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# A new monoterpenoid indole alkaloid from Ervatamia chinensis

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[ABSTRACT] AIM: To study the chemical constituents of the whole plants of E. chinensis, and find new or effective components. METHODS: Compounds were isolated by repeated silica gel and Sephadex LH-20 column chromatography. The structures were elucidated by NMR and HRESI-MS spectrometry. RESULTS: Six alkaloids were isolated from whole plants of E. chinensis, and their structures were elucidated as ervachinine E (1), rutaecarpine (2), 1-methyl-2-nonyl-4(1H)-quinolone (3), dihydroevocarpine (4), evocarpine (5), and 1-methyl-2-[(Z)-6-undecenyl]-4(1H)-quinolone (6), respectively. CONCLUSION: Compound 1 is a new monoterpenoid indole alkaloid and with moderate anti-tumor activity, while compounds 2-6 are reported from this species for the first time. [KEY WORDS] Eravatamia chinensis; Monoterpenoid indole alkaloid; Anti-tumor

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#### Introduction 1

Monoterpenoid indole alkaloids are structurally diversified alkaloids elaborated by the plant of the Apocynaceae family <sup>[1]</sup>. Some of these alkaloids, such as vinblastine, reserpine, and ibogamine, have important pharmaceutical applications <sup>[2-3]</sup>. The genus *Ervatamia* (Apocynaceae family) has fifteen plant species and five varieties growing in the south of China <sup>[2]</sup>, and many of them have been applied as traditional Chinese medicines for the treatment of hypertension and sore throat <sup>[4]</sup>. Previous chemical investigation on this genus reported the isolation of more than 300 indole alkaloids, some of which showed excellent antitumor activities <sup>[5]</sup>. A preliminary study on Ervatamia chinensis led to the isolation of five vobasinyl-ibogan type bisindole alkaloids and 11 terpenoid indole alkaloids [6]. As a part of our phytochemical investigation on plants in Apocynaceae family [6-8],

**Results and Discussion** Ervachinine E (1) was obtained as a white solid. Its molecular formula was deduced to be C<sub>20</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub> on the basis of the positive HRESI-MS at m/z 325.192 1 [M + H]<sup>+</sup> (calcd. 325.191 6). Its UV spectra at  $\lambda_{max}$  (log  $\varepsilon$ ) 319 (3.84), 283 (3.89), 248 (3.99), 212 (4.27) nm suggested a conjugated indole chromophore, while the IR absorptions at 1 640  $\text{cm}^{-1}$ 

one new monoterpenoid indole alkaloid, ervachinine E (1),

and five known alkaloids rutaecarpine (2) [9], 1-methyl-2nonyl-4(1*H*)-quinolone (3)  $^{[10]}$ , dihydroevocarpine (4)  $^{[11]}$ ,

evocarpine (5)<sup>[11]</sup>, and 1-methyl-2-[(Z)-6-undecenyl]-4(1H)-

quinolone  $(6)^{[10]}$  were isolated and identified from the whole

plants of Eravatamia chinensis. The present paper described

their isolation and structural elucidation.

indicated the presence of a conjugated carbonyl function. The <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) revealed that **1** possessed 20 carbon signals due to two methyl, five methylene, eight methine, and five quaternary carbons. The presence of the methine signals ( $\delta_C$  185.0,  $\delta_H$  9.94, 1H, s) revealed that the conjugated carbonyl observed in the IR spectra is due to an aldehyde function. Detailed comparison of the NMR data of 1 with those of lirofoline A [12] suggested that their structures were closely related, except for the pattern of substitution of methoxy group. The presence of a methine signal ( $\delta_{C}$  94.7,  $\delta_{H}$ 6.94, br d, J = 2.4) in 1 implied that the methoxy group was located at C-11 ( $\delta_C$  158.7) (Fig. 2), which was further

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| No.         | $\delta_{\rm H}$                 | $\delta_{C}$ | COSY               | HMBC (H $\rightarrow$ C) |
|-------------|----------------------------------|--------------|--------------------|--------------------------|
| 2           |                                  | 157.3        |                    |                          |
| 3α          | 2.74 (dt, 10.2, 2.4)             | 54.5         | 14                 | 5, 14, 15                |
| 3β          | 3.28 (dt, 10.2, 3.0)             |              | 14                 | 5, 14, 17, 21            |
| 5α          | 5.05 (d, 12.0)                   | 68.2         | 2, 3, 21           |                          |
| $5\beta$    | 4.95 (d, 12.0)                   |              |                    | 3                        |
| 6           | 9.94 (s)                         | 185.0        |                    | 7,8                      |
| 7           |                                  | 113.1        |                    |                          |
| 8           |                                  | 121.1        |                    |                          |
| 9           | 7.96 (d, 6.4)                    | 122.8        | 10                 | 11, 13                   |
| 10          | 6.88 (dd, 6.4, 2.4)              | 113.4        | 9                  | 8, 11, 12                |
| 11          |                                  | 158.7        |                    |                          |
| 12          | 6.94 (br d, 2.4)                 | 94.7         |                    | 8, 10, 11, 13            |
| 13          |                                  | 138.1        |                    |                          |
| 14          | $1.80 \ (m)^b$                   | 27.1         | 3, 15, 17 <i>α</i> | 15                       |
| 15α         | 1.93 (dddd, 10.8, 7.3, 4.8, 3.0) | 32.4         | 14, 20             | 3, 14                    |
| 15 <i>β</i> | 1.19 (ddt, 10.8, 4.2, 3.2)       |              | 14                 | 3, 17, 19, 20            |
| 16          | 3.63 (br dt, 12, 2.4)            | 30.9         | 17, 21             | 2, 14, 17                |
| $17\alpha$  | 1.65 (m)                         | 35.1         | 14, 16             | 15, 21                   |
| $17\beta$   | 2.27 (br t, 12.6)                |              | 16                 | 3, 14, 15                |
| 18          | 0.99 (t, 7.2)                    | 12.2         | 19                 | 19, 20                   |
| 19a         | 1.64 (m)                         | 28.6         | 18, 20             | 15, 18, 20, 21           |
| 19b         | 1.64 (m)                         |              | 18, 20             | 15, 18, 20, 21           |
| 20          | $1.80 (m)^{b}$                   | 39.3         | 15 <i>α</i> , 19   | 15, 16, 18,              |
| 21          | 2.95 (br s)                      | 52.9         | 16                 | 3, 5, 15, 17, 19         |
| 11-MeO      | 3.85 (s)                         | 56.2         |                    | 11                       |

 Table 1
 <sup>1</sup>H and <sup>13</sup>C NMR data of ervachinine E (1)<sup>a</sup> (J in Hz)

<sup>a</sup> Measured in CD<sub>3</sub>OD; <sup>b</sup> Overlapped.



Fig. 1 Structures of compounds 1–6

supported by the HMBC correlations of H<sub>3</sub>-OMe ( $\delta_{\rm H}$  3.85, s), H-9 ( $\delta_{\rm H}$  7.96, d, J = 6.4), H-10 ( $\delta_{\rm H}$  6.88, dd, J = 6.4, 2.4), and H-12 ( $\delta_{\rm H}$ , 6.94, br d, J = 2.4) to C-11. Detailed analysis of 2D NMR (HSQC, <sup>1</sup>H-<sup>1</sup>H COSY, and HMBC) confirmed that the other parts of the molecule were the same as those of lirofo-



# Fig. 2 ${}^{1}H{}^{-1}H$ COSY (bold) and selected HMBC (arrow, $H{\rightarrow}C$ ) correlations of 1

### line A [12].

The relative configuration of **1** established from the ROESY spectrum (Fig. 3) and coupling constant was consistent with that of lirofoline A <sup>[12]</sup>. Thus, it allowed the assignment of compound **1** to be ervachinine E, a new monoterpenoid indole alkaloid of the rearranged ibogan type.

#### **3** Experimental

#### 3.1 *General experimental procedures* Optical rotations were measured on a JASCO P-1020



Fig. 3 Key ROESY correlations of 1

digital polarimeter. IR spectra were obtained in a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded on a Shimadzu UV 2401PC spectrometer. NMR spectra were recorded on Bruker AM-400, DRX-500 and AV-600 spectrometers with TMS as internal standard. ESIMS was recorded on a Finnigan MAT 90 instrument. HRESIMS spectra were recorded on a VG Auto Spec-3000 spectrometer. Column chromatography was performed on silica gel (300–400 mesh; Qingdao Marine Chemical Inc., Qingdao, People's Republic of China), Sephadex LH-20 (40–70  $\mu$ m, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Lichroprep Rp-18 gel (40–63  $\mu$ m, Merck Darmstadt, Germany). Fractions were monitored by TLC and spots were visualized by Dragendorff's reagent.

#### 3.2 Plant material

The whole plants of *E. chinensis* were collected in November 2008 from Xishuangbanna area of Yunnan Province, China, and the plant sample was identified by Dr. WANG Zhi, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (KIB 08110613) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Science (CAS).

#### 3.3 Extraction and isolation

The powder of *E. chinensis* (7.5 kg) was extracted three times with 95% EtOH. The extract was concentrated under reduced pressure, followed by partitioning between EtOAc and 3% tartaric acid. The aqueous phase was adjusted to pH 9–10 with saturated Na<sub>2</sub>CO<sub>3</sub> and extracted with CHCl<sub>3</sub> to give crude alkaloids (40.0 g). The crude alkaloids were subjected to a Si gel column (petroleum ether-Me<sub>2</sub>CO, 1 : 0  $\rightarrow$  0 : 1) to obtain four major fractions A–D. Fraction A (3.5 g) was subjected to silica gel CC eluted with petroleum ether-Me<sub>2</sub>CO-Et<sub>2</sub>NH (30 : 1 : 0.1 $\rightarrow$ 15 : 1 : 0.1) to give **3** (20.0 mg), **4** (2.9 mg), **5** (68.2 mg), and **6** (6.6 mg). Fraction D was further chromatographed over a Si gel (300–400 mesh) column (petroleum ether-Me<sub>2</sub>CO-Et<sub>2</sub>NH, 10 : 1 : 0.2  $\rightarrow$  4 : 1 : 0.2) followed by Sephadex LH-20 CC eluted with CH<sub>3</sub>OH to afford **1** (3.7 mg), and **2** (2.4 mg).

**Ervachinine E (1)**:  $C_{20}H_{24}N_2O_2$ , white solid;  $[\alpha]_D^{25}$  -54.0

(c 0.25, MeOH); UV  $\lambda_{max}$  (log  $\varepsilon$ ): 319 (3.84), 283 (3.89), 248 (3.99), 212 (4.27) nm; CD (0.000926 M, MeOH)  $\lambda_{max}$  ( $\Delta \varepsilon$ ) 214 (+2.81), 229 (-2.39), 251 (-1.54), 299 (+0.46), 329 (-1.91); IR (KBr) v: 3 440, 2 926, 1 640, 1 450, 1 217, 1 168, 1 126, 1 046 cm<sup>-1</sup>; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; Positive ESI-MS *m/z* 325 [M + H]<sup>+</sup>; HR-ESI-MS *m/z* 325.192 1 [M + H]<sup>+</sup> (calcd. 325.191 6).

3.4 Cytotoxicity assay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) method in 96-well micro-plates. Briefly, 100 µL of adherent cells was seeded into each well of 96-well cell culture plates <sup>[13]</sup> and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of  $1 \times 10^5$  cells/mL. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.062 5, 0.32, 1.6, 8, and 40 µmol in triplicates for 48 h, with cisplatin (Sigma, USA) as positive controls. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC<sub>50</sub> were calculated by Reed and Muench's method [14].

Table 2 Cytotoxicity of compound ervachinine E (1)  $(IC_{50}/\mu mol \cdot L^{-1})$ 

| Compd.    | HL-60 | SMMC-7721 | A-549 | MCF-7 | SW480 |
|-----------|-------|-----------|-------|-------|-------|
| 1         | 6.59  | 13.61     | 11.22 | 14.44 | 14.70 |
| cisplatin | 1.00  | 17.05     | 26.75 | 16.97 | 18.32 |

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## 中国狗牙花中一个新单萜吲哚生物碱

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【摘 要】 目的:研究中国狗牙花的化学成分。方法:采用硅版、Sephadex LH-20、Rp-18 等多种层析柱分离手段,运用 NMR 和 HR-ESI-MS 等波谱技术鉴定化合物的结构。结果:从中国狗牙花全株中分离鉴定了 6 个生物碱: ervachinine E (1), rutaecarpine (2), 1-methyl-2-nonyl-4(1*H*)-quinolone (3), dihydroevocarpine (4), evocarpine (5), 1-methyl-2-[(*Z*)-6-undecenyl]-4(1*H*)-quinolone (6)。结论: 化合物 1 为新的单萜吲哚生物碱,显示中等强度的抗肿瘤活性; 化合物 2-6 为首次从该植物 中分离得到。

【关键词】 中国狗牙花; 单萜吲哚生物碱; 抗肿瘤

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