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TWO NEW INDOLOQUINAZOLINE ALKALOIDS FROM THE UNRIPE FRUITS OF *EVODIA RUTAECARPA*

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Abstract – Two new indoloquinazoline alkaloids, wuzhuyurutine A and wuzhuyurutine B, together with three known indoloquinazoline alkaloids, were isolated from the unripe fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae). Their structures were elucidated by the analysis of their NMR, MS, IR, UV spectra.

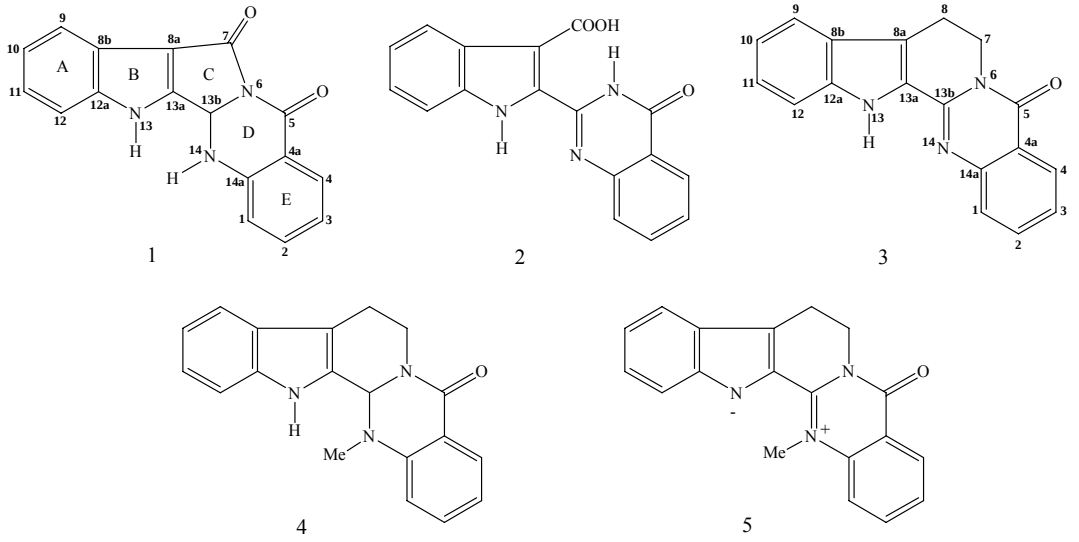
INTRODUCTION

The dried unripe fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), popularly known in China as Wu-zhu-yu, have been prescribed for the treatment of gastrointestinal disorders, headache, abdominal pain, dysentery and postpartum haemorrhage.[1] It contains alkaloids and limonoids as its main components.[2-5] A number of pharmacological effects have been attributed to the alkaloids related to body-temperature maintaining effects,[6] cardioprotection,[7] nociceptive and anti-nociceptive action.[8] In the present study, we isolated two new indoloquinazoline alkaloids (compounds **1** and **2**), together with three known compounds rutaecarpine (**3**), evodiamine (**4**) and dehydroevodiamine (**5**).[9] This paper describes the isolation and structural elucidation of two new indoloquinazoline alkaloids in detail.

RESULTS AND DISCUSSION

The 95% aqueous ethanolic extract of the unripe fruits of *E. rutaecarpa* were acidified by HCl after suspended in water and then extracted with cyclohexane and EtOAc to obtain cyclohexane extracts, and EtOAc extracts I and residual water layer I. The water layer I was basified by aqueous ammonia and then was successively partitioned with EtOAc to give EtOAc extracts II. The EtOAc extract II was applied on column chromatography on silica gel, and then purified further. Two new indoloquinazoline alkaloids, wuzhuyurutine A (**1**) and wuzhuyurutine B (**2**) were obtained.

Figure 1. Chemical structures of indoloquinazoline alkaloids from *E. rutaecarpa*



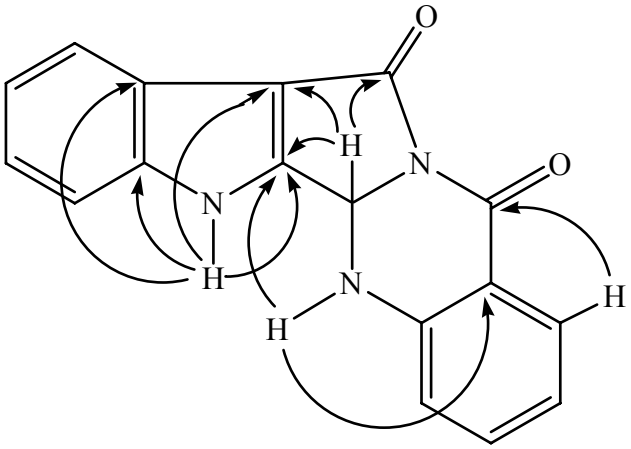
Compound (**1**) was isolated as an amorphous solid. Its molecular formula was deduced to be $C_{17}H_{11}N_3O_2$ by HR-SI-MS (*m/z* = 290.0926 $[M+H]^+$) and ^{13}C-NMR data (Table **1**). It had 14 degrees of unsaturation. The EI-MS showed a molecular ion at m/z 289 (100) and a [M-CO] fragment ion at m/z 261 (88). The IR spectrum displayed characteristic absorption for *N*-H (3202cm^{-1}), carbonyl groups (1676cm^{-1}) and aromatic rings (1604, 1530, 1480, 1448 cm^{-1}). The UV spectrum showed absorption maxima at 223, 239, 264, and 323 nm due to an indole chromophore, which was characteristic diagnostics of unsubstituted moiety in aromatic ring of indole ring.[10] In its ^{1}H NMR spectrum, eleven proton signals including eight aromatic singlets (δ 7.24 ~ 7.96), one methine singlet signal [δ 6.60 (1H, s)] and two *N*-H protons [δ 9.27 (1H, s), 11.87 (1H, s)] were observed. Analysis of the ^{1}H-^{1}H COSY spectrum of **1** indicated that signals at δ 7.24, 7.65, 7.91 and 7.96 formed the first aromatic spin system and signals at δ 7.26, 7.32, 7.69 and 7.77 made up the second aromatic spin system. The nine carbon signals bearing protons were easily determined by the HSQC experiment, which establishes one bond correlation. The ^{1}H and ^{13}C NMR spectra displayed signals for two spin systems for two 1,2-disubstitued aromatic rings, two *N*-H and two amide carbonyls (δ 162.3 and 163.7). Considering the structure of alkaloids isolated from the unripe fruits of *E. rutaecarpa*, 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 7.91 and an amide carbonyl carbon at δ 163.7 indicated the quinazoline type substructure included the first aromatic spin system. In the HMBC spectrum (Figure. **2**) of **1**, ^{1}H-^{13}C long range correlations were observed between the *N*-H (δ 11.87) and four quaternary carbons (δ 111.4, 120.7, 141.0 and 150.4). It suggested that the second aromatic spin system belonged to the indole type substructure with 6 degrees of unsaturation. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of evodiamine (**4**) [9] suggested that compound (**1**) is similar to **4** except for H-14*N* and the C ring (Figure.**1**). In the HMBC of **1**, stronger correlation between the

proton resonating at δ 9.27 (H-14*N*) and two quaternary carbons at δ 119.0 (C-4a) and δ 150.4 (C-13a) indicated that methyl group at *N*-14 (present in **4**) was substituted by a proton (present in **1**). The quinazoline type substructure with 6 degrees of unsaturation was easily deduced. Further analysis of the HMBC of **1, t**he characteristic of the methine singlet signal at δ 6.60 coupling with the other amide carbonyl at δ 162.3 and two quaternary carbons, C-13a (δ 150.4) and C-8a (δ 111.4), suggested that the unit (-CH_2-CH_2-) between *N*-6 and C-8a (present in **4**) was replaced by an amide carbonyl group (present in **1**). This was a closed C ring with 2 degrees of unsaturation. The above conclusions were further supported by the observation that EI-MS showed fragments for loss of a carbonyl from the molecular ion at *m/z* 261. Consequently, complete assignments of proton and carbon signals of compound (**1**) were performed by ^{1}H-^{1}H COSY, HSQC, and HMBC experiments. It is a novel compound, and has been given the trivial name wuzhuyurutine A.

Table 1. ^{1}H NMR and ^{13}C NMR spectra data in DMSO-d_6 at 500/125 MHz for compounds (1) and (2)

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	119.5	7.96 (d, 8.0)	126.8	7.76 (d, 7.0)
2	133.4	7.65 (ddd, 1.0, 7.0, 8.0)	133.8	7.79 (brd, 7.0)
3	123.3	7.24 (ddd, 1.0, 7.0, 8.0)	125.6	7.45 (brd, 7.0)
4	128.1	7.91 (dd, 1.0, 8.0)	126.0	8.13 (d, 7.0)
4a	119.0		121.9	
5	163.7		162.6	
7	162.3		169.7	
8a	111.4		114.7	
8b	120.7		129.2	
9	119.3	7.77 (d, 7.5)	123.8	8.53 (d, 7.5)
10	121.9	7.26 (ddd, 1.0, 7.0, 8.0)	120.5	7.11 (t, 8.0)
11	123.3	7.32 (ddd, 1.0, 7.0, 8.0)	123.9	7.23 (t, 8.0)
12	113.9	7.69 (d, 7.5)	112.2	7.58 (d, 7.5)
12a	141.0		135.7	
13		11.87 (s)		11.96 (s)
13a	150.4		131.5	
13b	63.2	6.60 (s)	149.5	
14		9.27 (s)		
14a	138.6		149.2	

Figure. 2 The key HMBC correlations for 1



Compound (**2**) was obtained as a colorless amorphous solid; R_f 0.40, silica gel F_{254} TLC plate, $CHCl_3$/MeOH (7:3). This appeared as bright yellow spot on a dark blue background on the TLC plate after using bromocresol green stain (To 100 mL absolute ethanol is added 0.04 g of bromocresol green, and then a 0.1M solution of aqueous NaOH is added dropwise until a blue color just appears in solution), [11] that imply **2** may have carboxyl group. Its molecular formula was established as $C_{17}H_{11}N_3O_3$ by a combination of HR-SI-MS and ^{13}C NMR data (Table **1**), with 14 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3428 cm^{-1}), *N*-H (3384 cm^{-1}), carboxyl (1750 cm^{-1}) and carbonyl (1650 cm^{-1}) groups. The UV spectrum showed absorption maxima at 220, 240, 347, and 364 nm due to an indole chromophore, which was characteristic diagnostics of unsubstituted moiety in aromatic ring of indole ring.[10] The ^1H and ^{13}C NMR spectra displayed signals for two spin systems for 1,2-disubstitued aromatic rings, one *N*-H (δ 11.96 in the ^1H NMR), one amide carbonyl (δ 162.6 in the ^{13}C NMR) and one carboxyl carbon signal (δ 169.7 in the ^{13}C NMR). Based on the analysis of its IR, ^1H and ^{13}C NMR spectra and comparison with NMR data of compound (**1**) and rutaecarpine (**3**), [9] compound (**2**) was recognized as an indoloquinazoline alkaloid. Further analysis of the HMBC correlation between the aromatic proton at δ 8.13 (H-C_4) and the amide carbonyl carbon at δ 162.6 (C-5) indicated the quinazoline type substructure included one of the aromatic spin systems. In the HMBC spectrum of **2**, ^1H-^{13}C long range correlations were observed between the *N*-H (δ 11.96) to two quaternary carbons (δ 114.7, C-8a, and δ 129.2, C-8b). It suggested that the other aromatic spin system belonged to the indole type substructure. So, compound (**2**) was shown to be an analogue of rutaecarpine and bouchardatine [2-(2-[3-formylindolyl])-(3H)-quinazolin-4-one]. [12] The fact that the high field region of ^1H NMR spectra of **2** did not show two methylene groups (present in **3**) indicated that **2** was similar to **3** except for C ring (Fig.**1**). The ^{13}C NMR spectra of **2** exhibited the carboxyl carbon singlet at δ_C 169.7. Thus **2** was similar to bouchardatine which has not a closed C ring and its NMR data were in agreement with the reported data for bouchardatine nearly except the aldehyde. The ESI-TOF-MS (positive mode) of **2** showed an

$[M\text{-}OH]^+$ ion peak at *m/z* 288, and the EI-MS showed fragment peak at *m/z* 261 (100) for loss of a $[\text{-}COO+H]^+$ fragment from the mother molecular ion. The above data suggested that the aldehyde linking at C-8a (present in bouchardatine) was replaced by one carboxylic acid group (present in **2**). Compound **2** is probably a catabolic product of rutaecarpine (**3**) derived through oxidative fission of the 7/8 bond in 7,8-dehydrorutaecarpine. On this basis structure (**2**) can be assigned. It is a new compound, and named wuzhuyurutine B.

Along with the new compound, three know indoloquinazoline alkaloids were also isolated from the fruits of *E. rutaecarpa*. By direct comparison their R_f of TLC, ^1H and ^{13}C NMR with the corresponding authentic samples that were obtained before in our lab, [9] respectively, they were identified as rutaecarpine (**3**), evodiamine (**4**) and dehydroevodiamine (**5)**.

All of the compounds isolated from *E. rutaecarpa* are typical constituents of the genus *Evodia*. It is interesting to note that indoloquinazoline alkaloids only occur in rutaceous plants.[13] Previous reports of indoloquinazoline alkaloids are restricted to the Rutaceae and include the genera *Araliopsis*, *Euxylophora*, *Hortia*, *Phellodendron*, *Teradium* (*Evodia*), *Vepris*, *Zanthoxylum*, *Leptothyrsa* and *Bouchadatia*.[12-15] Indoloquinazoline alkaloids particular converging on Rutaceae should be of significance regarding the chemotaxonomy, although they are not the dominant class of alkaloid in nature. *E. rutaecarpa* is a good natural sources of indoloquinazoline alkaloids. Recently, some novel indoloquinazoline derivatives were isolated unceasing from this plant.[16, 17]

EXPERIMENTAL

General Method

UV spectrum was recorded on a Varian Cary Eclipse 300 spectrophotometer using MeOH as the solvent. IR spectrum (KBr) was determined on a Thermo Nicolet Nexus 470 FT-IR spectrophotometer. EI-MS spectrum was performed on Finnigan TRACE MS spectrometer, ESI-TOF-MS was performed on MDS SCIEX API QSTAR mass spectrometer, and HR-SI-MS spectrum was performed on Bruker APEX II mass spectrometer. NMR spectra were performed on a Varian INOVA 500 spectrometer. All the NMR experiments were recorded at room temperature, operating at 499.89 MHz for ^1H and 125.71 MHz for ^{13}C in DMSO-d_6 at room temperature with tetramethylsilane (TMS) as internal standard. The chemical shift values (δ) were given in the parts per million (ppm), and the coupling constants were in hertz (Hz). Standard pulse sequences were used for HSQC, HMBC, and ^1H-^1H COSY experiments. Silica gel (200-300 mesh) used in column chromatography was provided by Tsingtao Marine Chemistry Co. Ltd. in Shandong Province, China.

Plant Material

The dried unripe fruits of *E. rutaecarpa* (Juss.) Benth. were obtained in Xiangtan City, Hunan Province of

China, in December 2000, and authenticated by Professor Xiu-wei Yang. The voucher specimen of this plant is deposited in the Herbarium of School of Pharmaceutical Sciences, Peking University.

Extraction and Isolation

The dried and powdered fruits of *E. rutaecarpa* (11 kg) were extracted with 95%EtOH (4.4 L) under reflux for 4 × 1 h to give ethanolic extracts 2.3 kg. The ethanolic extract (1.5 kg) was further suspended in water (1.5 L) and acidified then by HCl to pH 3, and extracted successively with cyclohexane (3.0 L × 4 times) and EtOAc (3.0 L × 4 times) to obtain cyclohexane extracts (150 g), EtOAc extracts I (150 g) and residual water layer I, respectively. The water layer I was basified by aqueous ammonia to pH 10, and further extracted with EtOAc (3.0 L × 4 times) to give EtOAc extracts II (217.2 g) and residual water layer II. The EtOAc extracts II was separated by silica gel column chromatography, eluted with cyclohexane – EtOAc (2:1, 1:1 and 0:1, 300 mL each) and EtOAc - MeOH (3:1, 1:1, and 0:1, 300 mL each) to afford eight major fractions (F1 – F8). Fraction F1 was applied to a silica gel column eluted with cyclohexane-EtOAc (12:1, 2:1, 1:1, and 0:1, 100 mL each) to yield compounds (**3**) (15.2 g) and (**4**) (1.1 g), respectively. Compound (**5**) (100 mg) was deposited from F7 by using MeOH. Fraction F4 (20.0 g) was subjected to silica gel column chromatography using a cyclohexane – EtOAc gradient system (3:1, 2:1, and 1:1, 800 mL each) following MeOH to give seven fractions (Fr. 4-I – Fr. 4-VII). Fr. 4-V (4.6 g) was separated by silica gel column chromatography using $CHCl_3$-MeOH gradient system (150:1, 99:1, 9:1, 7:3 and 3:2, 50 mL each) as elute to give 10 fractions (Fr. 4-V-1 – Fr. 4-V-10). Compound (**1**) (7 mg) was deposited from Fr. 4-V-5 (0.5 g) by using MeOH. Compound (**2**) (10 mg) was deposited from Fr. 4-VI (1.0 g) by using MeOH.

Wuzhuyurutine A (1). Colorless amorphous solid. UV λ_{max} (MeOH): 323, 264, 239, 223 nm. IR ν_{max} (KBr): 3202, 3104, 2925, 1676, 1604, 1530, 1480, 1448, 1380, 1350, 1244, 1157, 740 cm^{-1}. ^1H NMR, ^{13}C NMR, see Table **1**. EI-MS *m/z* (%): 289 (100), 261 (88), 119 (44). HR-SI-MS (positive) *m/z* 290.0926 $[M + H]^+$ (calcd. for $C_{17}H_{12}N_3O_2$, 290.0924).

Wuzhuyurutine B (2). Colorless amorphous solid. UV λ_{max} (MeOH): 364, 347, 240, 220 nm. IR ν_{max} (KBr): 3428, 3384, 3052, 2925, 2856, 1750, 1650, 1602, 1578, 1548, 1509, 1469, 1444, 1395, 1329, 1235 cm^{-1}. ^1H NMR and ^{13}C NMR, see Table **1**. EI-MS *m/z* (%): 261 (81), 119 (78). ESI-TOF-MS (positive) *m/z* 350 $[M+2Na-H]^+$, 328 $[M+Na]^+$, 306 $[M+H]^+$, 288 $[M-OH]^+$. HR-SI-MS (negative) *m/z* 304.0726 $[M-H]^-$ (calcd. for $C_{17}H_{10}N_3O_3$, 304.0728).

Rutaecarpine (3). White cylindrical crystals, mp 259-260 ºC (EtOAc) (lit.[18] 258-259ºC). UV λ_{max} (EtOH): 362, 345, 331, 289, 213 nm. IR ν_{max} (KBr): 3339, 1647, 1597, 1544, 1487, 1466, 1325, 1227, 766, 758 cm^{-1}. EI-MS *m/z* (%): 287 (100), 258 (6), 144 (19), 77 (4). NMR data were in agreement with

the reported data for rutaecarpine.[9]

Evodiamine (4). Pale yellow crystals, mp 269-272 ºC (EtOAc) (lit.,[18] 269-271ºC). UV λ_{max} (EtOH): 268, 225, 203 nm. IR ν_{max} (KBr): 3218, 2909, 2540, 1626, 1603, 1508, 1446, 746, 734 cm^{-1}. EI-MS *m/z* (%): 303 (100), 288 (10), 274 (11), 169 (68), 161 (23) 134 (76). NMR data were in agreement with the reported data for evodiamine.[9]

Dehydroevodiamine (5). Yellow crystals, mp 190-192 ºC (MeOH) (lit.,[19] 194-196ºC). UV λ_{max} (EtOH): 366, 314, 247, 227, 207 nm. IR ν_{max} (KBr): 3215, 1706, 1608, 1549, 1497, 1130, 790, 720 cm^{-1}. EI-MS *m/z* (%): 301 (57), 286 (100), 270 (14), 244 (12), 168 (15), 143 (13), 77 (12). NMR data were in agreement with the reported data for dehydroevodiamine.[9]

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