A New Cytotoxic Evodiamine Derivative From *Tetradium ruticarpum* (A. Jussieu) T. G. Hartley

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**Abstract**

1-Hydroxymethyl goshuyuamide II (I), a new derivative of evodiamine with a quinazolinocarboline skeleton, along with nine known evodiamine derivatives were isolated from the dried and nearly ripe fruits of *Tetradium ruticarpum* (A. Jussieu) T. G. Hartley using different chromatographic separations. Their structures were elucidated on the basis of extensive spectroscopic techniques, including 1D and 2D NMR spectra. Putative biosynthetic pathways toward I are proposed. Compounds 1 and 2 and 4 to 10 exhibited cytotoxic activity against six human tumor lines, and compounds 4 and 7 to 10 exhibited moderate inhibitory activity against nitric oxide production in LPS-activated RAW264.7 cells.

**Keywords**

*Tetradium ruticarpum* (A. Jussieu) T. G. Hartley, evodiamine derivatives, 1-hydroxymethyl goshuyuamide II, cytotoxic activity, anti-inflammatory activity

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*Tetradium ruticarpum* (A. Jussieu) T. G. Hartley, known as “Wuzhuyu” in China, grows mainly in mainland China. The dried and nearly ripe fruits of *T. ruticarpum* are commonly used as a traditional Chinese medicine for the treatment of headache, epigastric distension, hyperbaropathy, dysentery, eczema, and rhinitis (Flora of China, 2004). Phytochemical studies have revealed that the main active constituents of *T. ruticarpum* are alkaloids, limonoids, coumarins, and flavonoids. Evodiamine (EV), an indole alkaloid, and EV derivatives, have attracted much scientific attention because of their broad range of biological activities and unique structural characteristics. Thus, the isolation of more EV derivatives from medicinal plants is a worthwhile objective.

In the course of screening for cytotoxic and anti-inflammatory EV derivatives from the fruits of *T. ruticarpum*, a new derivative, named 1-hydroxymethyl goshuyuamide II (I), along with nine known EV derivatives (2–10) were isolated (Figure 1). These compounds were also screened in vitro for their cytotoxic and anti-inflammatory activities. Reported herein are the isolation, structural elucidation, and biological evaluation of these compounds.

**Results and Discussion**

1-Hydroxymethyl goshuyuamide II (I), a white amorphous powder, showed a quasi-molecular ion peak at *m/z* 372.1319 ([M + Na]<sup>+</sup>) by HRESIMS, corresponding to a molecular formula of C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>. Comprehensive analysis of the <sup>1</sup>H- and <sup>13</sup>C-NMR data (Table 1) revealed that the structure of I was in close correspondence to that of goshuyuamide II (2), except for the presence of a hydroxymethyl group at N-1 in I instead of a hydrogen atom in the latter, indicating that I was a derivative of evodiamine with C2–C3 ring opening. The hydroxymethyl group at N-1 was confirmed by HMBC correlations of C-2 to C-13 (Figure 2). Thus, compound I was determined and named 1-hydroxymethyl goshuyuamide II.

It is worth mentioning that hydroxymethyl goshuyuamide II (I) is a new derivative of evodiamine with C2–C3 ring opening. We think that the evodiamine (4) might be a precursor of I. So, putative biosynthetic pathways towards I are proposed (Scheme 1).

The nine known compounds were identified as goshuyuamide II (2), N-(2-methylaminobenzoyl) tryptamine (3), evodiamine (4), N-ethylevodiamine (5), 14-formyl-13b, [M + Na]<sup>+</sup> (calcd *m/z* 372.1324) by HRESIMS, corresponding to a molecular formula of C<sub>20</sub>H<sub>19</sub>N<sub>3</sub>O<sub>3</sub>. Comprehensive analysis of the <sup>1</sup>H- and <sup>13</sup>C-NMR data (Table 1) revealed that the structure of I was in close correspondence to that of goshuyuamide II (2), except for the presence of a hydroxymethyl group at N-1 in I instead of a hydrogen atom in the latter, indicating that I was a derivative of evodiamine with C2–C3 ring opening. The hydroxymethyl group at N-1 was confirmed by HMBC correlations of C-2 to C-13 (Figure 2). Thus, compound I was determined and named 1-hydroxymethyl goshuyuamide II.

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14-dihydrorutaecarpine (6), rutacarpine (7), 10-hydroxyrutaecarpine (8), 3-hydroxyrutaecarpine (9), and 7-hydroxyrutaecarpine (10), by comparing their NMR spectroscopic data with literature values.

Compounds 1–10 were tested for their cytotoxicity against HL-60, Hela, HepG-2, A-549, AGS, and MDA-MA-468 cell lines. As shown in Figure 3, compounds 1 to 2 and 4 to 10 possessed cytotoxic activities, but 3 was non-cytotoxic in these test systems (IC50 > 4.0 μM), with evodiamine (4) possessing obvious cytotoxic activities against HL-60, HepG-2, A-549, and AGS cells with IC50 values of 0.24, 3.01, 1.46, and 3.90 μM, respectively.

The above results suggest that the closed ring between C2 and C3 maybe a functional group for cytotoxic activity. The cytotoxic activities of 4 to 10 were better than those of 1 and 2, which suggested that the intensity of cytotoxic activity maybe weakened because of the ring opening of C2–C3. Furthermore, the cytotoxic activity of 3 was markedly decreased, maybe connected with its two opened-rings of C2–C3 and C3–C4.

Considering the traditional use of T. ruticarpum, all isolates were tested for their inhibitory activity against NO production in LPS-stimulated RAW264.7 cells. Compounds 4 and 7 to 10 exhibited moderate inhibitory activity against nitric oxide production in LPS-activated RAW264.7 cells (Table 2).

Analysis of the anti-inflammatory activity and the structures of the tested compounds 4 and 7 to 10 exhibited moderate activity, but 1 to 3 were inactive (IC50 > 25 μM), which suggested that closed-rings maybe important functional units for the anti-inflammatory activity of evodiamine derivatives.

### Experimental

#### General Experimental Procedures

Melting points, X-4 micro melting point apparatus; UV, Shimadzu UV-2401A spectrophotometer; IR, Bruker Tensor 27 spectrophotometer with KBr pellets; and optical rotations, JASCO-20C digital polarimeter. ESIMS, API QSTAR time-of-flight spectrometer. HREIMS, Waters AutoSpec

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Table 1. 1H NMR (600 MHz) and 13C NMR (125 MHz) Data for 1 and 2 in C6D6N (δ in ppm and J in Hz).

| Position | δH (mult., J, Hz) | δC (mult.) | Position | δH (mult., J, Hz) | δC (mult.) |
|----------|-------------------|------------|----------|-------------------|------------|
| 2        | 7.33 (m)          | 126.9 (d)  | 2        | 7.38 (d, 2.2)     | 123.5 (d)  |
| 3        | 151.4 (s)         | 3          | 4        | 4.65 (t, 8.1)     | 42.9 (t)   |
| 5        | 4.57 (t, 8.4)     | 43.2 (t)   | 5        | 3.46 (t, 8.1)     | 24.5 (t)   |
| 6        | 3.35 (t, 8.4)     | 24.8 (t)   | 6        | 6                 | 112.5 (s)  |
| 7        | 113.2 (s)         | 7          | 7        | 129.9 (s)         | 8          |
| 8        | 129.9 (s)         | 8          | 9        | 1204 (d)          | 9          |
| 10       | 8.28 (dd, 8.4, 1.5) | 120.3 (d) | 10       | 8.33 (m)          | 119.5 (d)  |
| 11       | 7.32 (m)          | 122.7 (d)  | 11       | 7.30 (m)          | 121.9 (d)  |
| 12       | 7.76 (d, 7.8)     | 111.1 (d)  | 12       | 7.60 (m)          | 112.0 (d)  |
| 13       | 137.5 (s)         | 13         | 13       | 140.9 (s)         | 15         |
| 14       | 116.5 (s)         | 15         | 15       | 150.9 (s)         | 16         |
| 16       | 7.16 (d, 8.4)     | 114.0 (d)  | 16       | 7.13 (d, 8.8)     | 114.2 (d)  |
| 17       | 7.63 (dt, 8.4, 1.5) | 135.9 (d) | 17       | 7.60 (m)          | 135.0 (d)  |
| 18       | 7.23 (m)          | 123.4 (d)  | 18       | 7.21 (dd, 8.1, 6.3) | 122.7 (d) |
| 19       | 8.40 (dd, 8.4, 1.5) | 129.1 (d) | 19       | 8.40 (dd, 8.1, 1.5) | 128.6 (d) |
| 20       | 141.4 (s)         | 20         | 21       | 162.5 (s)         | 21         |
| 21       | 162.5 (s)         | 21         | 1-N-CH3OH | 5.85 (s)          | 70.2 (t)   |
| 14-NCH3  | 3.50 (s)          | 30.9 (q)   | 14-NCH3  | 3.49 (s)          | 30.5 (q)   |
Premier P776 spectrometer. 1D NMR and 2D NMR, Bruker AM-400, DRX-500 or AVANCE III-600 spectrometers with TMS as an internal standard. MPLC, Lisui EZ Purify III System including pump manager P03, detector modules P02, fraction collector P01, and columns packed with MCI gel. Semipreparative HPLC, Agilent 1260 apparatus equipped with an UV detector and a Zorbax SB-C-18 column. Silica gel and Sephadex LH-20 were used for column chromatography (CC). Fractions were monitored by TLC and compounds were visualized by heating the silica gel plates after spraying with 10% H₂SO₄ in EtOH.

**Plant Material**

The fruits of *T. ruticarpum* were purchased in December 2014 from the Herb Material Market of Juhuacun, Kunming, Yunnan Province, P.R. China, and identified by one of the authors (Jiang-Bo He). A voucher specimen (20141202) was deposited at the Phytochemistry Laboratory (Medicine in Kunming University).

**Extraction and Isolation**

The fruits of *T. ruticarpum* (10.0 kg) were extracted with 80% methanol (3 × 50 L, 24 h, each). The EtOH extracts were evaporated to dryness under reduced pressure, suspended in distilled H₂O, and partitioned with EtOAc consecutively. The EtOAc fraction (500 g) was subjected to reversed-phase MPLC (MCI) (MeOH/H₂O, 50%→100%) to give fractions A–F. Fr.D (52 g) was chromatographed on a silica gel column with a gradient elution of light petroleum–acetone (10:1, 8:2, 7:3, 6:4, 5:5, and 0:1) to give fractions D–I–D–IV. Fraction D–I was purified by recrystallization from light petroleum–acetone at 25°C to obtain 3 (22 mg), 4 (1100 mg), and 7 (850 mg). Subfraction D–II was fractionated on Sephadex LH-20 (MeOH) by repeated silica gel CC and semipreparative HPLC (MeCN-H₂O) to yield 6 (21 mg) and 8 (12 mg). Compound 5 (7 mg) was obtained from Fr.D–III by recrystallization from light petroleum–acetone at 25°C. Fr.D–IV was subjected to Sephadex LH-20 (MeOH) followed by silica gel CC (light petroleum–acetone, 10:1) to yield 1 (8 mg), 2 (10 mg), 10 (15 mg), and 9 (18 mg).

**Scheme 1.** Plausible biogenetic pathway of 1.

1-Hydroxymethyl goshuyuamide II (1). White amorphous powder; [α]₂₀.₁ D = −11.71° (c 0.35, MeOH); UV (MeOH) λ max (log ε): 313 (3.00), 291 (3.06), 221 (4.11), 195 (3.75) nm; IR (KBr) ν max 3441, 2925, 1699, 1650, 1487 cm⁻¹; 1H NMR (C₅D₅N, 600 MHz) and 13C NMR (C₅D₅N, 150 MHz) see Table 1; HRESIMS [M + Na]⁺ m/z 372.1319 (calcd for C₂₀H₁₉N₃O₃, 372.1324).

**Cytotoxicity Assay**

The cytotoxicity of compounds 1 to 10 against human myeloid leukemia (HL-60), human cervical cancer (Hela), human hepatoma (HepG-2), human non-small cell lung cancer (A-549), human gastric cancer (AGS), and human breast cancer (MDA-MA-468) cell lines was assessed using the MTT method." Cells were plated in 96-well plates for 12 h before treatment and continuously exposed to different concentrations of compounds. After 48 h, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution was added to each well and incubated for another 4 h. Then, 20%
SDS (100 μL) was added to each well. After 12 h at room temperature, the OD value of each well was recorded at 595 nm. The IC_{50} value of each compound was calculated by the Reed and Muench method.\textsuperscript{12}

**Anti-Inflammatory Assay**

Murine monocytic RAW264.7 macrophages were dispensed into 96-well plates (2 × 10^5 cells / well) containing RPMI-1640 medium (Hyclone) with 10% FBS under a humidified atmosphere of 5% CO_2 at 37°C. After 24 h preincubation, the cells were treated with serial dilutions of the compounds, with the maximum concentration of 25 μM in the presence of 1 μg/mL LPS for 18 h. Each compound was dissolved in DMSO and further diluted in medium to produce different concentrations. NO production in each well was assessed by adding 100 μL of Griess reagent (reagent A and reagent B, respectively, Sigma) to 100 μL of each supernatant from either LPS (Sigma)-treated or LPS- and compound-treated cells in triplicate. After 5 min incubation, the absorbance was measured at 570 nm with a 2104 Envision Multilabel Plate Reader (Perkin-Elmer Life Sciences, Inc., Boston, MA, USA). MG-132 was used as a positive control.\textsuperscript{13}

The cytotoxicity of the compounds was evaluated using an MTS assay.\textsuperscript{14} Briefly, RAW264.7 cells, 2 × 10^5 cells/well, were seeded in 96-well plates. After 24 h incubation, the cells were treated either with or without test compounds at given concentrations for 18 h. Then, MTS was added to each well and the plates were kept for 4 h. Test compounds were dissolved in DMSO, and the absorbance was measured at 490 nm. The cytotoxicity was calculated by the cell viability of cells without test compounds as 100%.

**Conclusions**

From the fruits of *T. ruticarpum*, one new compound (1) and nine known ones (2–10) were isolated. Compound 1 is a new derivative of evodiamine, and putative biosynthetic pathways toward 1 are proposed.

The cytotoxic activity of all isolates against HL-60, Hela, HepG-2, A-549, AGS, and MDA-MA-468 cell lines was evaluated. Compounds 1 and 2 and 4 to 10 exhibited cytotoxic activity against six human tumor lines, and compound 4 showed significantly selective cytotoxic activity to HL-60, HepG-2, A-549, and AGS cells with IC_{50} values of 0.24, 3.01, 1.46, and 3.90 μM, respectively.

Compounds 4 and 7 to 10 possessed moderate anti-inflammatory activity with IC_{50} values of 11.5, 22.5, 18.4, 12.5, and 18.1 μM, respectively (Table 2).

### Table 2. Inhibitory Effects of Compounds From *T. ruticarpum* on LPS-Activated NO Production in RAW264.7 Cells.

| Compound | IC_{50} (μM) | Compound | IC_{50} (μM) |
|----------|-------------|----------|-------------|
| 1        | > 25        | 7        | 22.5 ± 1.62 |
| 2        | > 25        | 8        | 18.4 ± 1.53 |
| 3        | > 25        | 9        | 12.5 ± 1.41 |
| 4        | 11.5 ± 2.13 | 10       | 18.1 ± 0.37 |
| 5        | > 25        | MG-132\textsuperscript{a} | 0.1 ± 0.018 |
| 6        | > 25        |          |             |

\textsuperscript{a}Positive control substance.
Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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