

忧遁草化学成分

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[摘要] **目的:**研究忧遁草 *Clinacanthus nutans* 枝叶乙酸乙酯部位的化学成分。**方法:**采用反复硅胶柱色谱法, Sephadex LH-20 柱色谱法, MCI 柱色谱法等分离纯化手段相结合对忧遁草进行分离纯化, 并通过波谱数据 (¹H-NMR, ¹³C-NMR) 鉴定其化学结构。**结果:**从忧遁草枝叶中分离鉴定了 10 个化合物, 分别鉴定为吡啶(1), loliolide(2), (3*S*, 5*R*, 6*S*, 7*E*)-5, 6-环氧-3-羟基-7-大柱香波龙烯-9-酮(3), 植醇(4), (-)- α -tocospirone(5), α -nigerose(6), clerspide A(7), (2*R*)-1-*O*-glyceryl- β -*D*-galactoside(8), 豆甾醇(9), 胡萝卜苷(10)。**结论:**化合物 2~8 为首次从忧遁草枝叶中分离得到, 为阐明忧遁草的药效物质基础提供了有力证据, 为海南产的忧遁草资源的开发利用提供了科学依据。

[关键词] 忧遁草; 枝叶; 乙酸乙酯部位; 化学成分

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Chemical Constituents of *Clinacanthus nutans*

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[Abstract] **Objective:** To study on chemical constituents from the ethyl acetate extraction of the twigs and leaves of *Clinacanthus nutans*. **Method:** The compounds were isolated by chromatographic techniques, such as silica gel and Sephadex LH-20. The structures of these compounds were identified on the basis of organic spectra (¹H-NMR, ¹³C-NMR). **Result:** Ten compounds were isolated from *C. nutans* and identified as indazole (1), loliolide (2), (3*S*, 5*R*, 6*S*, 7*E*)-5, 6-epoxy-3-hydroxy-7-megastigmen-9-one (3), phytol (4), (-)- α -tocospirone (5), α -nigerose (6), clerspide A (7), (2*R*)-1-*O*-glyceryl- β -*D*-galactoside (8), stigmasterol (9), daucosterol (10), respectively. **Conclusion:** The compounds 2-8 were obtained from the twigs and leaves of *C. nutans* for the first time. This research could provide powerful therapeutic material basis for *C. nutans* and provide a scientific basis for further research and development on the resource of *C. nutans* in Hainan.

[Key words] *Clinacanthus nutans*; twigs and leaves; ethyl acetate extraction chemical constituents

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床科鳄嘴花属植物, 广布于中国南部至西南部地区以及马来西亚、印度尼西亚、爪哇和加里曼丹等国

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家^[1]。忧遁草的药用价值一直未被受到重视,直至 2011 年马来西亚某淋巴瘤末期患者服用忧遁草康复后,鳄嘴花的药用价值受到了世界各国医药科研工作者的广泛关注^[2]。忧遁草在不同国家用于治疗疾病的种类相差甚远,在我国忧遁草全株入药,有调经、消肿、祛瘀、止痛、接骨之效^[3];在马来西亚用其治疗各种癌症,包括淋巴瘤、肝癌、肺癌、肾癌、乳腺癌、鼻咽癌、子宫癌、卵巢癌、血癌、脑癌、前列腺癌等;印度尼西亚用其治疗各种肾炎、肾萎缩、肾衰竭及肾结石;而泰国则用其治疗疱疹、湿疹、皮疹、虫及蛇咬伤,并已被列为泰国基本卫生保健药用植物^[4]。目前,忧遁草中报道的化合物结构类型丰富,包括三萜类^[5-7]、甾醇类^[6]、黄酮碳苷^[7-8]、含硫糖苷类^[9-11]、叶绿素类^[12-13]、其他类型化合物^[11, 14-16],具有抗病毒^[17-24]、抗氧化^[15, 25-27]、抗肿瘤^[15, 28]、抗炎^[15, 29]、抗菌^[25, 30]及免疫调节^[11, 29]等广泛的药理活性。为了更好的开发利用海南的忧遁草资源,也为了寻找新的生物活性成分,本研究对海南产的忧遁草枝叶的乙醇提取物的化学成分进行了研究,从中分离鉴定了 10 个化合物,分别是吡啶(1),loliolide(2), (3*S*, 5*R*, 6*S*, 7*E*)-5,6-环氧-3-羟基-7-大柱香波龙烯-9-酮(3),植醇(4), (-)- α -tocospirone(5), α -nigerose(6), clerspide A(7), (2*R*)-1-*O*-glyceryl- β -*D*-galactoside(8), 豆甾醇(9), 胡萝卜苷(10)。其中化合物 2~8 首次从该植物中分离得到。

1 材料

实验用忧遁草的枝叶于 2015 年 10 月采自海南省五指山市,由中国热带农业科学院热带生物技术研究代正福副研究员鉴定为爵床科植物忧遁草 *Clinacanthus nutans* 的枝叶,凭证标本存放于中国热带农业科学院热带生物技术研究(标本编号 YDC20151020)。AV-500 型超导核磁共振仪, Autospec-3000 型质谱仪(Bruker);GF₂₅₄ 高效薄层板和柱色谱用硅胶(200~300, 60~80 目)均为青岛海洋化工厂生产;LH-20 型羟丙基葡聚糖凝胶(Sephadex)和 RP-18(Merck), CHP20P(MCI, 日本三菱化学公司),所有试剂均为分析纯。

2 提取与分离

忧遁草枝叶鲜重 3.0 kg 切碎,用 95% 乙醇冷浸提取 3 次,室温,每次 7 d;过滤,合并滤液后经真空减压浓缩得粗浸膏,将其分散于水中成悬浊液,依次用乙酸乙酯、正丁醇萃取,分别得乙酸乙酯萃取物 22.8 g,正丁醇萃取物 10.6 g。乙酸乙酯萃取物

(22.8 g)经 MCI 柱,以甲醇-水(30:70~100:0)梯度洗脱,分段收集得到 11 个流分,然后 Fr. 2(200.0 mg)经 Sephadex LH-20(石油醚-三氯甲烷-甲醇 2:1:1)得到 Fr. 2A~Fr. 2B, Fr. 2B(20.5 mg)经硅胶柱色谱(三氯甲烷-甲醇 20:1)得到化合物 1(1.1 mg);Fr. 3(480.0 mg)经 Sephadex LH-20(甲醇)得到 Fr. 3A~Fr. 3C, Fr. 3B(205.0 mg)通过硅胶柱色谱(石油醚-乙醇 30:1)得到 2(1.0 mg), 3(1.3 mg);Fr. 6(685.0 mg)通过 Sephadex LH-20(甲醇)得到 Fr. 6A~Fr. 6C, Fr. 6A(203.0 mg)经由 Sephadex LH-20(三氯甲烷-甲醇 1:1)得到 Fr. 6A1~Fr. 6A3, Fr. 6A1(56.5 mg)通过硅胶柱色谱(三氯甲烷-甲醇 30:1)得到化合物 7(2.7 mg),由 Fr. 6A3(33.2 mg)得到化合物 6(3.4 mg);Fr. 10(7.9 g)经 Sephadex LH-20(甲醇)得到 Fr. 10A~Fr. 10D, Fr. 10D(1.08 g)再经 Sephadex LH-20(三氯甲烷-甲醇 1:1)得到 Fr. 10D1~Fr. 10D3, Fr. 10D3 经硅胶柱色谱(石油醚-三氯甲烷 10:1~8:1)得到 Fr. 10D3A~Fr. 10D3D, Fr. 10D3A~Fr. 10D3D 分别经硅胶柱色谱分离纯化得到化合物 4(4.0 mg), 5(3.7 mg), 8(8.2 mg), 9(2.7 mg), 10(1.8 mg)。

3 结构鉴定

化合物 1 淡黄色粉末, C₇H₆N₂; ESI-MS m/z 141 [M + Na]⁺; ¹H-NMR(CDCl₃, 500 MHz) δ : 8.05(1H, d, J = 7.5 Hz, H-5), 7.92(1H, s, H-3), 7.41(1H, d, J = 7.7 Hz, H-8), 2.37(2H, m, H-6, 7); ¹³C-NMR(CDCl₃, 125 MHz) δ : 133.3(C-3), 122.3(C-4), 122.0(C-5), 127.6(C-6), 112.9(C-7), 138.2(C-8), 123.5(C-9)。以上数据与文献[31]报道基本一致,故可鉴定为吡啶。

化合物 2 无色油状物, C₁₁H₁₆O₃; ESI-MS m/z 197 [M + H]⁺; ¹H-NMR(500 MHz, CDCl₃) δ : 5.69(1H, s, H-7), 4.33(1H, m, H-3), 2.46(1H, dt, J = 14.1, 2.5 Hz, H-2b), 1.97(1H, dt, J = 14.5, 2.5 Hz, H-4a), 1.78(3H, s, H-9), 1.77(1H, dd, J = 14.5, 3.5 Hz, H-4b), 1.52(1H, dd, J = 14.5, 3.5 Hz, H-2a), 1.46(3H, s, H-11), 1.27(3H, s, H-10); ¹³C-NMR(125 MHz, CDCl₃) δ : 36.1(C-1), 47.4(C-2), 67.0(C-3), 45.8(C-4), 86.8(C-5), 182.5(C-6), 113.1(C-7), 172.0(C-8), 30.8(C-9), 26.6(C-10), 27.2(C-11)。以上数据与文献[32]报道一致,故鉴定为 loliolide。

化合物3 无色油状物, $C_{13}H_{20}O_3$; ESI-MS m/z 247 $[M + Na]^+$; 1H -NMR (500 MHz, $CDCl_3$) δ : 7.02 (1H, d, $J = 15.6$ Hz, H-7), 6.29 (1H, d, $J = 15.6$ Hz, H-8), 3.90 (1H, m, H-2b), 2.37 (1H, m, H-4b), 2.28 (3H, s, H-10), 1.63 (1H, dd, $J = 14.4, 9.2$ Hz, H-4a), 1.61 (1H, ov, H-2a), 1.26 (1H, ov, H-2b), 1.18 (6H, s, H-11, 13), 0.97 (3H, s, H-12); ^{13}C -NMR (125 MHz, $CDCl_3$) δ : 35.2 (C-1), 40.7 (C-2), 64.2 (C-3), 46.8 (C-4), 67.4 (C-5), 69.6 (C-6), 142.5 (C-7), 132.7 (C-8), 197.6 (C-9), 28.4 (C-10), 29.5 (C-11), 25.1 (C-12), 20.0 (C-13)。以上数据与文献报道^[33]一致,故鉴定为(3*S*, 5*R*, 6*S*, 7*E*)-5,6-环氧-3-羟基-7-大柱香波龙烯-9-酮。

化合物4 无色油状物, $C_{20}H_{40}O$; ESI-MS m/z 319 $[M + Na]^+$; 1H -NMR ($CDCl_3$, 500 MHz) δ : 5.41 (1H, t, $J = 6.9$ Hz, H-2), 4.15 (2H, d, $J = 6.9$ Hz, H-1), 1.99 (2H, m, H-4), 1.67 (3H, s, H-20), 0.86 (6H, d, $J = 6.9$ Hz, H-16, 20), 0.84 (6H, d, $J = 6.5$ Hz, H-18, 19); ^{13}C -NMR ($CDCl_3$, 125 MHz) δ : 59.6 (C-1), 123.2 (C-2), 140.5 (C-3), 39.5 (C-4), 25.3 (C-5), 36.8 (C-6), 32.8 (C-7), 37.5 (C-8), 24.6 (C-9), 37.4 (C-10), 32.9 (C-11), 37.6 (C-12), 24.9 (C-13), 39.5 (C-14), 28.1 (C-15), 22.9 (C-16), 22.8 (C-17), 19.9 (C-18), 19.9 (C-19), 16.3 (C-20)。以上数据与文献报道^[34]一致,故鉴定化合物为植醇。

化合物5 无色油状物, $C_{29}H_{50}O_4$; ESI-MS m/z 485 $[M + Na]^+$; 1H -NMR ($CDCl_3$, 500 MHz) δ : 3.82 (1H, s, OH), 2.06 (3H, s, H-6a), 2.05 (3H, s, H-5a), 2.01 (1H, m, H-7b), 1.94 (1H, m, H-8b), 1.70 (1H, m, H-7a), 1.65 (1H, m, H-10b), 1.62 (1H, m, H-8a), 1.57 (1H, m, H-10a), 1.38 (3H, s, H-3a), 1.33 (3H, s, H-9a), 0.86 (3H, d, $J = 6.8$ Hz, H-22), 0.86 (3H, d, $J = 6.8$ Hz, H-21a), 0.84 (3H, d, $J = 6.8$ Hz, H-13a), 0.83 (3H, d, $J = 6.8$ Hz, H-17a); ^{13}C -NMR (125 MHz, $CDCl_3$) δ : 199.0 (C-1), 93.5 (C-2), 81.4 (C-3), 24.2 (C-3a), 201.9 (C-4), 142.0 (C-5), 13.2 (C-5a), 147.1 (C-6), 13.6 (C-6a), 32.2 (C-7), 36.6 (C-8), 87.2 (C-9), 25.9 (C-9a), 41.5 (C-10), 22.5 (C-11), 37.7 (C-12), 33.0 (C-13), 19.9 (C-13a), 37.6 (C-14), 24.8 (C-15), 37.4 (C-16), 32.9 (C-17), 19.9 (C-

17a), 37.5 (C-18), 24.4 (C-19), 39.5 (C-20), 28.1 (C-21), 22.6 (C-21a), 22.7 (C-22)。以上数据与文献报道^[35]一致,故鉴定化合物为(-)- α -tocospirone。

化合物6 白色粉末, $C_{12}H_{22}O_{11}$; ESI-MS m/z 365 $[M + Na]^+$; 1H -NMR (CD_3OD , 500 MHz) δ : 5.10 (1H, d, $J = 3.7$ Hz, H-1), 4.45 (1H, d, $J = 7.8$ Hz, H-1'), 3.84 ~ 3.08 (10H, m, H-2-6, 2'-6'); ^{13}C -NMR (125 MHz, CD_3OD) δ : 98.2 (C-1), 94.0 (C-1'), 78.1 (C-3'), 78.0 (C-3), 76.3 (C-5), 74.8 (C-2), 73.9 (C-5'), 73.0 (C-4'), 71.9 (C-2'), 71.7 (C-4), 62.9 (C-6), 62.8 (C-6')。以上数据与文献^[36]报道一致,故鉴定化合物为 α -nigerose。

化合物7 无色胶状物, $C_{19}H_{34}O_{10}$; ESI-MS m/z 445 $[M + Na]^+$; 1H -NMR (CD_3OD , 500 MHz) δ : 5.85 (1H, ddd, $J = 17.4, 10.4, 7.2$ Hz, H-2), 5.20 (1H, dd, $J = 17.5, 1.1$ Hz, H-1a), 5.11 (1H, dd, $J = 10.1, 1.2$ Hz, H-1b), 5.00 (1H, d, $J = 2.2$ Hz, H-1''), 4.28 (1H, d, $J = 7.9$ Hz, H-1'), 4.07 (1H, dt, $J = 13.2, 6.7$ Hz, H-3), 3.94 (1H, d, $J = 9.6$ Hz, H-4''a), 3.91 (1H, dd, $J = 11.0, 2.5$ Hz, H-6'a), 3.87 (1H, d, $J = 2.4$ Hz, H-2''), 3.74 (1H, d, $J = 9.6$ Hz, H-4''b), 3.57 (2H, s, H-5''), 3.55 (1H, dd, $J = 11.0, 6.0$ Hz, H-6'b), 3.33 (1H, m, H-3'), 3.31 (1H, m, H-5'), 3.30 (1H, dd, $J = 9.5, 9.0$ Hz, H-4'), 3.23 (1H, dd, $J = 9.1, 7.6$ Hz, H-2'), 1.67 (1H, m, H-4a), 1.50 (1H, m, H-4b), 1.41 (2H, m, H-5), 1.32 (2H, m, H-7), 1.26 (2H, m, H-6), 0.89 (3H, t, $J = 7.0$ Hz, H-8); ^{13}C -NMR (125 MHz, $CDCl_3$) δ : 116.4 (C-1), 140.8 (C-2), 83.1 (C-3), 35.7 (C-4), 25.7 (C-5), 33.0 (C-6), 23.7 (C-7), 14.4 (C-8), 103.2 (C-1'), 75.3 (C-2'), 78.2 (C-3'), 71.7 (C-4'), 76.8 (C-5'), 68.4 (C-6'), 110.8 (C-1''), 78.0 (C-2''), 80.6 (C-3''), 75.0 (C-4''), 65.7 (C-5'')。以上数据与文献^[37]报道一致,故鉴定化合物为 clerspide A。

化合物8 白色粉末, $C_9H_{18}O_8$; ESI-MS m/z 277 $[M + Na]^+$; 1H -NMR (500 MHz, CD_3OD) δ : 4.23 (1H, d, $J = 7.5$ Hz, H-1'), 3.89 (1H, dd, $J = 10.4, 5.3$ Hz, H-1a), 3.81 (1H, dd, $J = 3.2, 1.0$ Hz, H-4'), 3.78 (1H, m, H-2), 3.72 (1H, dd, $J = 10.4, 4.5$ Hz, H-6'a), 3.68 (1H, dd, $J =$

10.4, 4.5 Hz, H-6'b), 3.67 (1H, dd, $J = 10.4$, 4.5 Hz, H-1b), 3.60 (1H, dd, $J = 11.3$, 5.5 Hz, H-3a), 3.56 (1H, m, H-2'), 3.55 (1H, dd, $J = 11.3$, 5.5, H-3b), 3.53 (1H, m, H-5'), 3.51 (1H, m, H-3'); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD): δ 72.0 (C-1), 72.2 (C-2), 64.0 (C-3), 105.2 (C-1'), 72.6 (C-2'), 74.8 (C-3'), 70.3 (C-4'), 76.7 (C-5'), 62.5 (C-6')。以上数据与文献[38]报道一致,故鉴定化合物为(2*R*)-1-*O*-glyceryl- β -*D*-galactoside。

化合物 9 白色粉末, $\text{C}_{29}\text{H}_{48}\text{O}$; ESI-MS m/z 413 $[\text{M} + \text{H}]^+$; $^1\text{H-NMR}$ (CDCl_3 , 500 MHz) δ : 5.35 (1H, s, H-6), 5.15 (1H, dd, $J = 15.2$, 8.6 Hz, H-22), 5.02 (1H, dd, $J = 15.2$, 8.6 Hz, H-23), 3.52 (1H, m, H-3); $^{13}\text{C-NMR}$ (125 MHz, CDCl_3) δ : 37.4 (C-1), 32.0 (C-2, 8, 25), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 31.8 (C-7), 50.3 (C-9), 36.7 (C-10), 21.2 (C-11, 27), 39.8 (C-12), 42.4 (C-13), 56.1 (C-14), 24.5 (C-15), 28.9 (C-16), 57.0 (C-17), 12.2 (C-18), 19.6 (C-19), 40.6 (C-20), 21.4 (C-21), 138.5 (C-22), 129.4 (C-23), 51.4 (C-24), 19.1 (C-26), 25.6 (C-28), 12.4 (C-29)。以上数据与文献[39]报道一致,故鉴定化合物为豆甾醇。

化合物 10 白色粉末, $\text{C}_{35}\text{H}_{60}\text{O}_6$; 难溶于石油醚、三氯甲烷、乙酸乙酯、丙酮等有机溶剂,易溶于三氯甲烷甲醇混合溶剂,与胡萝卜苷对照品在不同溶剂系统下共薄层显示 R_f 均一致,故鉴定为胡萝卜苷。

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