg)、**6**(7.4 mg)、**7**(9.1 mg)和**9**(3.8 mg)。Fr.4(5.1 g)经反相RP-18柱色谱、Sephadex LH-20凝胶柱色谱(以甲醇为洗脱系统)和反复硅胶柱色谱(以石油醚-乙酸乙酯,氯仿-甲醇为洗脱系统)分离得到化合物**2**(7.2 mg)。

1.4 体外肿瘤细胞生长抑制实验(MTT法)

以K562和SGC-7901细胞为指示瘤株,采用MTT法^[12~13]测定化合物细胞毒体外活性。收集对数生长期细胞,并将其制成单细胞悬浮液,于96孔板上按50000个mL⁻¹接种每孔90 μL,SGC-7901培养24 h后加入用DMSO配制一定浓度的样品溶液10 μL[浓度为1 mg(100 μL)⁻¹],K562直接加样品10 μL;接着连续培养72 h后取出在显微镜下观察每孔的细胞形态。粗筛和复筛时加入的细胞悬液用量不同,粗筛为每孔加入2.0 μL,复筛为每孔加入10 μL。然后再加入15 μL用0.01 mol L⁻¹ PBS配制的浓度为5 mg mL⁻¹的MTT溶液(pH=7.4),37℃培养4 h后,弃去上清液,每孔再加入100 μL DMSO,轻轻吹打使化合物充分溶解。在波长为490 nm下,用酶标仪测量各孔的吸光度(A),按公式计算抑制率和IC₅₀值。

$$\text{生长抑制率}(\%) = \left(1 - \frac{\text{用药组平均 } A \text{ 值}}{\text{阴性对照组平均 } A \text{ 值}}\right) \times 100\%$$

以样品浓度为横坐标,抑制率为纵坐标,利用origin软件拟合出抑制率的曲线图。样品活性结果以IC₅₀(半数抑制浓度)表示,当抑制率为50%时的样品浓度也就是细胞毒活性的IC₅₀值。

1.5 结构鉴定

7-Hydroxycadalene (1) 白色粉末, ESI-MS *m/z*: 213 [M - H]⁻, ¹H NMR (CDCl₃, 500 MHz): δ 1.37 (6H, d, *J* = 6.8 Hz, CH₃-12, 13), 2.47 (3H, s, CH₃-15), 2.56 (3H, s, CH₃-14), 3.67 (1H, m, H-11), 5.00 (1H, s, OH-7), 7.15 (1H, d, *J* = 7.3 Hz, H-3), 7.20 (1H, d, *J* = 7.3 Hz, H-2), 7.26 (1H, s, H-8), 7.90 (1H, s, H-5); ¹³C NMR (CDCl₃, 125 MHz): δ 17.0 (C-15), 19.7 (C-14), 23.8 (C-12, 13), 28.6 (C-11), 107.0 (C-8), 119.3 (C-3), 125.2 (C-6), 125.7 (C-2), 126.3 (C-5),

127.0 (C-10), 130.2 (C-9), 133.2 (C-1), 142.3 (C-4), 152.2 (C-7)。上述¹H NMR 数据与文献[14]报道基本一致, 鉴定为 7-hydroxycadalene。

Dregeana-1 (2) 白色油状, ESI-MS *m/z*: 651 [M + Na]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 0.82 (3H, t, *J* = 6.6 Hz, CH₃-5'), 0.89 (3H, s, CH₃-18), 0.91 (3H, d, *J* = 6.6 Hz, 3'-Me), 0.99 (1H, m, H-4'a), 1.16 (1H, m, H-4'b), 1.07 (3H, s, CH₃-19), 1.54 (1H, m, H-3'), 2.05 (3H, s, CH₃-28), 2.27 (1H, d, *J* = 12.4 Hz, H-6a), 2.60 (1H, m, H-6b), 2.40 (1H, m, H-16a), 2.80 (1H, d, *J* = 5.3 Hz, H-9), 2.90 (1H, m, H-16b), 2.95 (1H, m, H-5), 3.03 (1H, dd, *J* = 9.0, 14.3 Hz, H-2a), 3.24 (1H, dd, *J* = 9.0, 14.3 Hz, H-2b), 3.35 (1H, d, *J* = 3.0 Hz, H-2'), 3.85 (1H, t, *J* = 9.0 Hz, H-1), 3.90 (1H, t, *J* = 9.6 Hz, H-17), 4.15 (1H, d, *J* = 11.8 Hz, H-29a), 4.23 (1H, d, *J* = 11.8 Hz, H-29b), 5.40 (1H, dd, *J* = 5.3, 10.8 Hz, H-11), 5.54 (2H, s, CH₂-30), 6.15 (1H, d, *J* = 10.8 Hz, H-12), 6.24 (1H, s, H-22), 7.25 (1H, s, H-21), 7.42 (1H, s, H-23), 8.14 (1H, s, COOH); ¹³C NMR (CDCl₃, 125 MHz): δ 11.6 (C-5'), 12.5 (C-18), 15.3 (3'-Me), 22.4 (C-19), 23.2 (C-4'), 29.3 (C-28), 32.9 (C-6), 37.0 (C-17), 38.2 (C-3'), 38.5 (C-2), 40.9 (C-11), 41.2 (C-16), 49.3 (C-13), 50.1 (C-10), 55.4 (C-9), 72.2 (C-11), 73.7 (C-2), 74.4 (C-29), 74.5 (C-12), 75.2 (C-2'), 78.7 (C-4), 87.4 (C-14), 110.4 (C-22), 119.5 (C-30), 121.9 (C-20), 134.3 (C-8), 140.8 (C-21), 143.5 (C-23), 160.5 (COOH), 167.7 (C-3), 172.5 (C-7), 175.0 (C-1'), 205.2 (C-15)。上述波谱数据与文献[15]报道基本一致, 鉴定为 dregeana-1。

4-Oxopinoresinol (3) 白色粉末, ESI-MS *m/z*: 395 [M + Na]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 3.27 (1H, m, H-1), 3.49 (1H, dd, *J* = 3.8, 9.2 Hz, H-5), 3.93 (6H, s, OCH₃-3', 3''), 4.07 (1H, dd, *J* = 4.6, 9.4 Hz, H-8a), 4.36 (1H, dd, *J* = 6.8, 9.4 Hz, H-8b), 5.36 (1H, d, *J* = 3.8 Hz, H-6), 5.38 (1H, d, *J* = 3.8 Hz, H-2), 6.87~6.95 (6H, m, Ar-H); ¹³C NMR (CDCl₃, 125 MHz): δ 50.1 (C-1), 53.4 (C-5), 56.2 (OCH₃-3', 3''), 72.8 (C-8), 83.5 (C-2), 84.8 (C-6), 107.9 (C-2'), 108.2 (C-2''), 114.6 (C-5'), 114.8 (C-5''), 118.1 (C-6'), 118.5 (C-6''), 131.2 (C-1'), 132.4 (C-1''), 145.4 (C-4'), 146.2 (C-4''), 146.9 (C-3'), 147.1 (C-3''), 177.1 (C-4)。上述波谱数据与文献[16]报道基本一致, 鉴定为 4-oxopinoresinol。

4-Ketopinoresinol (4) 白色粉末, ESI-MS *m/z*: 395 [M + Na]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 3.54 (1H, m, H-1, H-5), 3.54 (1H, m, H-8a), 3.87 (1H, m, H-8b), 3.92 (3H, s, OCH₃-3'), 3.94 (3H, s, OCH₃-3''), 5.36 (1H, d, *J* = 3.9 Hz, H-6), 5.76 (1H, d, *J* = 4.8 Hz, H-2), 6.89~6.96 (6H, m, Ar-H); ¹³C NMR (CDCl₃, 125 MHz): δ 45.4 (C-1), 54.8 (C-5), 56.0 (OCH₃-3'), 56.1 (OCH₃-3''), 68.7 (C-8), 80.4 (C-2), 83.7 (C-6), 107.5 (C-2'), 108.2 (C-2''), 114.7 (C-5'), 114.7 (C-5''), 117.8 (C-6'), 118.1 (C-6''), 127.9 (C-1'), 132.4 (C-1''), 145.5 (C-3'), 146.7(C-3''), 146.8 (C-4'), 146.8 (C-4''), 177.2 (C-4)。上述波谱数据与文献[17]报道基本一致, 鉴定为 4-ketopinoresinol。

6-Deoxyjacareubin (5) 黄色针状结晶, ESI-MS *m/z*: 309 [M - H]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 1.49 (6H, s, CH₃-4', 5'), 5.62 (1H, d, *J* = 10.1 Hz, H-1'), 6.37 (1H, s, H-4), 6.73 (1H, d, *J* = 10.1 Hz, H-2'), 7.24 (1H, t, *J* = 7.9 Hz, H-7), 7.31 (1H, d, *J* = 7.9 Hz, H-6), 7.76 (1H, d, *J* = 7.9 Hz, H-8); ¹³C NMR (CDCl₃, 125 MHz): δ 28.6 (C-4', 5'), 78.6 (C-3'), 95.1 (C-4), 103.6 (C-9a), 105.2 (C-2), 115.4 (C-2'), 117.0 (C-8), 120.3 (C-6), 121.2 (C-8a), 124.3 (C-7), 127.9 (C-1'), 144.3 (C-5), 144.4 (C-10a), 156.4 (C-4a), 158.1 (C-3), 161.0 (C-1), 180.9 (C-9)。上述波谱数据与文献[18]报道基本一致, 鉴定为 6-deoxyjacareubin。

Schleicheol 1 (6) 浅黄色油状, ESI-MS *m/z*: 445 [M + H]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 0.70 (3H, s, CH₃-18), 0.94 (3H, d, *J* = 6.3 Hz, CH₃-21), 1.05 (3H, s, CH₃-19), 3.29 (3H, s, OCH₃-7) , 3.38 (1H, m, H-7) , 3.59 (1H, m, H-3) , 5.45 (1H, s, H-6); ¹³C NMR (CDCl₃, 125 MHz): δ 12.0 (C-18), 12.1 (C-29), 18.9 (C-21), 19.0 (C-19), 19.2 (C-26), 20.0 (C-27), 21.4 (C-11), 23.2 (C-28), 25.8 (C-15), 26.1 (C-23), 28.6 (C-16), 29.3 (C-25), 31.7 (C-2), 34.1 (C-22), 36.2 (C-20), 36.6 (C-10), 37.1 (C-1) , 37.3 (C-8), 39.8 (C-12), 42.1 (C-4), 43.0 (C-13), 46.0 (C-24), 48.6 (C-9), 55.0 (OCH₃-7), 55.6 (C-17), 56.6 (C-14), 71.6 (C-3), 82.0 (C-7), 121.6 (C-6), 144.1 (C-5)。上述波谱数据与文献[19]报道基本一致, 鉴定为 schleicheol 1。

豆甾醇 (7) 浅黄色油状, ESI-MS *m/z*: 413 [M + H]⁺; ¹H NMR (CDCl₃, 500 MHz): δ 3.54 (1H, m, H-3), 5.03 (1H, dd, *J* = 15.1, 8.7 Hz, H-23), 5.17 (1H,

dd, $J = 15.1, 8.7$ Hz, H-22), 5.37 (1H, d, $J = 5.0$ Hz, H-6); ^{13}C NMR (CDCl_3 , 125 MHz): δ 12.0 (C-18), 12.4 (C-29), 19.2 (C-19), 19.5 (C-21), 20.0 (C-26), 21.2 (C-11), 23.2 (C-27), 24.4 (C-15), 25.6 (C-28), 29.0 (C-16), 29.1 (C-2), 31.8 (C-7), 32.0 (C-8), 33.8 (C-25), 36.6 (C-10), 37.4 (C-1), 39.8 (C-12), 40.6 (C-20), 42.3 (C-4), 42.4 (C-13), 50.2 (C-9), 51.4 (C-24), 56.1 (C-17), 56.9 (C-14), 71.9 (C-3), 121.9 (C-6), 129.4 (C-23), 138.5 (C-22), 140.9 (C-5)。上述波谱

数据与文献[20]报道基本一致, 鉴定为豆甾醇。

β -谷甾醇(8) 白色粉末; Libenann-Burchard 反应呈阳性。与 β -谷甾醇对照品共薄层层析, 在3种溶剂展开系统下 R_f 值相同, 故确定化合物8为 β -谷甾醇^[21-22]。

胡萝卜苷(9) 白色粉末; Libenann-Burchard 反应呈阳性。与胡萝卜苷对照品共薄层层析, 在3种溶剂展开系统下 R_f 值相同, 故确定化合物9为胡萝卜苷^[21]。

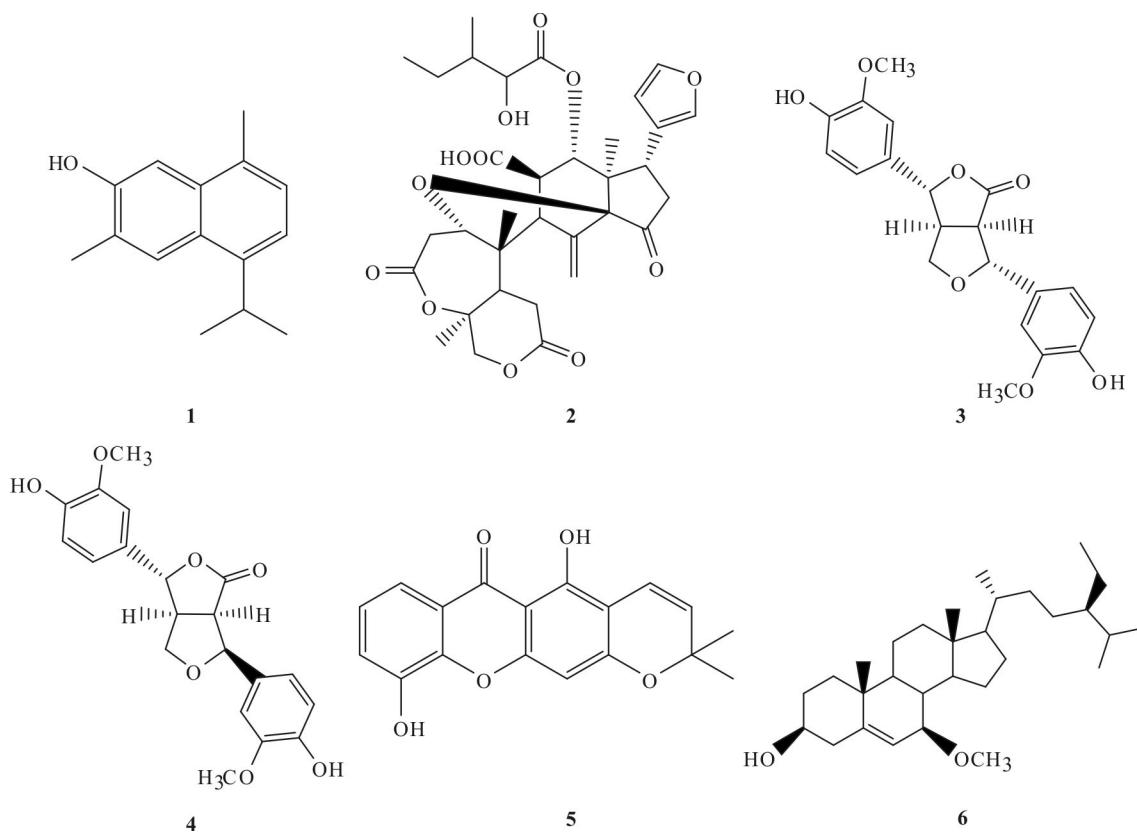


图1 化合物1~6的结构

Fig. 1 Structures of compounds 1–6

2 结果和讨论

本研究从大叶山棟根中分离鉴定了9个化合物, 分别是7-hydroxycadalene(1)、dregeana-1(2)、4-oxopinoresinol(3)、4-ketopinoresinol(4)、6-deoxyjacareubin(5)、schleicheol 1(6)、豆甾醇(7)、 β -谷甾醇(8)和胡萝卜苷(9), 包括倍半萜、三萜、木脂素和甾体等化学成分, 其中化合物1~6为首次从山棟属植物中分离得到。采用MTT法测定了化合物1~6的体外抗肿瘤活性, 结果表明化合物1和5对

慢性髓原白血病细胞K562有生长抑制活性, IC_{50} 值分别为10.6和12.7 mg mL⁻¹。化合物1、5和6对人胃癌细胞SGC-7901均有生长抑制活性, IC_{50} 值分别为26.7、9.3和28.0 mg mL⁻¹。本研究结果丰富了大叶山棟的化学成分和生物活性成分, 为大叶山棟的开发和利用提供了科学依据。

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