狗牙花生物碱成分分析

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［摘要］ **目的**：对夹竹桃科狗牙花属植物中狗牙花的生物碱类化学成分进行分离并对分离得到的化合物进行结构鉴定。**方法**：将干燥狗牙花 3.5 kg 粉碎，加甲醇室温渗漉提取，收集渗滤液，将渗滤液减压浓缩得到浸膏。浸膏用 2 mol·L^{-1}硫酸调节 pH 2，加入乙酸乙酯萃取得到非碱性脂溶性成分浓缩物；萃取后的酸水液用氢氧化钠溶液调节 pH 9～10，然后用三氯甲烷萃取，得到总生物碱 10 g。通过硅胶柱色谱，小孔凝胶（MCI）柱色谱和 LH-20 羟丙基葡聚糖凝胶（Sephadex LH-20）柱色谱进行分离和重结晶技术纯化，应用 TLC 监测，分离得到纯度较高的单体化合物。利用现代波谱法结合理化分析对其化学结构进行进一步鉴定。**结果**：从狗牙花中共分离得到并鉴定了 6 个单吲哚生物碱，分别鉴定为 ervaramine 型：ervatamine（**1**），20-epiervatamine（**3**）；vobasine 型：tabernaemontanine（**2**），dregamine（**4**）；aspidosperma 型：（－）-mehranine（**5**），tetrahydroalstonine（**6**）。**结论**：化合物 **4**，**6** 为首次从该植物中分离得到。MTS 法测定化合物的肿瘤细胞毒活性，化合物 **1**～**6** 对 5 种人体肿瘤细胞株（人早幼粒白血病细胞 HL-60，人肝癌细胞 SMMC-7721，人肺癌细胞 A-549，人乳腺癌细胞 MCF-7，人结肠癌细胞 SW-480）未显示出细胞毒活性。

［关键词］ 狗牙花；生物碱；结构鉴定
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Alkaloids from *Tabernaemontana divaricata*

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［**Abstract**］ **Objective**: To isolate alkaloids from *Tabernaemontana divaricata*. **Method**: The air-dried powdered leaves of *T. divaricata* were extracted with CH_3OH at room temperature. The CH_3CH_2OH extract was partitioned between ethyl acetate and 2 mol·L^{-1} H_2SO_4, and the acidic aqueous phase was basified with NaOH to pH 9-10 and the alkaloids were extracted with $CHCl_3$. The crude alkaloids were subjected to silica gel chromatography, MCI chromatography, and Sephadex LH-20 column to isolate alkaloids, and their structures were established on the basis of spectroscopic methods. including NMR and mass spectrometry and comparison with literature. Cytotoxicity of the compounds was evaluated by the MTT method. **Result**: Six monoterpenoid indole alkaloids were isolated and their structures were elucidated as ervaramine-type: ervatamine (**1**), 20-epiervatamine (**3**); vobasine-type: tabernaemontanine (**2**), dregamine (**4**), and aspidosperma-type: (－) -mehranine (**5**) and tetrahydroalstonine (**6**). **Conclusion**: Compounds **4** and **6** were isolated from *T. divaricata* for the first time. All the alkaloids were assessed for their cytotoxicity against five human tumour lines (HL-60, SMMC-7721, A-549, MCF-7 and SW-480), and the result showed that none had activity.

［**Key words**］ *Tabernaemontana divaricata*; alkaloids; structural identification

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狗牙花又名白狗花、狮子花,为夹竹桃科狗牙花属植物,在亚洲热带和亚热带(孟加拉国、不丹、印度、缅甸、尼泊尔、泰国等)均有野生及栽培。狗牙花根茎含生物碱、苷类等成分。茎、叶的甲醇提取物可用于治疗胃癌疼痛、降低血压功效[1-2]。在广东、广西等省区民间用根、叶和花治疗毒蛇咬伤等。现代医学研究发现根可以用来治疗高血压、头痛、和疥疮等疾病[3-5]。目前对狗牙花研究的热点几乎都集中在生物碱类化学成分,单吲哚类生物碱包括 coronaridine, voacangine, voaphylline, lochnericine, tabernaricatines F and G, 19, 20-didehydro-6α-hydroxyervatamine 等,而双吲哚类则有 tabernaricatines A ~ E,19,20-dihydrotabernamine,19,20-dihydroervahanine, conodurine, tabernaelegantine 等[6-10]。文献报道显示,狗牙花的生物碱提取物和生物碱类单体成分具有显著的生物活性,包括乙酰胆碱酯酶抑制活性、细胞毒活性[10]。鉴于狗牙花中生物碱类化学成分多变的骨架结构和多样的生物活性,为全面研究其药理作用的物质基础,开发狗牙花的药用价值,对其生物碱成分进行了研究。通过硅胶柱色谱,MCI 柱色谱和 LH-20 羟丙基葡聚糖凝胶(Sephadex LH-20)柱色谱分离纯化方法从总碱中分离并鉴定了 6 个单吲哚生物碱,利用现代波谱法结合理化分析,并与文献对照鉴定其结构分别为 ervaramine-types (**1**, **3**), vobasine-types (**2**, **4**), aspidosperma-types (**5**,**6**)。化合物 **4**,**6** 为首次从该植物中分离得到。化合物 **1** ~ **6** 进行 5 种人体肿瘤细胞株(人早幼粒白血病细胞 HL-60,人肝癌细胞 SMMC-7721,人肺癌细胞 A-549,人乳腺癌细胞 MCF-7,人结肠癌细胞 SW-480)抑制活性筛选,但未显示出细胞毒活性。

1 材料

AM-500 型核磁共振仪(瑞士 Bruker 公司),N-1100 型系列旋转蒸发仪(上海爱朗仪器有限公司),A-1000S 型水流抽气机(上海爱朗仪器有限公司)。

高效薄层色谱硅胶 G 板(烟台化工研究院),80 ~ 100 目柱色谱硅胶,200 ~ 300 目柱色谱硅胶和色谱硅胶 H(青岛海洋化工厂),反相 RP-18 (40 ~ 60 μm,德国 Merck 公司),薄层色谱硅胶 GF_{254}(青岛海洋化工厂),Sephadex LH-20 (20 ~ 80 μm;美国 Pharmacia 精细化工公司)。所用洗脱溶剂均为工业纯,使用前经过重蒸,其他试剂为分析纯。

狗牙花枝、叶采自云南勐腊,由中国科学院西双版纳热带植物园周仕顺实验员鉴定为夹竹桃科植物狗牙花 *Tabernaemontana divaricata*,标本保存于云南师范大学化学化工学院(No. 130412)。

2 提取分离

提取分离过程流程见图 1。

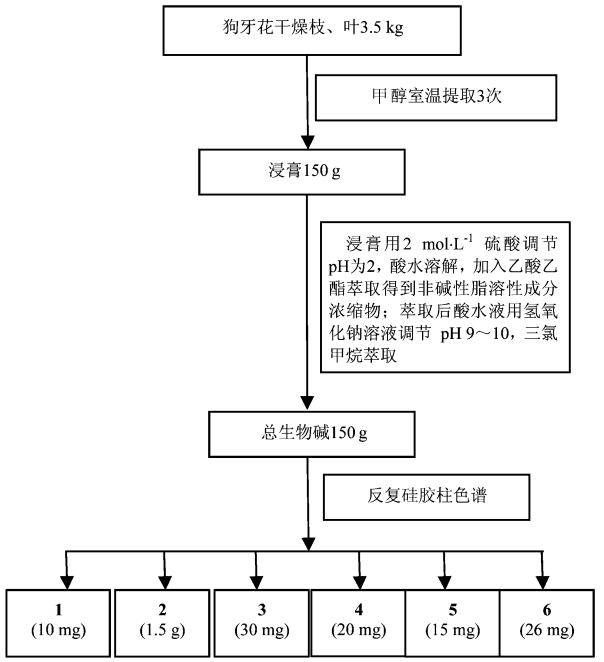

图 1 狗牙花提取分离流程

Fig. 1 Extraction separation process of *Tabernaemontana divaricata*

3 结构鉴定

化合物 **1** 淡黄色晶体性粉末,改良碘化铋钾试剂显色呈阳性;ESI-MS *m/z* 354 $[M+H]^+$;^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 8.87 (1H, s, N-H), 7.73 (1H, d, J = 8.0 Hz, H-9), 7.37 (1H, d, J = 8.0 Hz, H-12), 7.34 (1H, d, J = 8.0 Hz, H-11), 7.15 (1H, t, J = 8.0 Hz, H-10), 3.72 (2H, dd, J = 17.0, 18.0 Hz, H-6), 3.53 (3H, s, $COOCH_3$), 2.91 (2H, d, J = 5.0 Hz, H-14), 2.88 (1H, m, H-21), 1.81 (1H, m, H-21), 2.84 (1H, d, J = 11.0 Hz, H-5), 2.44 (1H, d, J = 11.0 Hz, H-5), 2.34 (s, 3H, N-CH_3), 2.25 (m, 1H, H-15), 1.74 (1H, m, H-20), 1.65 (1H, m, H-19), 1.22 (1H, m, H-19), 0.87 (3H, t, J = 8.0 Hz, H-18)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 132.5 (s, C-2), 192.6 (s, C-3), 65.1 (t, C-5), 29.4 (t, C-6), 122.3 (s, C-7), 128.1 (s, C-8), 121.3 (d, C-9), 120.1 (s, C-10), 126.6 (d, C-11), 111.9 (d, C-12), 136.6 (s, C-13), 43.2 (t, C-14), 41.0 (d, C-15), 51.0 (s, C-16), 10.7 (q, C-18), 23.8 (t, C-19), 37.8 (d, C-20), 60.4 (t, C-

21), 175.7 (s, $COOCH_3$), 52.1 (q, $COOCH_3$), 46.3 (q, N-CH_3)。以上数据与文献[9-10]报道一致,故鉴定化合物 **1** 为 ervatamine (20*R*)。

化合物 **2** 灰白色粉末,改良碘化铋钾试剂显色呈阳性; ESI-MS *m/z* 354 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.09 (1H, s, N-H), 7.71 (1H, d, *J* = 8.0 Hz, H-9), 7.36 (1H, d, *J* = 7.5 Hz, H-12), 7.34 (1H, d, *J* = 7.5 Hz, H-11), 7.16 (1H, d, *J* = 8.0 Hz, H-10), 3.96 (1H, t, *J* = 7.5 Hz, H-5), 3.46 (1H, dd, *J* = 12.5, 8.0 Hz, H-6), 3.31 (1H, dd, *J* = 15.0, 8.0 Hz, H-6), 3.42 (1H, t, *J* = 9.8 Hz, H-14), 2.78 (2H, t, *J* = 9.8 Hz, H-14), 3.21 (1H, d, *J* = 13.0 Hz, H-21), 2.51 (1H, d, *J* = 13.0 Hz, H-21), 3.03 (1H, s, H-16), 2.72 (1H, d, *J* = 10.0 Hz, H-15), 2.63 (3H, s, $COOCH_3$), 2.58 (3H, s, N-CH_3), 1.75 (1H, m, H-19), 1.55 (1H, m, H-19), 1.53 (1H, m, H-20), 0.99 (3H, t, *J* = 7.5 Hz, H-18)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 134.0 (s, C-2), 190.8 (s, C-3), 56.9 (d, C-5), 18.6 (t, C-6), 120.7 (d, C-7), 128.6 (s, C-8), 120.9 (d, C-9), 120.3 (s, C-10), 126.6 (d, C-11), 111.9 (d, C-12), 136.5 (s, C-13), 45.7 (t, C-14), 31.8 (d, C-15), 43.5 (d, C-16), 12.7 (q, C-18), 25.4 (t, C-19), 42.6 (d, C-20), 46.6 (t, C-21), 172.0 (s, $COOCH_3$), 50.3 (q, $COOCH_3$), 43.0 (q, N-CH_3)。以上数据与文献[11]报道一致,故鉴定化合物 **2** 为 tabernaemontanine (20*R*)。

化合物 **3** 白色无定形性粉末,改良碘化铋钾试剂显色呈阳性; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.17 (1H, s, N-H), 7.57 (1H, d, *J* = 8.0 Hz, H-9), 7.42 (1H, d, *J* = 8.0 Hz, H-12), 7.35 (1H, t, *J* = 7.5 Hz, H-11), 7.15 (1H, t, *J* = 8.0 Hz, H-10), 3.64 (3H, s, $COOCH_3$), 3.51 (1H, d, *J* = 12.0 Hz, H-6), 2.79 (1H, d, *J* = 16.0 Hz, H-6), 3.49 (1H, d, *J* = 12.0 Hz, H-5), 2.11 (1H, d, *J* = 8.0 Hz, H-5), 2.67 (1H, d, *J* = 11.0 Hz, H-21), 1.63 (1H, d, t, *J* = 16.0 Hz, H-21), 2.62 (1H, d, *J* = 16.0 Hz, H-14), 2.49 (1H, d, *J* = 16.0 Hz, H-14), 2.58 (1H, dd, *J* = 11.0, 4.0 Hz, H-15), 2.34 (3H, s, N-CH_3), 1.85 ~ 1.91 (1H, m, H-20), 1.32 ~ 1.45 (2H, m, H-19), 0.91 (3H, t, *J* = 7.5 Hz, H-18)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 132.8 (s, C-2), 194.2 (s, C-3), 60.7 (t, C-5), 31.8 (t, C-6), 119.5 (s, C-7), 127.4 (s, C-8), 120.2 (d, C-9), 120.6 (d, C-10), 126.5 (d, C-11), 112.4 (d, C-12), 136.8 (s, C-13), 36.6 (t, C-14), 36.2 (d, C-15), 49.3 (s, C-16), 11.4 (q, C-18), 23.9 (t, C-19), 38.9 (d, C-20), 57.5 (t, C-21), 175.6 (s, $COOCH_3$), 52.5 (q, $COOCH_3$), 46.2 (q, N-CH_3)。以上数据与文献[12-13]报道一致,故鉴定化合物 **3** 为 20-epiervatamine (20*S*)。

化合物 **4** 白色无定形性粉末,改良碘化铋钾试剂显色呈阳性; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 9.24 (1H, s, N-H), 7.70 (1H, d, *J* = 8.0 Hz, H-9), 7.36 (1H, t, *J* = 7.0 Hz, H-12), 7.33 (1H, d, *J* = 8.0 Hz, H-11), 7.15 (1H, t, *J* = 7.0 Hz, H-10), 3.98 (1H, t, *J* = 7.5 Hz, H-5), 3.37 (1H, dd, *J* = 15.0, 8.0 Hz, H-6), 3.27 (1H, dd, *J* = 15.0, 10.0 Hz, H-6), 3.04 (1H, t, *J* = 12.0 Hz, H-14), 2.67 (1H, d, *J* = 7.0 Hz, H-14), 2.88 (1H, m, H-15), 2.85 (1H, s, H-16), 2.67 (1H, m, H-21), 2.59 (1H, m, H-21), 2.64 (3H, s, $COOCH_3$), 2.61 (3H, s, N-CH_3), 1.89 (1H, m, H-20), 1.31 ~ 1.39 (2H, m, H-19), 1.02 (3H, t, *J* = 7.5 Hz, H-18)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 134.1 (s, C-2), 191.5 (s, C-3), 56.7 (d, C-5), 20.2 (t, C-6), 120.3 (s, C-7), 128.5 (s, C-8), 120.8 (d, C-9), 120.2 (d, C-10), 126.6 (d, C-11), 111.9 (d, C-12), 136.5 (s, C-13), 39.2 (t, C-14), 30.6 (d, C-15), 49.0 (d, C-16), 11.5 (q, C-18), 23.4 (t, C-19), 43.4 (d, C-20), 48.7 (t, C-21), 171.3 (s, $COOCH_3$), 50.3 (q, $COOCH_3$), 42.5 (q, N-CH_3)。以上数据与文献[14-15]报道一致,故鉴定化合物 **4** 为 dregamine (20*S*)。

化合物 **5** 白色无定形性粉末,改良碘化铋钾试剂显色呈阳性; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.11 (1H, d, *J* = 8.0 Hz, H-11), 7.04 (1H, d, *J* = 7.0 Hz, H-9), 6.67 (1H, d, *J* = 7.0 Hz, H-10), 6.42 (1H, d, *J* = 8 Hz, C-12), 3.57 (1H, d, *J* = 13.0 Hz, H-3), 2.39 (1H, d, *J* = 13.0 Hz, H-3), 3.23 (1H, d, *J* = 4.0 Hz, H-14), 2.99 (1H, d, *J* = 4.0 Hz, H-15), 2.77 (3H, s, N-CH_3), 3.17 (1H, m, H-5), 2.19 (1H, m, H-5), 2.29 (1H, m, H-6), 1.65 (1H, m, H-6), 1.77 (1H, m, H-16), 1.13 (1H, m, H-16), 1.75 (1H, m, H-17), 1.51 (1H, m, H-17), 1.24 (1H, m, H-19), 1.32 (1H, m, H-19), 3.36 (1H, dd, *J* = 4.0, 6.0 Hz, H-2), 2.25 (1H, s, H-21), 0.84 (3H, t, *J* = 7.5 Hz, H-18)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 73.3 (d, C-2), 53.7 (t, C-3), 53.2 (t, C-5), 41.2 (t, C-6), 51.4 (s, C-7), 136.7 (s, C-8), 121.4 (d,

C-9),117.1 (d,C-10),127.7 (d,C-11),106.6 (d,C-12),150.2 (s,C-13),53.1 (d,C-14),57.3 (d,C-15),20.0 (t,C-16),23.6 (t,C-17),7.6 (q,C-18),27.8 (t,C-19),34.7 (s,C-20),67.8 (d,C-21),31.6 (q,N-CH_3)。以上数据与文献[16-18]报道一致,故鉴定化合物**5**为(-)-mehranine。

化合物**6** 淡黄色粉末,改良碘化铋钾试剂显色呈阳性;^{1}H-NMR ($CDCl_3$, 500 MHz) δ: 7.95 (1H,s,N-H), 7.46 (1H,d,J=7.5 Hz,H-9),7.28 (1H,d,J=10.0 Hz,H-12),7.12 (1H,t,J=7.5 Hz,H-10),7.08 (1H,t,J=7.5 Hz,H-11),4.51 (1H,m,H-19),3.77 (3H,s,$COOCH_3$),3.36 (1H,d,J=10.0 Hz,H-3),3.12 (1H,m, H-21),2.73 (1H,m,H-21),2.98 (1H,m,H-5),2.74 (1H,m,H-5),2.88 (1H,m,H-6),2.57 (1H,m,H-6), 2.51 (1H,m,H-14),2.78 (1H,m,H-14),1.56 (1H,q,J=9.0 Hz,H-15),7.58 (1H,s,H-17),1.41 (3H,d, J=10.0 Hz,H-18),1.72 (1H,m,H-20)。^{13}C-NMR ($CDCl_3$, 125 MHz) δ: 134.5 (s,C-2),53.6 (t,C-3),56.3 (t, C-5),21.8 (t,C-6),108.1 (s,C-7),127.2 (s,C-8),118.1 (d,C-9),119.4 (d,C-10),121.4 (d,C-11),110.8 (d, C-12),136.1 (s,C-13),34.2 (t,C-14),31.4 (d,C-15),109.5 (s,C-16),155.8 (d,C-17),18.5 (q,C-18),72.5 (d,C-19),38.5 (d,C-20),58.8 (d,C-21),168.0 (s,$COOCH_3$),51.1 (q,$COOCH_3$)。以上数据与文献[19-20]报道一致,故鉴定化合物**6**为 tetrahydroalstonine。

4 体外人体肿瘤细胞毒活性筛选

4.1 待测化合物 化合物**1**~**6**;人体肿瘤细胞株:人早幼粒白血病细胞 HL-60,人肝癌细胞 SMMC-7721,人肺癌细胞 A-549,人乳腺癌细胞 MCF-7,人结肠癌细胞 SW-480。

4.2 MTS 法检测细胞活性原理[21] MTS 为 MTT 类似物,全称为 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium。活细胞线粒体中琥珀酸脱氢酶能够代谢还原 MTS,生成可溶性的甲臜(formazan)化合物,该化合物的吸光度 A(490 nm)与活细胞数目成正比。检测以顺铂和紫杉醇作为阳性对照。化合物的半数抑制浓度(IC_{50})通过浓度效应生长曲线计算确定。

4.3 试验方法 用含 10% 胎牛血清的培养液(DMEM 或者 RMPI 1640)配成单个细胞悬液,以每孔 1 万~2 万个细胞接种到 96 孔板,每孔 100 μL,贴壁细胞提前 12 h 接种培养。加入待测化合物溶液(化合物单体固定浓度 40 μmol·L^{-1}初筛,粗提物 100 mg·L^{-1}初筛,在该浓度对肿瘤细胞生长抑制在 50% 附近的化合物设 5 个浓度进入梯度复筛),每孔终体积 200 μL,每种处理均设 3 个复孔。37 ℃ 培养 48 h 后,每孔加 MTS 溶液 20 μL。继续孵育 4 h,终止培养,小心吸弃孔内培养上清液 100 μL 避免细胞丢失,每孔加 20% 的十二烷基硫酸钠(SDS) 100 μL,过夜孵育(温度 37 ℃),使结晶物充分溶解。选择 490 nm 波长,酶联免疫检测仪(Bio-Red 680)读取各孔 A,记录结果,以浓度为横坐标,细胞存活率为纵坐标绘制细胞生长曲线,应用两点法计算化合物的 IC_{50}。实验结果表明,化合物**1**~**6** 进行 5 种人肿瘤细胞株未显示出细胞毒活性。

5 讨论

狗牙花为我国重要的夹竹桃科狗牙花属植物,其生物碱成分[22-23]和药理作用[24-28]值得深入研究。在结构类型上 6 个单吲哚生物碱鉴定分别为 ervaramine 型:化合物**1**,**3**;vobasine 型:化合物**2**,**4**;aspidosperma 型:化合物**5**,**6**。化合物**4**,**6** 为首次从该植物中分离得到。研究结果为深入研究狗牙花的生物碱成分积累了资料,丰富该植物中生物碱骨架类型,为狗牙花属植物的化学分类提供了参考依据。

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