阴地翠雀花的化学成分研究

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摘　要:为研究阴地翠雀花(*Delphinium umbrosum* Hand.-Mazz.)的化学成分,采用硅胶柱色谱从其 95% 乙醇提取物中分离得到 14 个化合物。通过 HR-ESI-MS、1D 和 2D NMR 等波谱技术鉴定了它们的结构,包括 13 个牛扁碱型 C_{19}-二萜生物碱:牛扁碱(**1**)、氨茴酰牛扁碱(**2**)、majusine A(**3**)、14-deacetylajadine(**4**)、ajacine(**5**)、14-deacetylnudicauline(**6**)、甲基牛扁碱(**7**)、德尔色明 A 和 B(**8**)、delavaine A free acid 和 delavaine B free acid(**9**)、德拉瓦印 A 和 B(**10**)、拉翠碱 A 和 B(**11**)、umbrosumine C(**12**)、umbrosumines A 和 B(**13**),以及 1 个异喹啉类生物碱:*S*-glaucine(**14**)。所有化合物均为首次从该植物中分离得到。测试了化合物(**3**~**8**、**10**~**13**)对脂多糖诱导小鼠 RAW 264.7 巨噬细胞产生 NO 的抑制作用,以及化合物 **1**~**14** 对小鼠乳腺癌 4T1 细胞的抗肿瘤作用,结果显示所有测试化合物均无明显抗炎及抗肿瘤活性。

关键词:阴地翠雀花;二萜生物碱;抗炎;抗肿瘤
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Study on the chemical constituents of *Delphinium umbrosum* Hand.-Mazz.

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Abstract: To study the chemical constituents of *Delphinium umbrosum* Hand.-Mazz., fourteen compounds were isolated from the ethanol extract of *D. umbrosum* by silica gel column chromatography. Their structures were identified by means of spectroscopic methods such as HR-ESI-MS, 1D and 2D NMR, including thirteen lycoctonine C_{19}-type diterpenoid alkaloids: lycoctonine (**1**), anthranoyllycoctonine (**2**), majusine A (**3**), 14-deacetylajadine (**4**), ajacine (**5**), 14-deacetylnudicauline (**6**), methyllycaconitine (**7**), delsemines A and B (**8**), delavaine A free acid and delavaine B free acid (**9**), delavaines A and B (**10**), gyalamines A and B (**11**), umbrosumine C (**12**), umbrosumines A and B (**13**), and one isoquinoline alkaloid: *S*-glaucine (**14**). All compounds were isolated from this plant for the first time. The inhibitory effects of compounds (**3-8**, **10-13**) on lipopolysaccharide-induced NO production in mouse RAW 264.7 macrophages, and the anti-tumor effects of compounds **1-14** on mouse breast cancer 4T1 cells were tested, and the results showed that all the tested compounds had no obvious anti-inflammatory and anti-tumor activities.

Key words: *Delphinium umbrosum* Hand.-Mazz.; diterpenoid alkaloids; anti-inflammatory; anti-tumor

翠雀属(*Delphinium*)为毛茛科(Ranuculaceae)多年生或一、二年生草本植物,在全球约有 300 种,广泛分布于北半球地区,我国有近 220 种[1]。在我国,翠雀属多种植物具有悠久的药用历史,可治疗跌打损伤、风湿、牙痛和肠炎等疾病[2]。二萜生物碱是一类结构复杂的天然产物,广泛存在于翠雀属植物中,基本骨架主要分为 C_{18}-、C_{19}-和 C_{20}-二萜生物碱[3];现代药理研究表明二萜生物碱具有抗炎、镇痛、抗肿瘤和抗心律失常等药理活性[4]。有研究证

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明乌头碱型 C_{19}-二萜生物碱 forrestline F,以及阿替生型 C_{20}-二萜生物碱 songorine 具有一定的抗炎活性[5,6];牛扁碱型 C_{19}-二萜生物碱 delbrunine 和 delpheline,以及阿替生型 C_{20}-二萜生物碱 delphatisine C 具有一定的抗肿瘤活性[7]。阴地翠雀花(*Delphinium umbrosum* Hand.-Mazz.)为毛茛科翠雀属植物,产于云南西北部(中甸至德钦一带),生长在海拔 3 500~3 900 m 间山地草坡或林下[8]。目前,未见阴地翠雀花的化学成分及生物活性研究报道。因此,为了补充阴地翠雀花的研究空白,为其开发利用提供理论指导。本文对阴地翠雀花的生物碱成分进行系统研究,并测试了部分化合物对脂多糖诱导小鼠 RAW 264.7 巨噬细胞产生 NO 的抑制作用,以及所有化合物对小鼠乳腺癌 4T1 细胞的抗肿瘤活性。

1　材料与方法

1.1　材料

1.1.1　仪器与试剂

核磁共振波谱仪(Bruker AV 400 和 600,TMS 为内标,$CDCl_3$ 为溶剂);超高效液相色谱(ACQUITY UPLCI-Class)与四级杆飞行时间质谱(Xevo G2-S QTof)联用仪(Waters 公司);RE-2000A 旋转蒸发仪(上海亚荣);ZF-20C 紫外光谱仪、电子分析天平(托利多上海仪器有限公司)。薄层层析硅胶 GF254(青岛海洋化工厂);柱层析硅胶 G 和 H(200~400 目,青岛海洋化工厂);显色剂为改良碘化铋钾溶液和碘蒸气;石油醚、二氯甲烷、乙酸乙酯、甲醇、二乙胺等试剂均为分析纯。

全自动酶标仪(Molecular 仪器公司);CO_2 培养箱(Panasonic 公司);旋涡混合器(金怡仪器科技有限公司);脱色摇床仪(百岛生物公司);显微镜(麦克奥迪实业集团有限公司)。培养基、胎牛血清和脂多糖(Natocor 公司);RAW 264.67 细胞(ATCC 细胞库);NO 检测试剂盒(碧云天生物公司)。

1.1.2　实验药材

所使用的阴地翠雀花样品于 2020 年 8 月采自云南省迪庆州香格里拉市虎跳峡镇,经云南中医学院李国栋副教授鉴定为阴地翠雀花(*Delphinium umbrosum* Hand.-Mazz.),标本(2020HS00002)留存于西南交通大学生命科学与工程学院。

1.2　方法

1.2.1　提取与分离

将干燥的阴地翠雀花全草部分(10.0 kg),粉碎后用 95% 乙醇浸提 5 天(4 次),回收滤液、减压浓缩得总浸膏 1.2 kg,用温水溶解浸膏后,加入稀释的盐酸水溶液,调节 pH 至 2~3,用石油醚(4.0 L)萃取 4 次,萃取液进行减压浓缩。随后水层用氨水调节 pH 至 9~10,二氯甲烷萃取至无生物碱,减压浓缩萃取液,得总生物碱 73.0 g。

阴地翠雀花的总生物碱经正相硅胶柱层析初步分离,选用洗脱剂(二氯甲烷∶甲醇100∶1→0∶1)进行梯度洗脱,得到 A~D 四个部分。B 部分通过硅胶柱层析(石油醚∶乙酸乙酯∶二乙胺50∶1∶0.03→0∶1∶0.03)洗脱得到 B_1 和 B_2 两个部分。B_1 部分通过硅胶柱层析(石油醚∶乙酸乙酯30∶1→0∶1)梯度洗脱,得到 $B_{1\text{-}1}$、$B_{1\text{-}2}$ 和 $B_{1\text{-}3}$ 三个部分;$B_{1\text{-}2}$ 部分通过硅胶柱层析(石油醚∶二乙胺15∶1→4∶1)梯度洗脱,得到化合物 **14**(120.0 mg);$B_{1\text{-}3}$ 部分通过硅胶柱层析(二氯甲烷∶甲醇80∶1→10∶1)梯度洗脱,得到化合物 **4**(13.0 mg),化合物 **5**(19.0 mg),化合物 **11**(230.0 mg)和化合物 **12**(50.0 mg)。B_2 部分通过硅胶柱层析(石油醚∶二乙胺20∶1→0∶1)梯度洗脱,得到化合物 **6**(16.0 mg)。C 部分通过硅胶柱层析(二氯甲烷∶甲醇60∶1→0∶1)洗脱得到 C_1、C_2、C_3 和 C_4 四个部分。C_1 部分经硅胶柱层析(石油醚∶二乙胺15∶1→3∶1)洗脱得到化合物 **10**(180.0 mg);C_2 部分通过硅胶柱层析(乙酸乙酯∶二乙胺50∶1→8∶1)梯度洗脱得到化合物 **2**(140.0 mg)和化合物 **9**(50.0 mg);C_3 部分经硅胶柱层析(石油醚∶乙酸乙酯∶二乙胺30∶1∶0.1→0∶1∶0.1)洗脱得到化合物 **1**(113.0 mg);C_4 部分通过硅胶柱层析(二氯甲烷∶甲醇40∶1→5∶1)梯度洗脱得到化合物 **3**(6.0 mg)。D 部分通过硅胶柱层析(二氯甲烷∶甲醇70∶1→0∶1)洗脱得到 D_1、D_2 和 D_3 三个部分。D_2 部分通过硅胶柱层析(乙酸乙酯∶二乙胺40∶1→5∶1)洗脱得到化合物 **7**(22.0 mg)和化合物 **13**(6.0 mg);D_3 部分通过硅胶柱层析(石油醚∶乙酸乙酯∶二乙胺30∶1∶1→5∶1∶1)洗脱得到化合物 **8**(287.0 mg)。

1.2.2　体外抗炎活性测试

采用 MTT 法测定所有化合物对小鼠单核巨噬 RAW 264.7 细胞的存活率[9]。将 RAW 264.7 细胞以 100 μL/孔(每孔含 6×10^3 个细胞)接种至 96 板孔中培养 12 h。加入含药培养基(40 μmol/L,100 μL/孔),正常组和空白组加入含 1% DMSO 的培养基(100 μL/孔)培养 24 h 后,加入 5 mg/mL MTT(20 μL/孔)培养 4 h,弃去上清液,加入 DMSO(150 μL/孔),摇床震荡 10 min 后,采用酶标仪在 492 nm 处

读取吸光值(A)。

细胞存活率 =

$[(A_{样品}-A_{空白})/(A_{正常对照}-A_{空白})]\times 100\%$

采用 Griess 法考察部分化合物的抗炎活性,测定其对由脂多糖诱导的小鼠单核巨噬 RAW 264.7 细胞炎症的抗炎作用[10,11],以塞来昔布(10 μmol/L)为阳性对照。将 RAW 264.7 细胞以 100 μL/孔(每孔含 2×10^4 个细胞)在 96 板孔中培养 12 h,用 LPS(1 μg/mL)和含药培养基(40 μmol/L,100 μL/孔)预处理 24 h,模型组与空白组加入培养基。按 NO 试剂盒说明书进行操作,并用酶免疫测定仪在 540 nm 处测量吸光值(A)。

NO 生成抑制率 =

$[(A_{模型}-A_{样品})/(A_{模型}-A_{空白})]\times 100\%$

1.2.3 体外抗肿瘤活性测试

采用 MTT 法测定化合物对小鼠乳腺癌 4T1 细胞的生长抑制率[12,13],以紫杉醇(10 μmol/L)为阳性对照。将 4T1 细胞以 100 μL/孔(每孔含 2×10^4 个细胞)在 96 板孔中培养 12 h,加入含药培养基(40 μmol/L,100 μL/孔),正常组和空白组加入含 1% DMSO 的培养基(100 μL/孔)培养 24 h,于 96 板孔中重新加入 5 mg/mL MTT(20 μL/孔)和培养基培养 4 h,弃去上清液,加入 DMSO(150 μL/孔),摇床震荡 10 min 后,采用酶标仪在 492 nm 处读取吸光值(A)。

细胞生长抑制率 =

$[(A_{正常对照}-A_{样品})/(A_{正常对照}-A_{空白})]\times 100\%$

2 实验结果

2.1 结构鉴定

化合物 1 白色无定形粉末,碘化铋钾溶液呈阳性反应;$[\alpha]_D^{25}$ +37.0(*c* 0.33, $CHCl_3$)。HR-ESI-MS:*m/z* 468.297 4 $[M+H]^+$(calcd for $C_{25}H_{42}NO_7$, 468.296 1),分子式 $C_{25}H_{41}NO_7$。^{1}H NMR(400 MHz, $CDCl_3$)δ:4.06(1H, s, OH-8),3.86(1H, s, H-6),3.42,3.38,3.31,3.24(各 3H, s, 4 × OCH_3),3.18(1H, t, *J* = 8.4 Hz, H-16),3.04(1H, m, *J* = 6.8 Hz, H-9),1.04(3H, t, *J* = 7.2 Hz, *N*-CH_2CH_3);^{13}C NMR(100 MHz, $CDCl_3$)δ:84.2(d, C-1),25.8(t, C-2),31.0(t, C-3),38.6(s, C-4),43.3(d, C-5),90.6(d, C-6),88.2(s, C-7),77.7(s, C-8),49.3(d, C-9),38.1(d, C-10),49.1(s, C-11),28.9(t, C-12),46.0(d, C-13),84.0(d, C-14),33.7(t, C-15),82.7(d, C-16),64.8(d, C-17),67.6(t, C-18),53.3(t, C-19),51.3(t, C-21),13.9(q, C-22),55.9(q, 1-OCH_3),57.9(q, 6-OCH_3),58.0(q, 14-OCH_3),56.4(q, 16-OCH_3)。以上数据与文献报道[14]一致,故确定该化合物为牛扁碱(结构见图 1)。

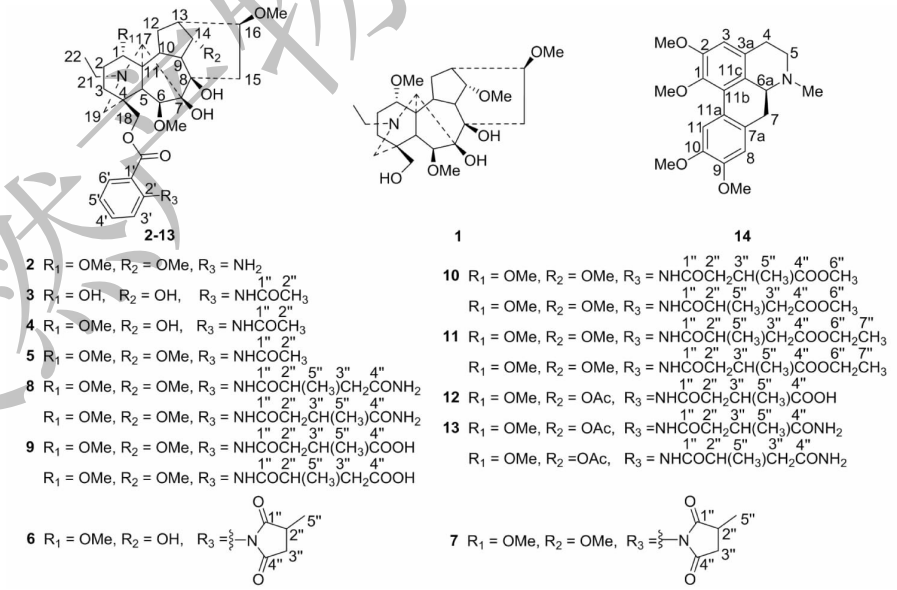

图 1 化合物 1 ~ 14 的化学结构

Fig. 1 The chemical structures of compounds **1-14**

化合物 2 白色无定形粉末,碘化铋钾溶液呈阳性反应;$[\alpha]_D^{25}$ +64.1(*c* 0.34, $CHCl_3$)。HR-ESI-MS:*m/z* 587.334 4 $[M+H]^+$(calcd for $C_{32}H_{47}N_2O_8$, 587.333 2),分子式 $C_{32}H_{46}N_2O_8$。^{1}H NMR(400

MHz, $CDCl_3$) δ: 6.64 ~ 7.81 (4H, m, Ar-H), 5.74 (2H, br s, NH_2), 3.60 (1H, t, J = 4.8 Hz, H_β-14), 3.41, 3.37, 3.34, 3.25 (各 3H, s, 4 × OCH_3), 1.06 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.2 (d, C-1), 26.3 (t, C-2), 32.4 (t, C-3), 37.7 (s, C-4), 43.4 (d, C-5), 91.1 (d, C-6), 88.7 (s, C-7), 77.7 (s, C-8), 50.5 (d, C-9), 38.4 (d, C-10), 49.2 (s, C-11), 28.9 (t, C-12), 46.3 (d, C-13), 84.1 (d, C-14), 33.8 (t, C-15), 82.7 (d, C-16), 64.7 (d, C-17), 68.8 (t, C-18), 52.6 (t, C-19), 51.1 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.1 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.0 (s, O*CO*Ar), 110.5 (s, C-1′), 150.9 (s, C-2′), 117.0 (d, C-3′), 134.5 (d, C-4′), 116.4 (d, C-5′), 130.9 (d, C-6′)。以上数据与文献报道[15]一致,故确定该化合物为氨茴酰牛扁碱。

化合物 3 白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ +24.2 (c 0.10, $CHCl_3$)。HR-ESI-MS: m/z 601.311 2 $[M + H]^+$ (calcd for $C_{32}H_{45}N_2O_9$, 601.312 5), 分子式 $C_{32}H_{44}N_2O_9$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.97 (1H, br s, NH), 7.12 ~ 8.73 (4H, m, Ar-H), 3.26, 3.41 (各 3H, s, 2 × OCH_3), 1.07 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 72.2 (d, C-1), 29.8 (t, C-2), 27.3 (t, C-3), 38.0 (s, C-4), 46.0 (d, C-5), 90.7 (d, C-6), 88.8 (s, C-7), 77.9 (s, C-8), 43.3 (d, C-9), 45.1 (d, C-10), 48.2 (s, C-11), 28.4 (t, C-12), 38.6 (d, C-13), 76.0 (d, C-14), 33.8 (t, C-15), 81.6 (d, C-16), 65.1 (d, C-17), 69.7 (t, C-18), 56.7 (t, C-19), 51.4 (t, C-21), 14.4 (q, C-22), 58.4 (q, 6-OCH_3), 56.2 (q, 16-OCH_3), 168.2 (s, O*CO*Ar), 114.5 (s, C-1′), 142.0 (s, C-2′), 120.8 (d, C-3′), 135.2 (d, C-4′), 122.7 (d, C-5′), 130.4 (d, C-6′), 169.2 (s, C-1″), 25.5 (t, C-2″)。以上数据与文献报道[16]一致,故确定该化合物为 majusine A。

化合物 4 白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ +22.5 (c 0.15, $CHCl_3$)。HR-ESI-MS: m/z 615.326 7 $[M + H]^+$ (calcd for $C_{33}H_{47}N_2O_9$, 615.328 2), 分子式 $C_{33}H_{46}N_2O_9$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.99 (1H, br s, NH), 7.10 ~ 8.73 (4H, m, Ar-H), 4.01 (1H, m, J = 4.4 Hz, H_β-14), 3.39, 3.37, 3.26 (各 3H, s, 3 × OCH_3), 2.23 (3H, s, NHCO*CH*$_3$), 1.07 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.9 (d, C-1), 25.5 (t, C-2), 32.4 (t, C-3), 38.0 (s, C-4), 50.5 (d, C-5), 90.6 (d, C-6), 89.4 (s, C-7), 76.4 (s, C-8), 45.3 (d, C-9), 46.2 (d, C-10), 48.5 (s, C-11), 27.6 (t, C-12), 36.4 (d, C-13), 75.4 (d, C-14), 33.3 (t, C-15), 81.8 (d, C-16), 65.2 (d, C-17), 69.8 (t, C-18), 52.5 (t, C-19), 51.3 (t, C-21), 14.4 (q, C-22), 56.2 (q, 1-OCH_3), 58.4 (q, 6-OCH_3), 56.7 (q, 16-OCH_3), 168.2 (s, O*CO*Ar), 114.6 (s, C-1′), 142.0 (s, C-2′), 120.7 (d, C-3′), 135.1 (d, C-4′), 122.7 (d, C-5′), 130.4 (d, C-6′), 169.2 (s, C-1″), 25.7 (t, C-2″)。以上数据与文献报道[17]一致,故确定该化合物为 14-deacetylajadine。

化合物 5 白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ +12.2 (c 0.13, $CHCl_3$)。HR-ESI-MS: m/z 629.342 3 $[M + H]^+$ (calcd for $C_{34}H_{49}N_2O_9$, 629.343 8), 分子式 $C_{34}H_{48}N_2O_9$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.98 (1H, br s, NH), 7.10 ~ 8.72 (4H, m, Ar-H), 3.60 (1H, br t, J = 4.0 Hz, H_β-14), 3.40, 3.37, 3.34, 3.25 (各 3H, s, 4 × OCH_3), 2.23 (3H, s, NHCO*CH*$_3$), 1.06 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.0 (d, C-1), 26.2 (t, C-2), 32.3 (t, C-3), 37.7 (s, C-4), 50.6 (d, C-5), 91.1 (d, C-6), 88.7 (s, C-7), 77.6 (s, C-8), 43.4 (d, C-9), 46.2 (d, C-10), 49.4 (s, C-11), 28.8 (t, C-12), 38.2 (d, C-13), 84.0 (d, C-14), 33.8 (t, C-15), 82.7 (d, C-16), 64.6 (d, C-17), 69.9 (t, C-18), 52.5 (t, C-19), 51.1 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 58.2 (q, 6-OCH_3), 58.0 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.2 (s, O*CO*Ar), 114.6 (s, C-1′), 142.0 (s, C-2′), 120.7 (d, C-3′), 135.1 (d, C-4′), 122.6 (d, C-5′), 130.4 (d, C-6′), 169.2 (s, C-1″), 25.6 (t, C-2″)。以上数据与文献报道[17, 18]一致,故确定该化合物为 ajacine。

化合物 6 白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ +11.5 (c 0.11, $CHCl_3$)。HR-ESI-MS: m/z 669.336 8 $[M + H]^+$ (calcd for $C_{36}H_{49}N_2O_{10}$, 669.338 7), 分子式 $C_{36}H_{48}N_2O_{10}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.28 ~ 8.05 (4H, m, Ar-H), 4.08 (1H, s, H_β-14), 3.84 (1H, s, H_α-6), 3.35 (6H, s, 2 × OCH_3), 3.25 (3H, s, OCH_3), 1.05 (3H, t, J =

7.2 Hz, *N*-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 85.0 (d, C-1), 25.5 (t, C-2), 32.3 (t, C-3), 38.0 (s, C-4), 45.2 (d, C-5), 90.4 (d, C-6), 89.3 (s, C-7), 76.4 (s, C-8), 50.3 (d, C-9), 46.1 (d, C-10), 48.4 (s, C-11), 27.6 (t, C-12), 36.5 (d, C-13), 75.4 (d, C-14), 33.2 (t, C-15), 81.8 (d, C-16), 65.2 (d, C-17), 69.6 (t, C-18), 52.4 (t, C-19), 51.3 (t, C-21), 14.3 (q, C-22), 56.2 (q, 1-OCH_3), 58.4 (q, 6-OCH_3), 56.6 (q, 16-OCH_3), 164.1 (s, O*CO*Ar), 127.1 (s, C-1′), 133.1 (s, C-2′), 129.5 (d, C-3′), 133.8 (d, C-4′), 131.2 (d, C-5′), 130.6 (d, C-6′), 180.0 (s, C-1″), 35.3 (d, C-2″), 37.1 (t, C-3″), 176.0 (s, C-4″), 16.5 (q, C-5″)。以上数据与文献报道[17, 19]一致,故确定该化合物为 14-deacetylnudicauline。

化合物 7 白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ +21.1 (*c* 0.18, $CHCl_3$)。HR-ESI-MS: *m/z* 683.353 6 [M + H]$^+$ (calcd for $C_{37}H_{51}N_2O_{10}$, 683.354 4), 分子式 $C_{37}H_{50}N_2O_{10}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25 ~ 8.04 (4H, m, Ar-H), 3.58 (1H, t, *J* = 4.4 Hz, H_β-14), 3.39, 3.34, 3.33, 3.24 (各 3H, s, 4 × OCH_3), 1.04 (3H, t, *J* = 7.2Hz, *N*-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.0 (d, C-1), 26.2 (t, C-2), 32.2 (t, C-3), 37.6 (s, C-4), 43.3 (d, C-5), 90.9 (d, C-6), 88.6 (s, C-7), 77.6 (s, C-8), 50.2 (d, C-9), 38.2 (d, C-10), 49.1 (s, C-11), 28.8 (t, C-12), 46.2 (d, C-13), 84.0 (d, C-14), 33.7 (t, C-15), 82.6 (d, C-16), 64.6 (d, C-17), 69.5 (t, C-18), 52.4 (t, C-19), 51.1 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.2 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 164.1 (s, O*CO*Ar), 127.1 (s, C-1′), 133.1 (s, C-2′), 129.5 (d, C-3′), 133.8 (d, C-4′), 131.1 (d, C-5′), 130.1 (d, C-6′), 179.9 (s, C-1″), 35.3 (d, C-2″), 37.1 (t, C-3″), 176.0 (s, C-4″), 16.5 (q, C-5″)。以上数据与文献报道[20]基本一致,故确定该化合物为甲基牛扁碱。

化合物 8 白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: *m/z* 700.381 8 [M + H]$^+$ (calcd for $C_{37}H_{54}N_3O_{10}$, 700.380 9), 分子式 $C_{37}H_{53}N_3O_{10}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.05/11.15 (1H, br s, NH), 7.09 ~ 8.68 (4H, m, Ar-H), 3.59 (1H, t, *J* = 4.8Hz, H_β-14), 3.38, 3.36, 3.32, 3.24 (各 3H, s, 4 × OMe), 1.04 (3H, t, *J* = 7.2Hz, *N*-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.0 (d, C-1), 26.1 (t, C-2), 32.3 (t, C-3), 37.6 (s, C-4), 43.3 (d, C-5), 91.0 (d, C-6), 88.6 (s, C-7), 77.6 (s, C-8), 50.5 (d, C-9), 38.1 (d, C-10), 49.1 (s, C-11), 28.7 (t, C-12), 46.1 (d, C-13), 84.0 (d, C-14), 33.7 (t, C-15), 82.6 (d, C-16), 64.6 (d, C-17), 69.9 (t, C-18), 52.4 (t, C-19), 51.1 (t, C-21), 14.2/14.3 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.2 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.0 (s, O*CO*Ar), 115.0 (s, C-1′), 141.8 (s, C-2′), 120.8 (d, C-3′), 134.9 (d, C-4′), 122.8 (d, C-5′), 130.4 (d, C-6′), 173.9/170.7 (s, C-1″), 39.5/42.0 (d/t, C-2″), 39.2/36.5 (t/d, C-3″), 174.8/178.1 (s, C-4″), 18.2/17.8 (q, C-5″)。以上数据与文献报道[21, 22]基本一致,故鉴定化合物 **8** 为一对区域异构体,德尔色明 A 和 B。

化合物 9 白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: *m/z* 701.365 8 [M + H]$^+$ (calcd for $C_{37}H_{53}N_2O_{11}$, 701.364 9), 分子式 $C_{37}H_{52}N_2O_{11}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.97/11.05 (1H, br s, NH), 7.06 ~ 8.73 (4H, m, Ar-H), 3.38, 3.34, 3.32, 3.23 (各 3H, s, 4 × OCH_3), 1.04 (3H, t, *J* = 7.2 Hz, *N*-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 83.9 (d, C-1), 26.2 (t, C-2), 32.3 (t, C-3), 37.6 (s, C-4), 43.3 (d, C-5), 91.0 (d, C-6), 88.6 (s, C-7), 76.6 (s, C-8), 50.5 (d, C-9), 38.2/37.9 (d, C-10), 49.1 (s, C-11), 28.7 (t, C-12), 46.2 (d, C-13), 84.0 (d, C-14), 33.8 (t, C-15), 82.6 (d, C-16), 64.6 (d, C-17), 69.5/69.8 (t, C-18), 52.4/53.5 (t, C-19), 51.1 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.1 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.0/167.9 (s, O*CO*Ar), 114.6 (s, C-1′), 142.0/142.3 (s, C-2′), 120.5 (d, C-3′), 134.9 (d, C-4′), 122.4/122.3 (d, C-5′), 130.4 (d, C-6′), 171.8 (s, C-1″), 42.0/38.6 (t/d, C-2″), 37.9/41.4 (d/t, C-3″), 177.8/176.0 (s, C-4″), 18.1/18.2 (q, C-5″)。以上数据与文献报道[21]基本一致,故鉴定化合物 **9** 为一对区域异构体, delavaine A free acid 和 delavaine B free acid。

化合物 10 白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: *m/z* 715.378 7 [M + H]$^+$ (calcd for $C_{38}H_{55}N_2O_{11}$, 715.380 6), 分子式 $C_{38}H_{54}N_2O_{11}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.15/11.03

(1H, br s, NH), 7.09 ~ 8.69 (4H, m, Ar-H), 3.39, 3.37, 3.33, 3.25 (各 3H, s, 4 × OCH_3), 1.05 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 84.0 (d, C-1), 26.2 (t, C-2), 32.3 (t, C-3), 37.6 (s, C-4), 43.3 (d, C-5), 91.0 (d, C-6), 88.6 (s, C-7), 77.6 (s, C-8), 50.5 (d, C-9), 38.2 (d, C-10), 49.2 (s, C-11), 28.8 (t, C-12), 46.2 (d, C-13), 84.0 (d, C-14), 33.8 (t, C-15), 82.6 (d, C-16), 64.6 (d, C-17), 69.8 (t, C-18), 52.5 (t, C-19), 51.1 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.2 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.1 (s, O*CO*Ar), 114.8/114.7 (s, C-1′), 142.0/141.7 (s, C-2′), 120.8 (d, C-3′), 135.0 (d, C-4′), 122.6 (d, C-5′), 130.4 (d, C-6′), 172.6/170.0 (s, C-1″), 41.5/39.1 (t/d, C-2″), 39.1/37.6 (d/t, C-3″), 174.2/176.1 (s, C-4″), 18.0/17.2 (q, 5″), 51.8/52.1 (q, C-6″)。以上数据与文献报道[23]基本一致,故鉴定化合物 **10** 为一对区域异构体,德拉瓦印 A 和 B。

化合物 11　白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: m/z 729.397 8 [M + H]$^+$ (calcd for $C_{39}H_{57}N_2O_{11}$, 729.396 2),分子式 $C_{39}H_{56}N_2O_{11}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.14/11.02 (1H, br s, NH), 7.08 ~ 8.70 (4H, m, Ar-H), 3.39, 3.36, 3.32, 3.24 (各 3H, s, 4 × OCH_3), 1.05 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 82.7 (d, C-1), 26.2 (t, C-2), 32.3 (t, C-3), 37.7 (s, C-4), 43.4 (d, C-5), 91.1 (d, C-6), 88.6 (s, C-7), 77.6 (s, C-8), 50.6 (d, C-9), 38.2 (d, C-10), 49.2 (s, C-11), 28.8 (t, C-12), 46.2 (d, C-13), 84.0 (d, C-14), 33.8 (t, C-15), 82.7 (d, C-16), 64.6 (d, C-17), 69.9/69.8 (t, C-18), 52.5 (t, C-19), 51.1 (t, C-21), 14.1 (q, C-22), 55.9 (q, 1-OCH_3), 57.9 (q, 6-OCH_3), 58.2 (q, 14-OCH_3), 56.4 (q, 16-OCH_3), 168.1 (s, O*CO*Ar), 114.8 (s, C-1′), 141.8/142.0 (s, C-2′), 120.8 (d, C-3′), 135.0 (d, C-4′), 122.6 (d, C-5′), 130.4 (d, C-6′), 172.1/170.1 (s, C-1″), 39.2/41.6 (d/t, C-2″), 38.0/36.1 (t/d, C-3″), 174.3/175.6 (s, C-4″), 18.0/17.2 (q, 5″), 60.7 (t, C-6″), 14.2 (q, C-7″)。以上数据与文献报道[24]基本一致,故鉴定化合物 **11** 为一对区域异构体,拉翠碱 A 和 B。

化合物 12　白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: m/z 729.359 1 [M + H]$^+$ (calcd for $C_{38}H_{53}N_2O_{12}$, 729.359 9),分子式 $C_{38}H_{52}N_2O_{12}$。^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.05 (1H, br s, NH), 7.09 ~ 8.68 (4H, m, Ar-H), 3.41, 3.36, 3.23 (各 3H, s, 3 × OCH_3), 1.06 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 83.8 (d, C-1), 25.9 (t, C-2), 32.0 (t, C-3), 37.7 (s, C-4), 50.2 (d, C-5), 90.8 (d, C-6), 88.5 (s, C-7), 77.3 (s, C-8), 43.6 (d, C-9), 45.7 (d, C-10), 49.0 (s, C-11), 28.2 (t, C-12), 37.9 (d, C-13), 74.8 (d, C-14), 33.1 (t, C-15), 83.3 (d, C-16), 64.6 (d, C-17), 69.8 (t, C-18), 52.5 (t, C-19), 51.1 (t, C-21), 14.1 (q, C-22), 55.9 (q, 1-OCH_3), 58.3 (q, 6-OCH_3), 57.9 (q, 16-OCH_3), 168.1 (s, O*CO*Ar), 114.8 (s, C-1′), 141.6 (s, C-2′), 120.8 (d, C-3′), 135.0 (d, C-4′), 122.8 (d, C-5′), 130.4 (d, C-6′), 170.4 (s, C-1″), 41.3 (t, C-2″), 36.0 (d, C-3″), 179.8 (s, C-4″), 17.1 (q, C-5″)。以上数据与文献报道[25]基本一致,故确定该化合物为 umbrosumine C。

化合物 13　白色无定形粉末,碘化铋钾溶液呈阳性反应; HR-ESI-MS: m/z 728.375 8 [M + H]$^+$ (calcd for $C_{38}H_{54}N_3O_{11}$, 728.375 8),分子式 $C_{38}H_{53}N_3O_{11}$。^{1}H NMR (600 MHz, $CDCl_3$) δ: 11.18/11.07 (1H, br s, *NH*), 7.12 ~ 8.70 (4H, m, Ar-H), 3.43, 3.38, 3.25 (各 3H, s, 3 × OCH_3), 1.07 (3H, t, J = 7.2 Hz, N-CH_2CH_3); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 84.0 (d, C-1), 26.1 (t, C-2), 31.6 (t, C-3), 37.8 (s, C-4), 50.5 (d, C-5), 91.0 (d, C-6), 88.7 (s, C-7), 77.4 (s, C-8), 43.7 (d, C-9), 45.9 (d, C-10), 49.1 (s, C-11), 28.2 (t, C-12), 37.9 (d, C-13), 74.8 (d, C-14), 33.2 (t, C-15), 83.4 (d, C-16), 64.6 (d, C-17), 70.0 (t, C-18), 52.5 (t, C-19), 51.2 (t, C-21), 14.2 (q, C-22), 55.9 (q, 1-OCH_3), 58.3 (q, 6-OCH_3), 57.9 (q, 16-OCH_3), 170.9 (s, 14-$OCOCH_3$), 168.1 (s, O*CO*Ar), 115.1 (s, C-1′), 141.8 (s, C-2′), 120.8 (d, C-3′), 135.0 (d, C-4′), 122.8 (d, C-5′), 130.5 (d, C-6′), 174.8 (s, C-1″), 42.1/39.6 (t/d, C-2″), 36.5/39.3 (d/t, C-3″), 177.8/173.7 (s, C-4″), 17.9/18.4 (q, C-5″)。以上数据与文献报道[26]基本一致,故鉴定化合物 **13** 为一对区域异构体, umbrosumines A 和 B。

化合物 14　白色无定形粉末,碘化铋钾溶液呈阳性反应; $[\alpha]_D^{25}$ + 95.0 (c 0.2, $CHCl_3$)。HR-ESI-

MS:*m/z* 356.186 9 [M + H]$^+$ (calcd for $C_{21}H_{26}NO_4$, 356.186 2),分子式 $C_{21}H_{25}NO_4$。^{1}H NMR(400 MHz, $CDCl_3$)δ: 8.07 (1H, s, H-11), 6.76 (1H, s, H-8), 6.57(1H,s,H-3),3.91,3.88,3.86,3.63(各 3H,s, 4 × OCH_3), 2.47 (3H, s, *N*-CH_3);^{13}C NMR (100 MHz,$CDCl_3$)δ:144.4(s,C-1),152.0(s,C-2),110.0 (d,C-3),129.4(s,C-3a),29.3(t,C-4),53.4(t,C-5),62.6(d,C-6a),34.6(t,C-7),128.9(s,C-7a), 110.5(d, C-8), 148.1 (s, C-9), 147.6 (s, C-10), 111.8 (d, C-11), 124.6 (s, C-11a), 127.2 (s, C-11b),127.0(s,C-11c),60.2(q,1-OCH_3),56.0(q, 2-OCH_3), 55.9 (q, 9-OCH_3), 55.8 (q, 10-OCH_3), 44.0(q,*N*-CH_3)。以上数据与文献报道[27]一致,故确定该化合物为 *S*-glaucine。

2.2 结构鉴定分析

经结构鉴定,确定以上化合物 **8**、**9**、**10**、**11**、**13** 均为一对区域异构二萜生物碱的混合物,二者碳谱十分相似,主要区别在于苯环侧链的甲基连接位置不同,分别连接在 C-2′′和 C-3′′位,进而导致 C-2′′和 C-3′′位的化学位移及峰型发生了变化,两者峰型相反。化合物 **8** 中区域异构体的比例可由氢谱 11 ppm 处 *NH* 的积分比例呈现,其比例为 3∶1,由于甲基(C-5′′)连接位置的变化[CH_3(C-2′′→C-3′′)],导致化合物 **8** 中区域异构体的 C-2′′、C-3′′的峰型截然相反,由此确定化合物 **8** 为一对区域异构二萜生物碱的混合物。同理,确定化合物 **9**、**10**、**11**、**13** 也均为一对区域异构二萜生物碱,其比例分别为 1.7∶1、1.2∶1、1.6∶1及 1.3∶1。

2.3 活性测试结果

化合物浓度在 40 μmol/L 时,测试了化合物(**3**~**8**、**10**~**13**)对脂多糖诱导小鼠 RAW 264.7 巨噬细胞产生 NO 的抑制作用,以及化合物(**1**~**14**)对小鼠乳腺癌 4T1 细胞的抗肿瘤作用,结果显示所有测试化合物均无明显抗炎及抗肿瘤活性。

3 结论

本研究对阴地翠雀花全草进行了生物碱成分的研究,共分离得到 14 个生物碱,其中包括 13 个牛扁碱型 C_{19}-二萜生物碱,1 个异喹啉类生物碱,化合物 **1**~**14** 均为首次从该植物中分离得到。本研究补充了阴地翠雀花的研究空白,为其植物化学分类提供了参考依据,为后续寻找活性生物碱成分提供了物质基础。测试了化合物对小鼠 RAW 264.7 巨噬细胞的抗炎作用,以及对小鼠乳腺癌 4T1 细胞的抗肿瘤作用,结果表明所有测试化合物均无明显抗炎及抗肿瘤活性。

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