Evollionines A – C, Three New Alkaloids Isolated from the Fruits of *Evodia rutaecarpa*

by **Yan-Hong Li**[a])[b]), **Juan He**[a]), **Yan Li**[a]), **Xing-De Wu**[a]), **Li-Yan Peng**[a]), **Ru-Nan Du**[a]), **Xiao Cheng**[a]), **Qin-Shi Zhao***[a]), and **Rong-Tao Li***[b])

[a]) State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, P. R. China (phone: +86-871-65223058; fax: +86-871-65215783; e-mail: qinshizhao@mail.kib.ac.cn)

[b]) Faculty of Life Science and Technology, Kunming University of Science and Technology (Chenggong Campus), Kunming 650500, P. R. China (phone: +86-871-65920569; fax: +86-871-65920570; e-mail: rongtaolikm@gmail.com)

A phytochemical study of the $CHCl_3$ extract of the dried unripe fruits of *Evodia rutaecarpa* (JUSS.) BENTH. (Rutaceae) resulted in the isolation of three new alkaloids, evollionines A – C (**1** – **3**, resp.), together with two known compounds, evodianinine and wuzhuyumide I. The structures of the new compounds were elucidated on the basis of detailed spectroscopic evidence and confirmed in the case of compound **1** by single-crystal X-ray analysis.

Introduction. – *Evodia rutaecarpa* (JUSS.) BENTH. is a small tree belonging to the family Rutaceae [1]. The dried and nearly ripe fruits (*Evodiae fructus*) are used as a traditional Chinese medicine for the treatment for headache, abdominal pain, migraines, chill limbs, postpartum hemorrhage, dysmenorrhea, diarrhea, nausea, and hypertension [2]. A number of limonins and quinolone alkaloids have been reported from *Evodiae fructus*; some of them exhibited significant cytotoxic activities in *in vitro* assays [3][4]. Evodiamine, a quinazolinocarboline alkaloid, the major component isolated, has been shown to possess various biological effects, such as testosterone secretion [5], catecholamine secretion [6], antinociceptive [7], anti-inflammatory [8], anti-obesity [9], vasodilatory [10], thermoregulatory [11] and uterotonic activities [12]. As part of our systematic search for bioactive constituents of Chinese herbal medicines, *Evodiae fructus* was investigated, leading to the isolation of three new alkaloids, evollionines A – C (**1** – **3**, resp.; *Fig. 1*), along with two known compounds, evodianinine [2] and wuzhuyumide I [13]. All compounds were evaluated for their cyctotoxic activities, and none of them proved active. Herein, we report the isolation and structure elucidation of these isolates.

Results and Discussion. – Compound **1** was obtained as colorless square crystals ($CHCl_3$/MeOH). Its molecular formula, $C_{19}H_{15}N_3O_2$, was deduced from the HR-EI-MS (*m*/*z* 317.1162 (M^+, calc. 317.1164)), corresponding to 15 degrees of unsaturation. The IR spectrum of **1** revealed the presence of a conjugated COOH function (1624 cm^{-1}) and aryl groups (1596 and 1443 cm^{-1}). The UV absorptions at 219 (4.28), 246 (4.36), 297 (3.81), and 346 (3.77) nm indicated an indole derivative with extended

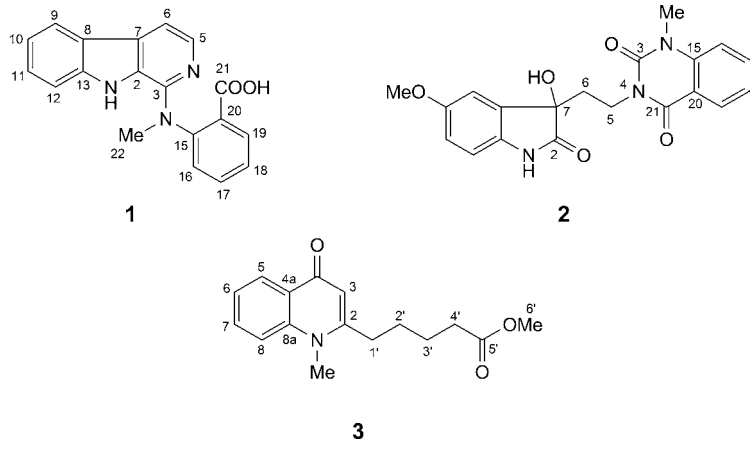

Fig. 1. *Compounds* **1**–**3**, *isolated from* Evodia rutaecarpa

conjugation [14]. The ^{1}H-NMR spectrum of **1** (*Table 1*) displayed a spin system (δ(H) 6.79 (*d*, *J* = 7.2, H–C(16)), 7.12 (*ddd*, *J* = 7.5, 7.2, 1.5, H–C(17)), 7.22 (*t*, *J* = 7.5, H–C(18)), and 7.53 (*dd*, *J* = 7.5, 1.5, H–C(19))), signals of a pair of aromatic H-atoms (δ(H) 7.99 (*d*, *J* = 5.6) and 7.65 (*d*, *J* = 5.6)) and of a MeN group (δ(H) 3.44). The ^{13}C NMR spectrum of **1** (*Table 1*) showed 19 C-atom signals, including those of one C=O group (δ(C) 178.1) and one Me group (δ(C) 41.9). Correlations in the ^{1}H,^{1}H-COSY and HSQC spectra (*Fig. 2*) revealed the presence of three different H-bearing structural fragments: **a** (CHCH), **b** (CHCHCHCH), and **c** (CHCHCHCH). Extensive evaluation of the HMBCs (*Fig. 2*) suggested **1** to be an evocarpine-type alkaloid, similar to evodianinine [2]; the only difference was that the amide bond in evodianinine was cleaved in **1** as deduced from the ^{13}C-NMR data and verified by the mass spectrum. Therefore, the structure of **1** was established as depicted in *Fig. 1*, and further confirmed by a single-crystal X-ray diffraction study of its *N*,*N*-diethylammonium salt (*Fig. 3*).

Compound **2**, isolated as white powder, had the molecular formula $C_{20}H_{19}N_3O_5$ as established by HR-EI-MS (*m/z* 381.1327 (calc. 381.1325)). The absorption at 3432 (OH), 1699 and 1656 cm^{-1} (C=O) in IR, and at 222, 246, 312 nm in UV spectra, together with a NH signal (δ(H) 11.50) in the ^{1}H-NMR spectrum (*Table 1*) implied the presence of a dihydroindolone derivative. In addition, signals of two CH_2 groups (δ(H) 4.87 (*td*, *J* = 9.7, 8.8), 4.72 (*d*, *J* = 10.0), 2.98 (*td*, *J* = 10.0, 8.8), and 2.86 (*d*, *J* = 9.7)), one MeN group (δ(H) 3.38), one MeO group (δ(H) 3.62), and seven aromatic H-atoms (δ(H) 6.82–8.27) were observed. The ^{13}C-NMR (DEPT) spectrum (*Table 1*) showed 20 C-atom signals of the indolequinazoline alkaloid skeleton in accordance with those of wuzhuyumide I [13], except for the presence of a MeO group at C(10), as supported by the HMBCs from MeO–C(10) to C(10). Compound **2** was optically inactive ($[\alpha]_D^{21.5} \approx 0$ (*c* = 0.8, pyridine)), indicating that it was a racemate. Thus, the structure of **2** was elucidated as shown in *Fig. 1*.

Compound **3** was obtained as white powder. Its molecular formula, $C_{16}H_{17}NO_3$, was deduced from the HR-EI-MS (*m/z* 271.1209 (M^+)), indicating eight degrees of

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.) *of* **1**[a]) *and* **2**[b]). δ in ppm, J in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	–	11.50 (*s*)	–
2	–	129.8 (*s*)	–	180.8 (*s*)
3	–	149.2 (*s*)	–	150.8 (*s*)
5	7.99 (*d*, *J* = 5.6)	136.2 (*d*)	4.87 (*td*, *J* = 9.7, 8.8) 4.72 (*d*, *J* = 10.0)	37.6 (*t*)
6	7.65 (*d*, *J* = 5.6)	110.5 (*d*)	2.98 (*td*, *J* = 10.0, 8.8) 2.86 (*d*, *J* = 9.7)	36.3 (*t*)
7	–	130.4 (*s*)	–	76.3 (*s*)
8	–	122.9 (*s*)	–	134.5 (*s*)
9	8.01 (*d*, *J* = 8.0)	122.0 (*d*)	7.51 (*d*, *J* = 1.9)	111.6 (*d*)
10	7.11 – 7.16 (*m*)	120.4 (*d*)	–	156.1 (*s*)
11	7.34 – 7.39 (*m*, overlapped)	128.4 (*d*)	6.82 (*d*, *J* = 8.4, 1.9)	114.4 (*d*)
12	7.34 – 7.39 (*m*, overlapped)	113.0 (*d*)	6.89 (*d*, *J* = 8.4)	110.7 (*d*)
13	–	141.9 (*s*)	–	136.2 (*s*)
15	–	145.7 (*s*)	–	140.9 (*s*)
16	6.79 (*d*, *J* = 7.2)	127.5 (*d*)	7.07 (*d*, *J* = 8.4)	114.2 (*d*)
17	7.12 (*ddd*, *J* = 7.5, 7.2, 1.5)	129.9 (*d*)	7.52 – 7.57 (*m*)	135.3 (*d*)
18	7.22 (*t*, *J* = 7.5)	127.3 (*d*)	7.14 (*t*, *J* = 7.8)	122.7 (*d*)
19	7.53 (*dd*, *J* = 7.5, 1.5)	128.5 (*d*)	8.27 (*d*, *J* = 7.8, 1.6)	128.6 (*d*)
20	–	141.4 (*s*)	–	115.9 (*s*)
21	–	178.1 (*s*)	–	161.7 (*s*)
22	3.44 (*s*)	41.9 (*q*)	3.38 (*s*)	30.5 (*q*)
MeO	–	–	3.62 (*s*)	55.6 (*q*)

[a]) Recorded in CD_3OD. [b]) Recorded in C_5D_5N.

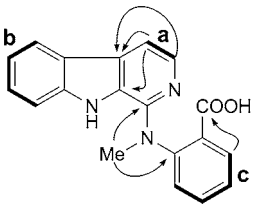

Fig. 2. *Key 2D-NMR correlations of compound* **1**

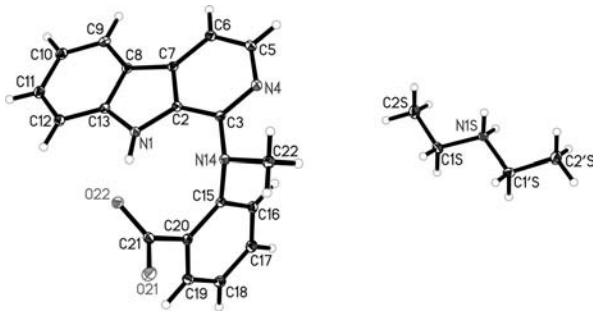

Fig. 3. *X-Ray crystal structure of the* N,N*-diethylammonium salt of* **1**

unsaturation. The UV spectrum of **3** displayed absorptions at λ_{max} 333, 322, 240, 213 nm, suggesting the presence of a *N*-methyl-quinolin-4(1*H*)-one skeleton [15]. The ^{1}H- and ^{13}C-NMR spectra of **3** (*Table 2*) displayed signals for one MeN group (δ(H) 3.83; δ(C) 34.6), one MeO group (δ(H) 3.66; δ(C) 51.7), one conjugated olefinic group (δ(H) 6.53; δ(C) 110.5 (C(3))), one conjugated CO group (δ(C) 176.6 (C(4))), four resonances in the aromatic region (δ(H) 8.40 (*dd*, *J* = 8.2, 1.5; δ(C) 126.4); 7.42 (*t*, *J* = 7.5; δ(C) 120.4); 7.72 (*ddd*, *J* = 8.7, 7.2, 1.5; δ(C) 132.6), and 7.59 (*d*, *J* = 8.7; δ(C) 115.6), as well as the signals at δ(C) 155.1 (C(2)), 125.6 (C(4a)), and 141.7 (C(8a)), suggesting the presence of an *ortho*-disubstitued benzene moiety that corresponds to an *N*-methyl-quinolin-4(1*H*)-one skeleton [16]. The side chain at C(2) was determined as $(CH_2)_4$COOMe according to ^{1}H,^{1}H-COSY correlations and HMBCs. So, the structure of **3** was elucidated as methyl 5-(1,4-dihydro-1-methyl-4-oxoquinolin-2-yl)pentanoate, named evollionine C.

All compounds isolated from *E. rutaecarpa* were tested for their cytotoxic activities *in vitro* on human tumor cell lines by using the MTS (3-(4,5-dimethylthiazol-2-yl)-5-[3-(methoxycarbonyl)phenyl]-2-(4-sulfophenyl-2*H*-tetrazolium inner salt) method. However, none of them exhibited any activity.

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 100 MHz, resp.; $CDCl_3$) *of* **3**. δ in ppm, *J* in Hz.

Position	**3**		Position	**3**	
	δ(H)	δ(C)		δ(H)	δ(C)
2	–	155.1 (*s*)	2′	1.76 – 1.78 (*m*)	27.8 (*t*)
3	6.53 (*s*)	110.5 (*d*)	3′	1.71 – 1.75 (*m*)	24.4 (*t*)
4	–	176.6 (*s*)	4′	2.38 (*t*, *J* = 6.7)	33.4 (*t*)
5	8.40 (*dd*, *J* = 8.2, 1.5)	126.4 (*d*)	5′	–	173.5 (*s*)
6	7.42 (*t*, *J* = 7.5)	124.0 (*d*)	4a	–	125.6 (*s*)
7	7.72 (*ddd*, *J* = 8.7, 7.2, 1.5)	132.6 (*d*)	8a	–	141.7 (*s*)
8	7.59 (*d*, *J* = 8.7)	115.6 (*d*)	MeN	3.83 (*s*)	34.6 (*q*)
1′	2.81 (*t*, *J* = 6.8)	34.6 (*t*)	MeO	3.66 (*s*)	51.7 (*q*)

This work was financially supported by the *National Natural Science Foundation of China* (No. 90813004 and No. 2009312311024) and the *973 Program* (No. 2009CB522300).

Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200 – 300 mesh, *Qingdao Marine Chemical Co., Ltd.*, P. R. China); *MCI* gel *CHP-20P* (75 – 150 μm, *Mitsubishi Chemical Corporation*, Japan); and prep. *SB-C18 HPLC* column (5 μm, 21.2 × 150 mm, *Agilent*, America). TLC: SiO_2 Plates *GF$_{254}$* (*Qingdao Marine Chemical Inc.*, P. R. China). M.p.: *X-4* micro melting-point apparatus. Optical rotations: *JASCO-20C* digital polarimeter. UV Spectra: *UV-2401A* spectrophotometer (*Shimadzu*). IR Spectra: *Tensor-27* spectrophotometer; KBr pellets; $\tilde{\nu}_{max}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm rel. to Me_4Si as internal standard; *J* in Hz. ESI- and HR-EI-MS: *VG Auto Spec-3000* instruments with glycerol as matrix for FAB-MS; *API-QSTAR-Pulsar-1* spectrometer; in *m/z*. X-Ray diffraction: *Bruker APEX DUO* diffractometer, with graphite-monochromated MoK_α *radiation.*

Plant Material. The dried and nearly ripe fruits of *E. rutaecarpa* were purchased from the Kunming Ju-Hua village pharmaceutical sale market, Yunnan province, P. R. China. The plant was identified by Prof. *Xiao Cheng* (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen

(201209R) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dried and nearly ripe fruits of *E. rutaecarpa* (20 kg) were extracted with MeOH (3×10 l) at r.t. for 24 h each time. The MeOH extracts were evaporated under reduced pressure. The residue was dissolved in 10% HCl, acidified to pH 1–2, and then partitioned with AcOEt (3×4 l). The acidic soln. was basified using 10% NH_3 to pH 9–10, followed by exhaustive extraction with $CHCl_3$ (3×4 l), to afford a $CHCl_3$ extract (36 g). The $CHCl_3$ extract was subjected to MPLC (*MCI* (500 g, 25 × 4 cm); MeOH/H_2O 40:60 → 100:0) to give *Frs. I–IV*. From *Fr. II* (10 g), wuzhuyumide I (630 mg) was precipitated and crystallized from MeOH. After repeated CC (SiO_2 (250 g, 50×3 cm); $CHCl_3$/acetone 9:1), the impure part of *Fr. II* was further purified by HPLC (*SB-C*$_{18}$ (5 μm, 21.2×150 mm); 40% MeOH/H_2O) to furnish **2** (8 mg) and evodianinine (10 mg). *Fr. III* (12 g) was subjected to repeated CC (SiO_2 (350 g, 60×5 cm); $CHCl_3$/MeOH/Et_2NH 8.8:0.2:1), and recrystallization yielded compound **1** (348 mg). Compound **3** (7.7 mg) was separated by CC (SiO_2 (100 g, 40×2.5 cm), $CHCl_3$/MeOH 9.8:0.2) from the residual part of *Fr. III*.

Evollionine A (*=2-[9*H*-β-Carbolin-1-yl(methyl)amino]benzoic Acid*; **1**). Colorless square crystals. M.p. 167.9–169.2°. $[\alpha]_D^{20.8} = -6.4$ ($c = 0.12$, MeOH). UV (MeOH): 346 (3.77), 297 (3.81), 246 (4.36), 219 (4.28). IR (KBr): 3448, 2989, 2958, 2863, 2793, 2719, 2489, 1624, 1596, 1443. ^{1}H- and ^{13}C-NMR (CD_3OD): *Table 1*. ESI-MS: 318 ($[M+H]^+$). HR-EI-MS: 317.1162 (M^+, $C_{19}H_{15}N_3O_2^+$; calc. 317.1164).

Crystal Data of Evollionine A (**1**). Empirical formula: $C_{19}H_{14}N_3O_2 \cdot C_4H_{12}N$; M_r 390.48; Crystal system, monoclinic; space group, $P_{2(1)}/n$; unit cell parameters, $a = 10.8930(12)$ Å, $b = 15.3389(16)$ Å, $c = 12.8358(14)$ Å, $\alpha = 90.00°$, $\beta = 103.772(2)°$, $\gamma = 90.00°$, $V = 2083.0(4)$ Å^3, $T = 100(2)$ K, $Z = 4$, $\mu(\text{Mo}K_\alpha) = 0.081$ mm^{-1}. A colorless square crystal of dimension $1.03 \times 0.37 \times 0.35$ mm was selected for X-ray analysis. A total of 21376 reflections, collected in the range $2.11° \leq \theta \leq 30.43°$, yielded 5830 independent reflections ($R_{int} = 0.0261$). The final R_1 values were 0.0492 (all data). The final wR (F^2) values were 0.1130 (all data). Crystallographic data for the structure of **1** have been deposited at the *Cambridge Crystallographic Data Centre* (deposition number CCDC-936217). Copies of the data can be obtained free of charge *via* www.ccdc.cam.ac.uk.

Evollionine B (*= 3-[2-(2,3-Dihydro-3-hydroxy-5-methoxy-2-oxo-1*H*-indol-3-yl)ethyl]-1-methylquinazoline-2,4(1*H*,3*H*)-dione*; **2**). White powder. $[\alpha]_D^{21.5} \approx 0$ ($c = 0.08$, pyridine). UV (MeOH): 312 (3.34), 246 (3.67), 222 (4.21), 196 (3.76). IR (KBr): 3432, 1699, 1656, 1613, 1488. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): see *Table 1*. ESI-MS: 404 ($[M+Na]^+$). HR-EI-MS: 381.1327 (M^+, $C_{20}H_{19}N_3O_5^+$; calc. 381.1325).

Evollionine C (*= Methyl 5-(1,4-Dihydro-1-methyl-4-oxoquinolin-2-yl)pentanoate*; **3**). White powder. $[\alpha]_D^{20.8} = -5.26$ ($c = 0.09$, MeOH). UV ($CHCl_3$): 333 (3.98), 322 (3.98), 240 (4.29), 213 (4.22), 197 (3.98). IR (KBr): 3425, 2948, 2934, 1743, 1633, 1597, 1568. ^{1}H- and ^{13}C-NMR (CD_3Cl): see *Table 2*. ESI-MS: 294 ($[M+Na]^+$). HR-EI-MS: 271.1209 (M^+, $C_{16}H_{17}NO_3^+$; calc. 271.1208).

REFERENCES

[1] Y.-X. Wang, M.-X. Gong, Z.-M. Wang, Q.-W. Zhang, H.-M. Gao, Y.-F. Song, *Chin. Pharm. J.* **2010**, *45*, 641.

[2] Q.-Z. Wang, J.-Y. Liang, *Acta Pharm. Sin.* **2004**, *39*, 605.

[3] H. Matsuda, M. Yoshikawa, M. linuma, M. Kubo, *Planta Med.* **1998**, *64*, 339.

[4] X. H. Han, S. S. Hong, D. Lee, J. J. Lee, M. S. Lee, D.-C. Moon, K. Han, K.-W. Oh, M. K. Lee, J. S. Ro, B. Y. Hwang, *Arch. Pharm. Res.* **2007**, *30*, 397.

[5] H. Lin, S.-C. Tsai, J.-J. Chen, Y.-C. Chiao, S.-W. Wang, G.-J. Wang, C.-F. Chen, P. S. Wang, *Metabolism* **1999**, *48*, 1532.

[6] M. Yoshizumi, H. Houchi, Y. Ishimura, M. Hirose, T. Kitagawa, K. Tsuchiya, K. Minakuchi, T. Tamaki, *J. Med. Invest.* **1997**, *44*, 79.

[7] Y. Kobayashi, *Planta Med.* **2003**, *69*, 425.

[8] Y. H. Choi, E. M. Shin, Y. S. Kim, X. F. Cai, J. J. Lee, H. P. Kim, *Arch. Pharm. Res.* **2006**, *29*, 293.

[9] Y. Kobayashi, Y. Nakano, M. Kizaki, K. Hoshikuma, Y. Yokoo, T. Kamiya, *Planta Med.* **2001**, *67*, 628.

[10] W.-F. Chiou, C.-J. Chou, A. Y.-C. Shum, C.-F. Chen, *Eur. J. Pharmacol.* **1992**, *215*, 277
[11] T. H. Tsai, T. F. Lee, C. F. Chen, L. C. H. Wang, *Pharmacol. Biochem. Behav.* **1995**, *50*, 293.
[12] C. L. King, Y. C. Kong, N. S. Wong, H. W. Yeung, H. H. Fong, U. Sankawa, *J. Nat. Prod.* **1980**, *43*, 577.
[13] G. Y. Zuo, X. S. Yang, X. J. Hao, *Chin. Chem. Lett.* **2000**, *11*, 127.
[14] Q. Z. Wang, J. Y. Liang, X. Feng. *Chin. Chem. Lett.* **2010**, *21*, 596.
[15] L. Huang, D.-Q. Yu, 'Application of Ultraviolet Spectrum in Organic Chemistry', Science Press, Beijing, 1988.
[16] X. Huang, W. Li, X.-W. Yang, *Fitoterapia* **2012**, *83*, 709.

Received December 19, 2013