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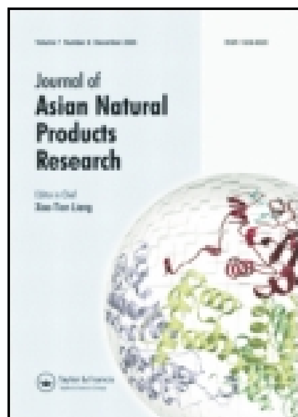

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Indoloquinazoline alkaloids from Euodia rutaecarpa and their cytotoxic activities

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Indoloquinazoline alkaloids from *Euodia rutaecarpa* and their cytotoxic activities

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Nine indoloquinazoline alkaloids (**1**–**9**) were isolated from the dried and nearly ripe fruits of *Euodia rutaecarpa* (Juss.) Benth. (Euodiae Fructus), along with limonin and β-sitosterol. Their structures were elucidated on the basis of their spectroscopic data. Among them, compounds **1** and **2** were new compounds and characterized as (7*R*,8*S*)-7-hydroxy-8-methoxy-rutaecarpine and (7*R*,8*S*)-7-hydroxy-8-ethoxy-rutaecarpine, respectively, and 1-hydroxy-rutaecarpine (**3**) and (7*R*,8*S*)-7,8-dihydroxy-rutaecarpine (**4**) were isolated from Euodiae Fructus for the first time. The nine indoloquinazoline alkaloids were evaluated for their cytotoxic activities against human promyelocytic leukemia HL-60 cells and human gastric carcinoma N-87 cells.

Keywords: *Euodia rutaecarpa*; indoloquinazoline alkaloids; (7*R*,8*S*)-7-hydroxy-8-methoxy-rutaecarpine; (7*R*,8*S*)-7-hydroxy-8-ethoxy-rutaecarpine; human promyelocytic leukemia HL-60 cell; human gastric carcinoma N-87 cells

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1. Introduction

The dried and nearly ripe fruits of *Euodia rutaecarpa* (Juss.) Benth. (Euodiae Fructus), a traditional Chinese drug 'Wu-Zhu-Yu', have been used for the treatment of headache accompanied by retching and cold limbs, abdominal colic, weakness and edema of the legs, dysmenorrhea, epigastric distension and pain with vomiting and acid regurgitation, diarrhea occurring before dawn daily, and hyperbaropathy [1]. In searching for potential anticancer drugs from natural products, indoloquinazoline alkaloids such as evodiamine, which are one of the main chemical constituents in Euodiae Fructus [2,3], exhibited significant cytotoxic activities against murine colon cancer cell line 26-L5 [4,5] and human prostate cancer cell lines DU145 and PC3 [6]. As part of our systematic search for bioactive constituents from Euodiae Fructus [3,7–11], we herein report the isolation and structural elucidation of two new alkaloids named (7*R*,8*S*)-7-hydroxy-8-methoxy-rutaecarpine (**1**) and (7*R*,8*S*)-7-hydroxy-8-ethoxy-rutaecarpine (**2**), and 1-hydroxy-rutaecarpine (**3**) and (7*R*,8*S*)-7,8-dihydroxy-rutaecarpine (**4**) isolated from Euodiae Fructus for the first time, as well as their potential cytotoxicities against human promyelocytic leukemia HL-60 cells and human gastric carcinoma N-87 cells.

2. Results and discussion

An ethanolic extract of Euodiae Fructus was partitioned with cyclohexane (CHA), ethyl acetate (EtOAc) and normal butanol (BuOH), and the CHA extract was

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separated by silica gel column chromatography (CC) and preparative high-performance liquid chromatography (HPLC) to yield compounds **1**–**11**. The structures of the known compounds were identified as 1-hydroxy-rutaecarpine (**3**) [12,13], (7*R*,8*S*)-7,8-dihydroxy-rutaecarpine (**4**) [14], rutaecarpine (**5**) [15], evodiamine (**6**) [15], wuchuyuamide-I (**7**) [9], 7β-hydroxy-rutaecarpine (**8**) [16,17], dehydroevodiamine (**9**) [15] (Figure 1), limonin [10], and β-sitosterol by comparison of their NMR, MS, and physical data with those described in the literature. Compounds **3** and **4** were isolated and identified for the first time from Euodiae Fructus.

Compound **1** was isolated as a white amorphous powder with $[\alpha]_{20}^{D}$ −89.7 ($c = 0.35$, MeOH), and assigned the molecular formula $C_{19}H_{15}N_3O_3$ on the basis of a quasi-molecular ion peak at *m*/*z* 334.1184 $[M + H]^+$ in the HR-ESI-MS. The IR spectrum displayed characteristic absorption for N–H and OH (3345 cm^{-1}), carbonyl group (1641 cm^{-1}) and aromatic rings (1618, 1598, and 1502 cm^{-1}). The UV spectrum showed absorption maxima at 234, 240, 330, 345, and 362 nm due to an indole chromophore, which was characteristic diagnostics of unsubstituted moiety in the aromatic ring of indole ring [18] and quite similar to that of **5** (rutaecarpine), suggesting that they had the same skeleton and **1** was also an indoloquinazoline alkaloid. Fourteen proton signals, including eight aromatic singlets (δ_H 7.14–8.20), one N–H proton signal [δ_H 12.21 (1H, s)], two methine proton signals [δ_H 4.89 (1H, d, $J = 2.0$ Hz), 6.62 (1H, d, $J = 2.0$ Hz)], and one characteristic *O*-methyl group proton signal [δ_H 3.33 (3H, s)], were observed in the ^{1}H NMR spectrum (Table 1), in which the signals at δ_H 7.82 (1H, br d, $J = 8.0$ Hz, H-1), 7.86 (1H, br t, $J = 8.0$ Hz, H-2), 7.55 (1H, br t, $J = 8.0$ Hz, H-3), and 8.19 (1H, br d, $J = 8.0$ Hz, H-4) formed the first aromatic spin system; the signals at δ_H 7.72 (1H, br d, $J = 8.0$ Hz, H-9), 7.16 (1H, br t, $J = 8.0$ Hz, H-10), 7.29 (1H, br t, $J = 8.0$ Hz, H-11), and 7.53 (1H, br d, $J = 8.0$ Hz, H-12) made up the second aromatic spin system. Ten carbon signals bearing protons were easily determined by the HSQC experiment, which established one-bond correlation. The ^{1}H and ^{13}C NMR spectra displayed signals for two spin systems with 1,2-disubstitued aromatic rings, one N–H, and one amide carbonyl function (δ_C 160.5) (Table 2). Considering the structure of alkaloids isolated from

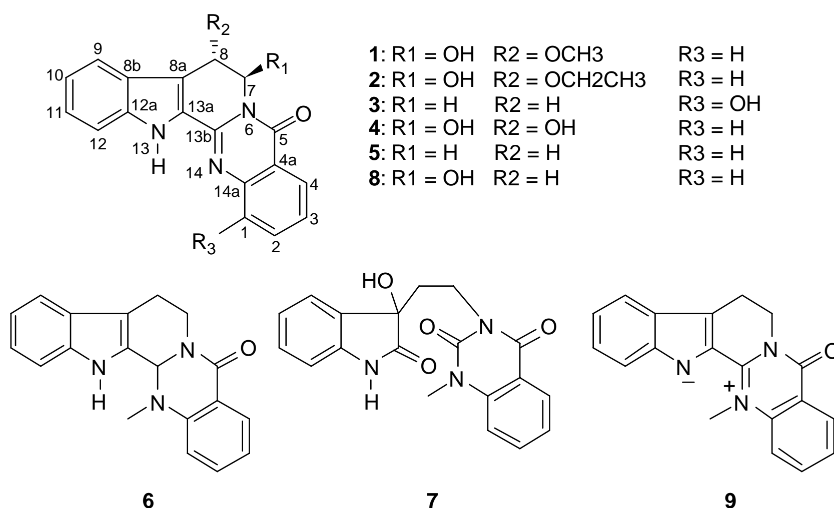

Figure 1. Structures of compounds **1**–**9**.

Table 1. ^{1}H NMR spectral data of compounds **1**, **2**, and **4** (DMSO-d_6, 400 MHz).

No. H	**1**	**2**	**4**
1	7.82, 1H, br d (8.0)	7.79, 1H, br d (8.0)	7.72, 1H, br d (8.0)
2	7.86, 1H, br d (8.0)	7.87, 1H, br d (8.0)	7.85, 1H, br d (8.0)
3	7.55, 1H, br t (8.0)	7.55, 1H, br t (8.0)	7.52, 1H, br t (8.0)
4	8.19, 1H, br d (8.0)	8.20, 1H, br d (8.0)	8.20, 1H, br d (8.0)
7	6.62, 1H, d (2.0)	6.62, 1H, d (2.0)	6.51, 1H, d (2.0)
8	4.89, 1H, d (2.0)	4.97, 1H, d (2.0)	5.03, 1H, d (2.0)
9	7.72, 1H, br d (8.0)	7.73, 1H, br d (8.0)	7.71, 1H, br d (8.0)
10	7.16, 1H, br d (8.0)	7.15, 1H, br d (8.0)	7.12, 1H, br d (8.0)
11	7.29, 1H, br d (8.0)	7.29, 1H, br d (8.0)	7.27, 1H, br d (8.0)
12	7.53, 1H, br d (8.0)	7.53, 1H, br d (8.0)	7.52, 1H, br d (8.0)
N–H	12.21, 1H, br s	12.18, 1H, br s	12.03, 1H, br s
OH	–	–	–
OCH_3	3.33, 3H, s	–	–
$1'_{eq}$	–	3.72, 1H, qd, (6.8, 9.2)	–
$1'_{ax}$	–	3.51, 1H, qd, (6.8, 9.2)	–
$2'$		1.05, 3H, t (6.8)	–

Note: All the signals were assigned by 1D and 2D NMR spectra; δ in ppm and J (parentheses) in Hz.

Euodiae Fructus, all the spectral data showed that the structure of **1** was an indoloquinazoline alkaloid. Further analysis of the HMBC correlation between the aromatic proton at δ_H 8.19 and an amide carbonyl carbon at δ_C 160.5 suggested that the quinazoline-type substructure included the first aromatic spin system. The signals at δ_H 6.62 (1H, d, $J = 2.0$ Hz) and 4.89 (1H, d, $J = 2.0$ Hz) formed the third spin system, which were assigned to two oxygenated methine proton signals and correlated with two carbons (δ_C 76.1 and 71.4) in the HSQC spectrum. In the HMBC experiment (Figure 2), the proton signal at δ_H 6.62 correlated with carbon signals at δ_C 160.5 (C-5), 143.3 (C-13b), and 114.2 (C-8a), and the proton at δ_H 4.89 correlated with carbon signals at δ_C 114.2 (C-8a) and 126.5 (C-8b), suggesting that the proton signal at δ_H 6.62 and 4.89 were assigned to H-7 and H-8, respectively. Therefore, carbon signals at δ_C 76.1 and 71.4 were assigned to C-7 and C-8, respectively. The location of the methoxyl group at C-8 was deduced on the basis of HMBC correlation from the methoxyl at δ_H 3.33 (3H, s) to the carbon at δ_C 71.4. In addition, the coupling constant between H-7 and H-8 was relatively small (2 Hz). Finally, the orientations of the H-7 and H-8 protons were studied by 2D NMR experiments, which were in agreement with those of (7*R*,8*S*)-7,8-dihydroxy-rutaecarpine (**4**) [14]. From the results mentioned above, the structure

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, and **4** (DMSO-d_6, 100 MHz).

No. C	**1**	**2**	**4**
1	126.7	127.2	127.0
2	135.0	135.5	135.2
3	126.7	126.9	126.8
4	126.7	127.7	127.1
4a	120.8	121.0	121.2
5	160.5	161.1	161.2
7	76.1	77.0	79.2
8	71.4	70.1	63.1
8a	114.2	115.4	117.6
8b	126.5	126.9	125.8
9	120.5	120.3	120.3
10	119.9	120.1	119.9
11	124.7	125.1	124.8
12	112.7	113.2	112.9
12a	138.3	138.8	138.8
13a	126.7	127.2	126.8
13b	143.3	143.9	144.0
14a	147.1	147.6	147.5
OCH_3	55.9	–	–
$1'$	–	64.2	–
$2'$	–	15.7	–

Note: All the signals were assigned by 1D and 2D NMR spectra.

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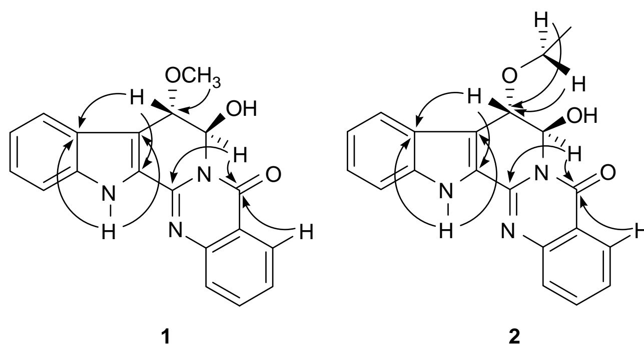

Figure 2. Key HMBC correlations of compounds **1** and **2**.

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of **1** was elucidated as (7*R*,8*S*)-7-hydroxy-8-methoxy-rutaecarpine. As far as we know, this compound has not been reported previously.

Compound **2** was isolated as a white amorphous power with $[\alpha]_{20}^{D}$ -52.8 ($c = 0.14$, MeOH). Its molecular formula was defined as $C_{20}H_{17}N_3O_3$ by the data of HR-ESI-MS at *m/z* 348.1342 $[M + H]^+$. The UV and IR spectra were very similar to those of **1**, suggesting that **2** also had a similar indoloquinazoline structure. The ^{1}H and ^{13}C NMR spectra were all almost the same as those of **1**, from which **2** differed only in the replacement of a methoxyl group by the signals of ethoxyl group [δ_H 3.72 (1H, qd, $J = 6.8$, 9.2 Hz, H_a-1′), 3.51 (1H, qd, $J = 6.8$, 9.2 Hz, H_b-1′), 1.05 (3H, t, $J = 6.8$ Hz, H-2′); δ_C 64.2 (C-1′), 15.7 (C-2′)]. The location of the ethoxyl group at C-8 was further deduced on the basis of HMBC correlation from the protons at δ_H 3.72 and 3.51 to the carbon at δ_C 70.1. The detailed assignment of ^{1}H and ^{13}C NMR spectral data of **2** (Tables 1 and 2) was based on HSQC and HMBC spectra. On the basis of the above data and comparison of ^{1}H and ^{13}C NMR spectral data (Tables 1 and 2) with those of **1**, the structure of **2** was concluded to be (7*R*,8*S*)-7-hydroxy-8-ethoxyl-rutaecarpine.

Compound **9** is a known alkaloid, its chemical structure is a rare special inner salt, and the R_f value was 0.39 on TLC plate ($CHCl_3$:MeOH:CH_3COOH = 6:1:0.05). To further confirm the structure, 2 mg of the compound was dissolved in 400 μl of MeOH containing 100 μl of HCl to gain its hydrochloride salt structure (**9A**). Compound **9A** could not be spread by MeOH on TLC plate, and had no absorption at 365 nm which was different compared with compound **9** having yellow fluorescence at the same wavelength. The EI-MS of **9A** gave a $[M]^+$ peak at the *m/z* 301, the same as compound **9**. The results mentioned above certified that the hydrogen on nitrogen atom (N_1) in the indole nucleus of dehydroevodiamine has strong acidity and could release H^+.

The nine indoloquinazoline alkaloids isolated were evaluated for their cytotoxic activities against human cancer cell lines HL-60 and N-87 by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [19]. These alkaloids all showed cytotoxic activities against the two cancer cell lines (Table 3). Compound **6** (evodiamine) showed significant cytotoxic activities against both HL-60 with IC_{50} of 5.88 μM and N-87 with IC_{50} of 7.30 μM. Compound **5** (rutaecarpine) exhibited selective activity against N-87 with IC_{50} of 8.41 μM. In comparison with IC_{50} values (19.8 μM) of **5** against HL-60, the cytotoxic activity was enhanced markedly when the 1-H of **5** was replaced by a hydroxyl group such as **3** (1-hydroxy-rutaecarpine) with IC_{50} of 10.1 μM. Inter-

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Table 3. Cytotoxicity of compounds **1**–**9** against two human cancer cell lines.

	IC_{50} values (μM)	
Compound	HL60	N-87
1	7.82	22.3
2	8.31	27.9
3	10.1	8.38
4	13.7	14.1
5	19.8	8.41
6	5.88	7.30
7	15.1	20.1
8	10.1	23.2
9	21.7	13.8
VCR[a]	0.11	3.62

[a] VCR was used as a positive compound.

estingly, compounds **1**, **2**, **4**, and **8**, which only differ from **5** by the replacement groups at C-7 and C-8, exhibited more activities than **5** against HL-60 (IC_{50} values: $\mathbf{5} < \mathbf{4} \le \mathbf{8} < \mathbf{1} \le \mathbf{2}$) but showed less activities against N-87 (IC_{50} values: $\mathbf{5} > \mathbf{4} > \mathbf{8} \ge \mathbf{1} \ge \mathbf{2}$). Although the structure–activity relationship between **5** and its analogs was not conclusively demonstrated only by these data, it is likely that the C-7 and C-8 of **5** would be an important site in a selective activity against two human carcinoma cell lines mentioned above.

3. Experimental

3.1 General experimental procedures

Optical rotation was measured on an Autopol III polarimeter (Rudolph Research Analytical, Flanders, NJ, USA) with MeOH as solvent. IR spectra were recorded on a Thermo Nicolet Nexus-470 FT-IR spectrometer as KBr disks. UV spectra were acquired on a Varian Cary 300 UV–Vis spectrophotometer in MeOH solution. Mass spectra were recorded on a Finnigan TRACE 2000 mass spectrometer (for EI-MS) and a Bruker DALTONICS APEX IV Fourier transform ICR high-resolution mass spectrometer (7.0 T, for HR-ESI-MS). 1D and 2D NMR spectra were run on a Bruker AV 400 spectrometer (400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) with TMS as an internal standard. A semi-preparative reversed-phase HPLC was carried out using a HPLC system including a LC P600 pump, a UV600 UV–Vis detector, a LabTech Chrom software (LabTech Co., Beijing, China), and a Phenomenex PRODIGY ODS column (250 mm × 21.2 mm i.d., 10 μm, Phenomenex, Inc., Torrance, CA, USA) equipped with a C_{18} guard column (8 mm × 4 mm i.d., 5 μm; Dikma, Beijing, China) cartridge system. Open CC separation was carried out using silica gel (200–300 mesh; Qingdao Marine Chemical Co., Qingdao, China). TLC was conducted on silica gel GF_{254} plates (Merck, Darmstadt, Germany).

3.2 Plant material

The dried and nearly ripe fruits of *E. rutaecarpa* (Juss.) Benth. were obtained in Xiangtan City, Hunan Province, China and authenticated by Prof. Xiu-Wei Yang, State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, China. The voucher specimen (19990901) was deposited in the Herbarium of School of Pharmaceutical Sciences, Peking University (Beijing, China).

3.3 Extraction and isolation

Powdered fruits of *E. rutaecarpa* (Juss.) Benth. (21 kg) were refluxed with 70% EtOH for seven times to afford an ethanolic extract. The extract was then suspended in water and partitioned with CHA, EtOAc, and BuOH to afford the corresponding extracts, 406.5 g (yield 1.93%), 532 g (2.53%), and 965 g (4.59%). The CHA extract (380 g) was subjected to silica gel CC, eluting with a gradient solvent system of CHA–$CHCl_3$ (100:0 → 0:100) to yield four fractions. Fraction 2 (43 g) was further subjected to silica gel CC, eluting with a solvent system of CHA–EtOAc (4:1) to give compounds **5** (3.3 g), **6** (120 mg), and **10** (1.2 g), and a subfraction (110 mg). The

subfraction was separated by semi-preparative reversed-phase HPLC using MeOH–H_2O (65:35) as a mobile phase to yield compounds **3** (12 mg) and **7** (11 mg). Fraction 3 (54.5 g) was purified by semi-preparative reversed-phase HPLC using MeOH–H_2O (60:40) as a mobile phase to afford compounds **8** (20 mg), **4** (6.8 mg), **1** (6.3 mg), and **2** (5.5 mg), and a subfraction (33.4 g). Fraction 4 was subjected to silica gel CC, eluting with a gradient solvent system of $CHCl_3$–MeOH (10:1 → 1:1) to give compounds **9** (1.6 g) and **11** (330 mg).

3.3.1 *(7R,8S)-7-Hydroxy-8-methoxy-rutaecarpine (**1**)*

A white amorphous powder; $[\alpha]_{20}^{D}$ −89.7 ($c = 0.35$, MeOH); UV λ_{MeOH} nm (log ε): 212 (4.39), 234 (4.31), 240 (4.26), 330 (4.27), 345 (4.31), 362 (4.14); IR (KBr) ν_{max} (cm^{-1}): 3345, 1641, 1618, 1598, 1554, 1502, 1468, 1329, 1268, 849, 788, 767; ^1H and ^{13}C NMR spectral data (Tables 1 and 2); EI-MS *m/z* 333 $[M]^+$ (7.2), 315 $[M–H_2O]^+$ (9.3), 304 (21.1), 301 (22.3), 285 (26.8), 272 (100); HR-ESI-MS *m/z* 334.1184 $[M + H]^+$ (calcd for $C_{19}H_{15}N_3O_3$, 334.1186).

3.3.2 *(7R,8S)-7-Hydroxy-8-ethoxy-rutaecarpine (**2**)*

A white amorphous powder; $[\alpha]_{20}^{D}$ −52.8 ($c = 0.14$, MeOH); UV λ_{MeOH} nm (log ε): 214 (4.35), 232 (4.25), 240 (4.22), 331 (4.25), 346 (4.32), 362 (4.15); IR (KBr) ν_{max} (cm^{-1}): 3271, 1643, 1622, 1597, 1466, 1329, 1220, 1196, 1057, 736; ^1H and ^{13}C NMR spectral data (Tables 1 and 2); EI-MS *m/z* 347 $[M]^+$ (4.8), 318 $[M–CH_3CH_2]^+$ (24.1), 301 $[M–CH_3CH_2OH]^+$ (17.8), 285 (15.0), 272 (100); HR-ESI-MS *m/z* 348.1342 $[M + H]^+$ (calcd for $C_{20}H_{17}N_3O_3$, 348.1343).

3.4 *Determination of cytotoxic activities*

Human promyelocytic leukemia cell line HL-60 and human gastric carcinoma cell line N-87 were used. Both cells were cultured in RPMI-1640 medium (GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (GIBCO) in 5% CO_2 at 37°C. The MTT method [19] was used to assay cytotoxicity in 96-well microplates. Briefly, 90 μl of the cell suspension was seeded into each well with an initial density of 5×10^4 cells/ml (HL-60) and 2×10^5 cells/ml (N-87), respectively. Each tumor cell line was cultured for 24 h before being exposed to each test compound at concentrations of 0.1, 1, 10, 20, and 40 μM in triplicate for 48 h, with Vincristine (VCR) used as a positive control. The absorbance was detected by a Microplate Reader at 490 nm, and IC_{50} values were calculated by the software origin 7.5.

Acknowledgements

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