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A novel alkaloid from the fruits of *Evodia officinalis*

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Studies on the fruits of *Evodia officinalis* yielded a new quinazolinedione alkaloid, wuchuyuamide III (**1**), together with known alkaloids, goshuyuamide II (**2**), evodiamine and rutaecarpine. Their structures were elucidated by means of 1D and 2D NMR spectroscopic analysis. Wuchuyuamide III (**1**) and goshuyuamide II (**2**) showed modest cytotoxicity against HeLa and HT1080 cell lines.

Keywords: *Evodia officinalis*; Wuchuyuamide; Goshuyuamide; Cytotoxicity

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1. Introduction

The fruits of *Evodia officinalis* have long been used as a traditional Chinese drug (Chinese name "Wu-Zhu-Yu") in the treatment of headache, abdominal pain, dysentery, postpartum haemorrhage, and amenorrhea [1]. There were many previous studies on the Evodia fruit [2]. Indolopyridoquinazoline alkaloids such as evodiamine, rutaecarpine, and dehydroevodiamine, quinolone alkaloids, limonoids and flavonoids were isolated [3,4]. Further chemical studies of this plant led us to isolate one new alkaloid (**1**). Here we describe the isolation and structure elucidation of the novel alkaloid, named wuchuyuamide III (**1**), and the cytotoxic effect of this compound on HeLa and HT1080 cell lines.

2. Results and discussion

Wuchuyuamide III (**1**) has the molecular formula $C_{18}H_{17}N_3O_3$ (HRFAB-MS). The ^{1}H NMR and ^{13}C NMR spectra indicated the presence of the three signals in the aliphatic region, including a three-proton singlet at δ 3.53 and two two-proton triplets ($J = 7.8$ Hz, each) at δ 3.24 and δ 4.26, as well as the presence of a 1,2-disubstituted benzene ring [δ 6.72 (1H, dd, $J = 8.4, 1.2$ Hz, H-3′), 7.22 (1H, ddd, $J = 8.4, 8.1, 1.2$ Hz, H-4′), 6.50 (1H, ddd, $J = 8.4, 8.1,$ 1.2 Hz, H-5′), and 7.71 (1H, dd, $J = 8.4, 1.2$ Hz, H-6′); δ 116.19 (C-1′), 151.13 (C-2′), 116.96

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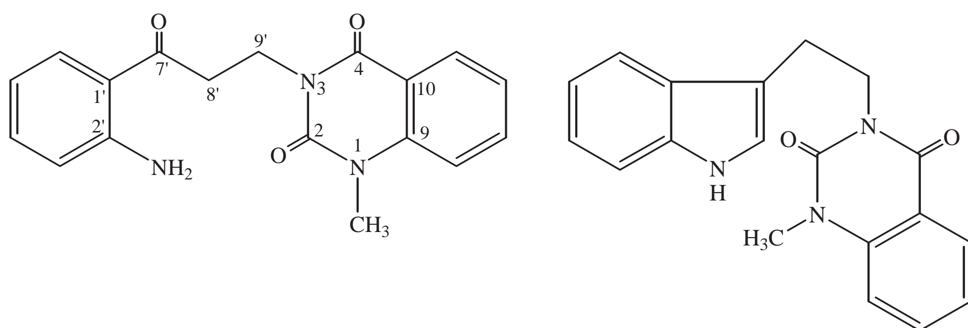

Figure 1. Structures of wuchuyuamide III (**1**) and goshuyuamide II (**2**).

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(C-3′), 134.23 (C-4′), 114.37 (C-5′), and 131.18 (C-6′)], and an 2,4-quinazolinedione group [δ 8.03 (1H, dd, $J = 7.8$, 1.5 Hz, H-5), 7.30 (1H, ddd, $J = 7.8$, 7.2, 1.5 Hz, H-6), 7.77 (1H, ddd, $J = 8.4$, 8.1, 1.5 Hz, H-7), and 7.41 (1H, dd, $J = 8.4$, 1.5 Hz, H-8); δ 127.70 (C-5), 122.70 (C-6), 135.26 (C-7), 114.53 (C-8), 140.34 (C-9), and 114.79 (C-10)] (figure 1 and table 1). HMQC analysis allowed the assignment of all protonated carbons observed in the ^{13}C NMR spectrum. The correlation of the three-proton singlet signal at δ 3.53 to δ 30.56 indicated the presence of an N-methyl group [5]. The aromatic ring and the mutually coupled methylene signals could be located around the ketone carbonyl at δ 199.98 based on HMBC correlations between H-6′ and C-7′, and H-9′ and C-7′ [6] (figure 2). The HMBC spectrum also revealed correlations between H-9′ and the two amide carbonyls δ 161.13 (C-4) and 150.18 (C-2) and between H-5 and C-4. From the above observations, **1** was deduced to be 3-[3-(2′-aminophenyl)-propanone]-1-methyl-2,4-quinazolinedione and named wuchuyuamide III.

Goshuyuamide II (**2**) was identified by comparison of its physical and spectral data with previous reports [7].

Table 1. ^{1}H NMR, ^{13}C NMR, HMBC, and ^{1}H–^{1}H COSY spectral data of **1**†.

No.	δ_C	δ_H	*HMBC (H → C)*	*COSY*
2	150.18			
4	161.13			
5	127.70	8.03 dd (7.8, 1.5)	C-4, 7, 9	6
6	122.70	7.30 ddd (7.8, 7.2, 1.5)	C-7, 8, 10	5, 7
7	135.26	7.77 ddd (8.4, 7.2, 1.5)	C-5, 9	6, 8
8	114.53	7.41 dd (8.4, 1.5)	C-6, 10	7
9	140.34			
10	114.79			
N-CH_3	30.56	3.53 s	C-2, 9	
1′	116.19			
2′	151.13			
3′	116.96	6.72 dd (8.4, 1.2)	C-1′, 5′	4′
4′	134.23	7.22 ddd (8.4, 8.1, 1.2)	C-2′, 3′, 6′	3′, 5′
5′	114.37	6.50 ddd (8.4, 8.1, 1.2)	C-1′, 3′	4′, 6′
6′	131.18	7.71 dd (8.4, 1.2)	C-1′, 2′, 4′, 7′	5′
7′	199.98			
8′	36.73	3.24 t (7.8)	C-7′, 9′	9′
9′	37.68	4.26 t (7.8)	C-2, 4, 7′	8′
NH_2		7.22 s		

† Recorded in $CDCl_3$ at 600 MHz (^{1}H NMR) and 150 MHz (^{13}C NMR).

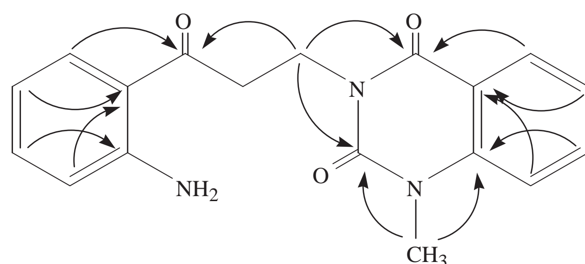

Figure 2. Key HMBC correlations of **1**.

Table 2. Cytotoxicities of **1** and **2**[†].

Compound	IC_{50} (μM)	
	HeLa cell	*HT1080 cell*
1	31.32 ± 0.51	24.51 ± 1.25
2	78.83 ± 1.28	70.34 ± 1.56
Doxorubicin	0.24 ± 0.02	0.48 ± 0.01

[†] Data are mean ± SD (IC_{50}, μM) from two separate experiments.

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Compounds **1** and **2** were examined for cytotoxicity against HeLa and HT1080 cell lines (table 2). In comparison with compound **2**, **1** showed stronger toxicity, with IC_{50} values of 31.32 μM and 24.51 μM respectively in the two cell lines.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP-370 polarimeter. Melting points were measured on an Electrothermal 9100 instrument without correction. UV spectra were obtained on a UV-1601 UV-visible spectrophotometer (Shimadzu). ^{1}H NMR, ^{13}C NMR, DEPT, and HMQC spectra were recorded on Bruker DMX 600 NMR spectrometer with $CDCl_3$ as a solvent. HRFAB-MS was obtained on a Platform quadrupole mass spectrometer. Preparative HPLC was carried out on J'sphere ODS-H80 (150 × 20 mm, YMC). Foetal bovine serum, media and supplement materials for cell culture were purchased from Gibco-BRL (Gaithersburg, MD, USA).

3.2 Extraction and isolation

The fruits of *Evodia officinalis* (1.8 kg) purchased from Ilsin Co. (Korea, June 2001), were extracted at room temperature with methanol. The MeOH extract (180 g) was partitioned between H_2O and EtOAc to obtain EtOAc extract (60 g). The EtOAc extract (60 g) was chromatographed on a silica gel column (5 × 60 cm) with mixtures of CH_2Cl_2/acetone (50:1, 30:1, 15:1, 10:1, 5:1, 1:1, acetone, MeOH) as eluents in a stepwise gradient mode. The fractions were combined on the basis of silica gel TLC, and 9 fractions (Fr. 1–Fr. 9) were obtained. Fraction 3 was dissolved in $CHCl_3$ and precipitated with MeOH. The supernatant was evaporated and subjected to silica gel chromatography eluting with

hexane/EtOAc (20:1, 10:1, 5:1, 1:1). Subfraction 3–4 was subjected to preparative TLC to give goshuyuamide II (**2**) (20 mg). Subfraction 3–5 (3 g) was eluted over silica gel column with hexane/EtOAc (5:1) to give seven fractions (3S-1–3S-7). Fraction 3S-3 was subjected to preparative HPLC (CH_3CN/H_2O, 40:60) to afford compound **1** (11 mg).

3.2.1 Wuchuyuamide III (1). Colourless prisms; $[\alpha]_D^{25}$ −17 (*c* 0.6, $CHCl_3$); m.p. 175.6–176.6°C; UV λ_{max} ($CHCl_3$) (log ε) 225.6 (3.97), 242.6 (4.33), 315.2 (3.38), 325.4 (3.39), 361.0 (3.40) nm; IR ν_{max} 3110, 1690, 1650, 1615 cm^{-1}; ^{1}H and ^{13}C NMR, see table; HRFAB-MS *m/z* 324.1345 $[M + H]^+$ (calcd for $C_{18}H_{18}N_3O_3$, 324.1348).

3.2.2 Goshuyuamide II (2). Colourless prisms; $[\alpha]_D^{25}$ + 3.7 (*c* 0.1, $CHCl_3$); m.p. 138–139°C; UV λ_{max} ($CHCl_3$) (log ε) 220.9 (4.45), 262.1 (3.78), 315.2 (3.76), 327.0 (3.68) nm; ESI-MS *m/z* 320.13 $[M + H]^+$.

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3.3 Cytotoxicity assay

A cytotoxicity assay was carried out according to Denizot and Lang [8]. Each cell (concentration of 1×10^4) was seeded in each well containing 100 μl DMEM. Subsequently, various concentrations of samples were added. The cells were incubated for 48 h at 37°C in an atmosphere containing 5% CO_2, then 10 μl FBS-free medium containing 5 mg/ml MTT was added to the wells. After 4 h of incubation at 37°C, the medium was discarded and the formazan blue formed in the cells was dissolved by adding 100 μl DMSO. Optical density was measured at 570 nm using a microplate reader (Molecular Devices Co., Menlo Park, CA, USA). Doxorubicin was used as a positive control.

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