

## Cytotoxicity and DNA Topoisomerase Inhibitory Activity of Constituents Isolated from the Fruits of *Evodia officinalis*

Ming-Lu Xu, Gao Li<sup>1,2</sup>, Dong-Cheol Moon<sup>3</sup>, Chong-Soon Lee<sup>4</sup>, Mi-Hee Woo<sup>5</sup>, Eung Seok Lee, Yurngdong Jahng, Hyeun-Wook Chang, Seung-Ho Lee, and Jong-Keun Son

College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea, <sup>1</sup>Key Laboratory of Natural Resources of the Changbai Mountain and Functional Molecules (Yanbian University), Ministry of Education, Yanji 133000, China, <sup>2</sup>College of Pharmacy, Yanbian University, Yanji 133000, China, <sup>3</sup>College of Pharmacy, Chungbuk National University, Cheongju 361-763, Korea, <sup>4</sup>Department of Biochemistry, College of Natural Sciences, Yeungnam University, Gyongsan 712-749, Korea, and <sup>5</sup>College of Pharmacy, Catholic University of Daegu, Gyongsan 712-702, Korea

(Received March 3, 2006)

Four alkaloids (1-4), three quinolone alkaloids (5-7), and three flavanoid glucosides (8-10) were isolated from the fruits of *Evodia officinalis* Dode, and their structures were determined from chemical and spectral data. Compounds 3, 8, 9 and 10 were isolated from this plant for the first time. Of these compounds, 1-3 and 5-7 exhibited moderate cytotoxicities against cultured human colon carcinoma (HT-29), human breast carcinoma (MCF-7), and human hepatoblastoma (HepG-2). Compound 8 showed strong inhibitory effects on DNA topoisomerases I and II (70 and 96% inhibition at a concentration of 20  $\mu$ M, respectively).

**Key words:** *Evodia officinalis*, DNA topoisomerases I and II inhibitors, Cytotoxicity

### INTRODUCTION

DNA topoisomerases are enzymes that relax DNA during a number of critical cellular processes, including replication, recombination, and transcription, by transiently breaking one or two strands of DNA, passing a single- or double-stranded DNA through the break, and finally resealing the break (Wang, 1996; Pommier, 1993; D'Arpa and Liu, 1989). Because of the important role of these enzymes, DNA topoisomerases have been established as important molecular targets for anticancer drugs (Liu, 1989). Currently, only the camptothecin (CPT) family of compounds have been introduced into the clinic as DNA topoisomerase I-directed drugs (Slichenmeyer *et al.*, 1993; Potmesil, 1994), although many topoisomerase II-directed drugs have been in clinical use for many years (Chen and Liu, 1994).

The dried fruits of *Evodia officinalis* Dode (Rutaceae), popularly known in China as "Wu-Chu-Yu", have been prescribed for the treatment of gastrointestinal disorders,

abdominal pain, dysentery, headache, postpartum hemorrhage, and amenorrhea, according to traditional Chinese medical practice. In this paper, as a continuing biochemical study of these isolated compounds (1-10) from the fruits of *E. officinalis*, their inhibitory activities against the DNA topoisomerases I and II and their cytotoxicity were evaluated.

### MATERIALS AND METHODS

#### General experimental procedures

Optical rotations were measured using a JASCO DIP-1000 (Tokyo, Japan) automatic digital polarimeter. The NMR spectra were recorded on a Bruker 250 MHz (DMX 250) spectrometer using Bruker's standard pulse program. Samples were dissolved in CD<sub>3</sub>OD-*d*<sub>4</sub>, CDCl<sub>3</sub>-*d*<sub>1</sub>, or pyridine-*d*<sub>5</sub>, and chemical shifts were reported in  $\delta$  (ppm) downfield from TMS. The FAB-MS spectra were measured by a VG TRIO 2A mass spectrometer. Silica gel 60 (70-230 and 270-400 mesh, Merck) and Lichroprep Rp-18 gel (40-63  $\mu$ m, Merck) TLC plates (Si-gel 60 F<sub>254</sub> and Rp-18 F<sub>254</sub>) were purchased from Merck KGaA (Darmstadt, Germany). Spots were detected under UV radiation and by spraying with 10% H<sub>2</sub>SO<sub>4</sub>, followed by heating. For preparative HPLC, an LC-10AD pump (Shimadzu), SPD-

Correspondence to: Jong-Keun Son, College of Pharmacy, Yeungnam University, Gyongsan 712-749, Korea  
Tel: 82-53-810-2817, Fax: 82-53-810-4654  
E-mail: jkson@yu.ac.kr

10A detector (Shimadzu) and Shim-Pack Prep-ODS (20 × 250 mm) column were used. Camptothecin (CPT) and etoposide were purchased from SIGMA Chemical Co. (St. Louis, MO, U.S.A.). Supercoiled pBR 322 plasmid DNA and calf thymus topoisomerase I were purchased from MBI Fermentas INC. (Hanover, MD, U.S.A.). Human topoisomerase II was purchased from TopoGEN INC. (Columbus, OH, U.S.A.). All other chemicals and solvents were analytical grade and used without further purification.

### Plant material

The fruits of *E. officinalis* were purchased in September 2000 from a folk medicine market "Yakryong-si" in Daegu, Republic of Korea. These materials were confirmed taxonomically by Professor Ki-Hwan Bae of Chungnam National University, Daejeon, Republic of Korea. A voucher specimen has been deposited at the College of Pharmacy, Yeungnam University.

### Extraction and isolation

The dried fruits (9 kg) of *Evodia officinalis* were extracted three times with MeOH (12 L) at 60°C for 12 hours. The combined MeOH extracts were concentrated under reduced pressure to obtain the MeOH extract (1.23 kg). The extract was suspended in H<sub>2</sub>O (1.5 L) and then fractionated sequentially with *n*-hexane, EtOAc, and water-saturated *n*-butanol, 3 times with each solvent. Removal of the solvents afforded 73.0 g, 308.68 g, 198.89 g, and 630.62 g of the *n*-hexane, EtOAc, *n*-butanol, and water fractions, respectively. A part of the EtOAc extract (26.4 g) was loaded on a silica gel column (6 × 70 cm, 230-400 mesh) and the column was eluted with *n*-hexane-EtOAc (gradient from 100% *n*-hexane to 100% EtOAc) and then EtOAc-MeOH (gradient from 100% EtOAc to 100% MeOH). The eluent was combined based on TLC results, yielding 22 fractions (EOE1~22). Fractions EOE-9 (1.2 g), EOE-10 (110 mg), EOE-14 (35 mg), and EOE-15 (48 mg) were recrystallized with 100% *n*-hexane to give compounds **1** (890 mg), **2** (26 mg), **3** (10 mg), and **4** (31 mg), respectively. Fractions EOE-18 and EOE-16 were chromatographed on a reverse phase column (2.0 × 60 cm, LiChroprep RP-18) with MeOH-H<sub>2</sub>O (gradient from 30 to 100% MeOH) eluent, affording compounds **5** (12 mg), **6** (12 mg), and **7** (20 mg).

The H<sub>2</sub>O extract (130 g) was loaded on a Diaion column (10 × 80 cm, Diaion HP-20) and eluted with H<sub>2</sub>O-MeOH (100% H<sub>2</sub>O, 75:25, 50:50, 25:75, 100% MeOH). The eluent was combined based on TLC results, giving 5 fractions (EOW1~5). Fraction 4 (2.0 g) was further purified on a reverse phase column (4.0 × 75 cm, LiChroprep RP-18) with MeOH-H<sub>2</sub>O (gradient from 2:8 to 9:1), the eluent was combined based on TLC, giving 15 subfractions (EOW4-1~4-15). Subfraction EOW4-8 (200 mg) was chro-

matographed again on a reverse phase column (2.0 × 60 cm, LiChroprep RP-18) and eluted with a MeOH-H<sub>2</sub>O gradient (from 70 to 100% MeOH), affording compounds **8** (10 mg), **9** (13 mg) and **10** (17 mg).

### Rutaecarpine (1)

Pale yellow amorphous solid; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz) 9.80 (1H, s, NH), 8.30 (1H, dd, *J* = 8.0, 1.2 Hz, H-19), 7.77 (1H, ddd, *J* = 8.2, 7.0, 1.2 Hz, H-17), 7.70 (1H, brd, *J* = 8.0 Hz, H-9), 7.64 (1H, brd, *J* = 8.2 Hz, H-16), 7.42 (1H, brd, *J* = 8.2 Hz, H-12), 7.32 (1H, brdd, *J* = 8.0, 7.0 Hz, H-18), 7.27 (1H, brdd, *J* = 8.2, 7.0 Hz, H-11), 7.15 (1H, brdd, *J* = 8.0, 7.0 Hz, H-10), 4.56 (2H, t, *J* = 7.0 Hz, H-5), 3.22 (2H, t, *J* = 7.0 Hz, H-6); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 62.9 MHz) δ 161.5 (C-5), 147.2 (C-1a), 145.3 (C-14a), 138.5 (C-12a), 134.4 (C-2), 127.3 (C-4), 127.2 (C-3a), 126.9 (C-1), 126.2 (C-3), 125.6 (C-9a), 125.4 (C-11), 121.0 (C-4a), 120.5 (C-1), 120.0 (C-9), 118.7 (C-8a), 112.2 (C-12), 41.2 (C-7), 19.6 (C-8); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Ikuta *et al.*, 1998; Wattanapiromsakul *et al.*, 2003), Positive FAB-MS *m/z* 288 [M+H]<sup>+</sup>.

### Evodiamine (2)

Pale yellow plates; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz) δ 2.48 (N-CH<sub>3</sub>, s), 2.94 (2H, m), 3.25 (1H, m), 4.87 (1H, m), 7.19 (4H, m), 7.43 (2H, m), 7.58 (1H, d, *J* = 7.8 Hz), 8.10 (1H, dd, *J* = 1.4, 7.8 Hz), 8.32 (1H, s); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 62.9 MHz) δ 164.8 (C-21), 150.7 (C-15), 136.7 (C-13), 133.0 (C-17), 129.0 (C-9), 128.2 (C-2), 126.2 (C-8), 124.0 (C-10), 123.7 (C-20), 123.0 (C-11), 122.3 (C-16), 120.0 (C-18), 118.9 (C-19), 113.6 (C-7), 111.3 (C-12), 68.8 (C-3), 39.5 (C-5), 37.2 (N-CH<sub>3</sub>), 20.1 (C-6); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Bergman *et al.*, 1985), Positive FAB-MS *m/z* 304 [M+H]<sup>+</sup>.

### 7-Hydroxyrutaecarpine (3)

Pale yellow powder (10.0 mg); [ $\alpha$ ]<sub>D</sub><sup>25</sup> +73° (c 0.0012, MeOH); <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>, 250 MHz) δ 13.3 (1H, s, 1-NH), 9.3 (1H, br, 5-OH), 8.4 (1H, dd, *J* = 8, 1.5 Hz, H-19), 7.7 (1H, ddd, *J* = 8, 7.5, 1.5 Hz, H-17), 7.6 (1H, d, *J* = 7.5 Hz, H-9), 7.6 (1H, d, *J* = 8 Hz, H-16), 7.5 (1H, dd, *J* = 5.5, 1.5 Hz, H-5), 7.4 (1H, d, *J* = 8.5 Hz, H-12), 7.4 (1H, dd, *J* = 8, 7.5 Hz, H-18), 7.3 (1H, ddd, *J* = 8.5, 7, 1 Hz, H-11), 7.2 (1H, ddd, *J* = 8, 7, 1 Hz, H-10), 3.77 (1H, dd, *J* = 16.8, 1.5 Hz, H-6eq), 3.4 (1H, dd, *J* = 16.8, 5.5 Hz, H-6ax); <sup>13</sup>C-NMR (pyridine-*d*<sub>5</sub>, 62.9 MHz) δ 161.6 (C-21), 148.6 (C-15), 145.1 (C-3), 139.9 (C-13), 134.6 (C-17), 127.7 (C-19), 127.5 (C-16), 127.2 (C-18), 127.1 (C-2), 126.0 (C-11), 125.2 (C-8), 122.0 (C-20), 120.4 (C-10), 120.3 (C-9), 115.5 (C-7), 112.8 (C-12), 74.6 (C-5), 28.5 (C-6); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Ikuta *et al.*, 1988). Positive FAB-MS *m/z* 304 [M+H]<sup>+</sup>.

**Wuchuyamide I (4)**

Amorphous powder;  $[\alpha]_D^{24} +0.6^\circ$  (c 0.2, C<sub>5</sub>H<sub>5</sub>N), {lit. (Zuo *et al.*, 2000),  $[\alpha]_D^{24} 0^\circ$  (c 0.24, C<sub>5</sub>H<sub>5</sub>N)}; <sup>1</sup>H-NMR (pyridine-*d*<sub>5</sub>, 250 MHz)  $\delta$  11.66 (1H, s, 1-NH), 8.26 (1H, dd, *J* = 6.4, 1.6 Hz, H-19), 7.77 (1H, d, *J* = 7.2 Hz, H-9), 7.50 (1H, m, H-17), 7.211 (1H, m, H-11), 7.15 (1H, t, *J* = 7.6 Hz, H-18), 7.07 (1H, d, *J* = 8.4 Hz, H-16), 7.02 (1H, t, *J* = 7.4 Hz, H-10), 6.99 (1H, d, *J* = 8.0 Hz, H-12), 5.05 (1H, br., 7-OH), 4.84, 4.76 (each 1H, m, H-5), 3.36 (3H, s, H-22), 2.90, 2.82 (each 1H, m, H-6); <sup>13</sup>C-NMR (pyridine-*d*<sub>5</sub>, 62.9 MHz)  $\delta$  180.8 (C-2), 161.6 (C-21), 150.8 (C-3), 142.9 (C-13), 140.8 (C-15), 134.7 (C-17), 133.3 (C-8), 129.4 (C-11), 128.5 (C-19), 124.8 (C-9), 122.6 (C-18), 122.2 (C-10), 115.9 (C-20), 114.1 (C-16), 110.2 (C-12), 75.7 (C-7), 37.5 (C-5), 36.3 (C-6), 30.4 (C-22). <sup>1</sup>H- and <sup>13</sup>C-NMR data are consistent with the literature values (Zuo *et al.*, 2000). Positive FAB-MS *m/z* 352 [M+H]<sup>+</sup>.

**1-Methyl-2-[(Z)-9-pentadecenyl]-4(1H)-quinolone and 1-methyl-2-[(Z)-10-pentadecenyl]-4(1H)-quinolone (5)**

Colorless oil; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.5 (1H, dd, *J* = 1.5 Hz, 8.0 Hz, H-5), 7.7 (1H, m, H-7), 7.6 (1H, d, *J* = 8.0 Hz, H-8), 7.4 (1H, t, *J* = 8.0 Hz, H-6), 6.4 (1H, s, H-3), 5.3 (2H, m, olefinic proton), 3.8 (3H, s, N-Me), 2.8 (2H, t, *J* = 7.6 Hz, H-1'), 2.0 (4H, m, allyl proton), 1.7 (2H, m, H-2'), 1.25-1.47 (16H, m), 0.9 (3H, *J* = 7.0 Hz, H-15'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 62.9 MHz)  $\delta$  177.5 (C-4), 154.7 (C-2), 141.7 (C-8a), 131.9 (C-7), 129.9 (C-10), 129.5 (C-9), 126.3 (C-5), 126.2 (C-4a), 123.1 (C-6), 115.3 (C-8), 110.8 (C-3), 34.6 (C-1'), 34.0 (N-CH<sub>3</sub>), 31.8 (C-13'), 29.1 (C-7'), 28.9 (C-6'), 28.5 (C-4'), 27.0 (C-5'), 26.9 (C-11'), 26.8 (C-12'), 22.5 (C-2',3',6',8'), 22.2 (C-14'), 14.0 (C-15'). <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Asano *et al.*, 1996; Sugimoto *et al.*, 1988). Positive FAB-MS *m/z* 367 [M+H]<sup>+</sup>.

**Evocarpine (6)**

Colorless oil; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.28 (1H, dd, *J* = 1.5, 8.0 Hz, H-5), 7.51 (1H, m, H-7), 7.33 (1H, d, *J* = 8.0 Hz, H-8), 7.21 (1H, t, *J* = 8.0 Hz, H-6), 6.03 (1H, s, H-3), 5.20 (2H, m, olefinic proton), 3.56 (3H, s, N-Me), 2.52 (2H, t, *J* = 7.6 Hz, H-1'), 1.92 (4H, m, allyl proton), 1.49 (2H, m, H-2'), 1.31-1.21 (12H, m), 0.82 (3H, *J* = 7.0 Hz, H-15'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 62.9 MHz)  $\delta$  177.5 (C-4), 154.5 (C-2), 141.8 (C-8a), 131.7 (C-7), 129.7 (C-8'), 129.3 (C-9'), 126.1 (C-5), 126.1 (C-4a), 122.9 (C-6), 115.2 (C-8), 110.6 (C-3), 34.4 (C-1'), 33.9 (N-CH<sub>3</sub>), 31.7 (C-11'), 29.4 (C-2'), 29.1 (C-3'), 28.9 (C-4'), 28.6 (C-5'), 28.1 (C-6'), 26.9 (C-7'), 26.6 (C-10'), 22.1 (C-12'), 13.8 (C-13'); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Asano *et al.*, 1996; Sugimoto *et al.*, 1988). Positive FAB-MS *m/z* 340 [M+H]<sup>+</sup>.

**Dihydroevocarpine (7)**

Colorless powder; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.38 (1H, dd, *J* = 1.5, 8.0 Hz, H-5), 7.60 (1H, m, H-7), 7.43 (1H, d, *J* = 8.0 Hz, H-8), 7.30 (1H, t, *J* = 8.0 Hz, H-6), 6.15 (1H, s, H-3), 3.67 (3H, s, N-Me), 2.63 (2H, t, *J* = 7.6 Hz, H-1'), 1.60 (2H, m, H-12'), 1.21-1.36 (20H, m), 0.83 (3H, *J* = 7.0 Hz, H-13'); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 62.9 MHz)  $\delta$  177.6 (C-4), 154.7 (C-2), 141.8 (C-8a), 131.9 (C-7), 126.4 (C-5), 126.4 (C-4'), 123.1 (C-6), 115.3 (C-8), 110.9 (C-3), 34.6 (C-1'), 34.0 (N-CH<sub>3</sub>), 31.8 (C-11'), 29.5-28.8 (C-3'-10'), 28.4 (C-2'), 22.6 (C-12'), 14.0 (C-13'); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Asano *et al.*, 1996; Sugimoto *et al.*, 1988). Positive FAB-MS *m/z* 342 [M+H]<sup>+</sup>.

**Limocitrin 3-O-rutinoside (8)**

Yellow solid; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  8.0 (1H, d, *J* = 2.5 Hz, H-2'), 7.8 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 7.7 (1H, d, *J* = 8.5 Hz, H-5'), 6.3 (1H, s, H-6), 5.3 (1H, dd, *J* = 7 Hz, H-glu-1''), 4.5 (1H, s, H-rham-1'''), 4.0 (3H, s, 3'-OCH<sub>3</sub>), 3.9 (3H, s, 8-OCH<sub>3</sub>), 1.1 (3H, d, *J* = 6.5 Hz, rham-CH<sub>3</sub>); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 62.9 MHz)  $\delta$  179.5 (C-4), 158.6 (C-7, 2), 157.9 (C-5), 151.0 (C-4'), 150.4 (C-9), 148.4 (C-3'), 135.5 (C-3), 129.1 (C-8), 124.0 (C-6'), 123.0 (C-1'), 116.2 (C-5'), 114.3 (C-2'), 105.6 (C-10), 104.4 (C-glu-1''), 102.6 (C-rham-1'''), 100.1 (C-6), 76.6 (C-3'''), 76.2 (C-5''), 74.5 (C-2''), 72.0 (C-4'''), 70.8 (C-3'''), 70.6 (C-2'''), 70.3 (C-4''), 68.5 (C-5''), 67.1 (C-6''), 62.1 (8-OCH<sub>3</sub>), 56.7 (3'-OCH<sub>3</sub>), 17.9 (C-6'''); <sup>1</sup>H, <sup>13</sup>C-NMR data are consistent with literature values (Harborne, 1981; Ulubelen *et al.*, 1984; Wang *et al.*, 1989; Ito *et al.*, 2000). Positive FAB-MS *m/z* 654.5 [M+H]<sup>+</sup>.

**Veronicafolin 3-rhamnosyl-glucoside (9)**

Light yellow solid; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  7.98 (1H, d, *J* = 2.5 Hz, H-2'), 7.7 (1H, dd, *J* = 2.5, 8.5 Hz, H-6'), 6.9 (1H, d, *J* = 8.5 Hz, H-5'), 6.24 (1H, s, H-8), 5.23 (1H, d, *J* = 7 Hz, glu-1''), 4.51 (1H, s, rham-1'''), 3.94 (3H, s, 3'-OCH<sub>3</sub>), 3.89 (6H, s, 6-OCH<sub>3</sub> and 7-OCH<sub>3</sub>), 3-4 (sugar protons), 1.08 (3H, d, *J* = 6.5 Hz, rham-CH<sub>3</sub>); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 62.9 MHz)  $\delta$  179.43 (C-4), 158.69 (C-7), 158.53 (C-2), 157.93 (C-5), 150.94 (C-4'), 150.34 (C-9), 148.36 (C-3'), 135.45 (C-3), 129.08 (C-8), 124.03 (C-6'), 123.0 (C-1'), 116.16 (C-5'), 114.3 (C-2'), 105.56 (C-10), 104.39 (C-glu-1''), 102.53 (C-rham-1'''), 100.18 (C-6), 60-80 (sugar carbons), 62.04 (C-3'-OCH<sub>3</sub>), 56.67 (C-8-OCH<sub>3</sub>), 49.85 (C-7-OCH<sub>3</sub>), 17.87 (C-rham-CH<sub>3</sub>); <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are in close agreement with previous reports (Ulubelen *et al.*, 1980). Positive FAB-MS *m/z* 668.6 [M+H]<sup>+</sup>.

**Isorhamnetin 3-O-rutinoside (10)**

Yellow solid; <sup>1</sup>H-NMR (CD<sub>3</sub>OD, 250 MHz)  $\delta$  7.85 (1H, s,

H-2'), 7.5 (1H, d,  $J = 8.5$  Hz, H-6'), 6.8 (1H, d,  $J = 8.5$  Hz, H-5'), 6.3 (1H, d,  $J = 2.5$  Hz, H-8), 6.1 (1H, d,  $J = 2.5$  Hz, H-6), 5.1 (1H, d,  $J = 7$  Hz, H-1''), 4.4 (1H, s, H-1'''), 3.85 (3H, s, OCH<sub>3</sub>), 1.0 (3H, d,  $J = 6.5$  Hz, H-6''); <sup>13</sup>C-NMR (CD<sub>3</sub>OD, 62.9 MHz)  $\delta$  179.3 (C-4), 166.0 (C-7), 162.9 (C-5), 158.9 (C-9), 158.5 (C-2), 150.8 (C-4'), 148.3 (C-3'), 135.4 (C-3), 123.9 (C-1'), 122.9 (C-6'), 116.1 (C-5'), 114.5 (C-2'), 105.7 (C-10), 104.4 (C-glu-1''), 102.5 (C-rham-1''), 100.0 (C-6), 94.4 (C-8), 78.2 (C-3''), 77.4 (C-5''), 75.9 (C-2''), 73.9 (C-4''), 72.3 (C-3''), 72.1 (C-2''), 71.6 (C-4''), 69.8 (C-5''), 68.6 (C-6''), 56.7 (C-3'-OCH<sub>3</sub>), 17.9 (C-rham-CH<sub>3</sub>); <sup>1</sup>H, <sup>13</sup>C-NMR data are consistent with literature values (Ahmed *et al.*, 1991; Buschi and Pomilio, 1982; Crawford, 1978). Positive FAB-MS  $m/z$  624.5 [M+H]<sup>+</sup>.

### *In vitro* assay for DNA topoisomerase I inhibition

DNA topoisomerase I inhibition assays were carried out according to the method reported by Fukuda *et al.* (1996) with minor modifications. The topoisomerase I activity was determined by measuring the relaxation of supercoiled pBR 322 plasmid DNA. The reaction mixture was comprised of 35 mM Tris-HCl (pH 8.0), 72 mM KCl, 5 mM MgCl<sub>2</sub>, 5 mM DTT, 2 mM spermidine, 0.01% bovine serum albumin (BSA), 250 ng pBR 322 plasmid DNA, and 0.3 U calf thymus DNA topoisomerase I. Test compound solutions (less than 0.25% DMSO) were added to the reaction mixture in a final volume of 10 mL. The reaction mixtures

were incubated for 30 min at 37°C and terminated by the addition of a dye solution comprised of 2.5% SDS, 15% ficoll-400, 0.05% bromophenol blue, 0.05% xylene cyanole, and 25 mM EDTA (pH 8.0). The reaction products were determined by electrophoresis on 1% agarose gels in TBE (Tris-borate-EDTA) running buffer, at 1.5 V/cm for 10 h. The gels were stained with ethidium bromide (0.5  $\mu$ g/mL) for 30 min and destained in water for 30 min. For the visualization and quantitative analyses of the topoisomerase I activity, the gels were directly scanned with an image analyzer and percent inhibition was calculated by comparing the calculated area for supercoiled DNA in treated lanes to that of the control sample (topoisomerase only).

### *In vitro* assay for DNA topoisomerase II inhibition

DNA topoisomerase II inhibition activity was measured by assessing relaxation of supercoiled pBR 322 plasmid DNA. The reaction mixtures contained 50 mM Tris-HCl (pH 8), 120 mM KCl, 10 mM MgCl<sub>2</sub>, 0.5 mM ATP, 0.5 mM dithiothreitol, 300 ng pBR 322 plasmid DNA, 0.3 U human DNA topoisomerase II, and the indicated test compounds (less than 0.25% DMSO) in a final volume of 20  $\mu$ L. The reaction mixtures were incubated for 30 min at 37°C and terminated by addition of 5  $\mu$ L of a termination mixture [0.77% SDS, 77 mM EDTA (pH 8.0), 30% sucrose, 0.5% bromophenol blue and 0.5% xylene cyanole]. The reaction products were electrophoresed on 1% agarose gel in TBE

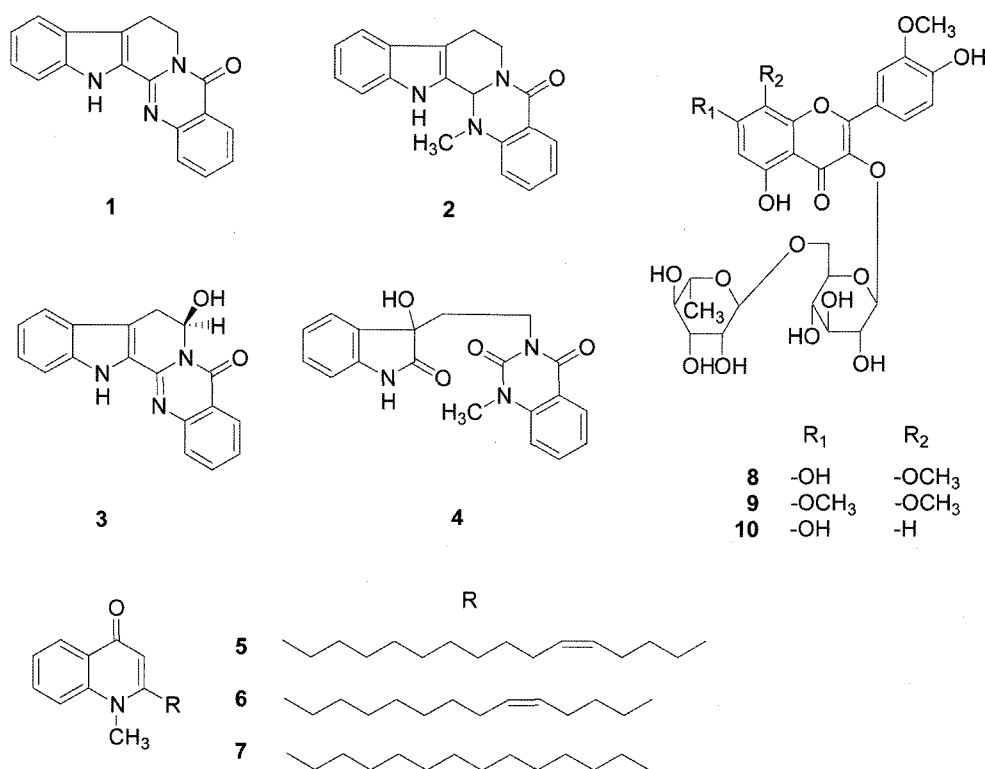


Fig. 1. Chemical structures of compounds 1-10

(Tris-borate-EDTA) running buffer at 1.5 V/cm for 10 h. Gels were stained with 0.5  $\mu\text{g/mL}$  ethidium bromide for 30 min and destained in water for 30 min. For visualization and quantitative analyses of topoisomerase II activity, the gels were directly scanned with an image analyzer and percent inhibition was calculated by comparing the calculated area for supercoiled DNA in treated lanes to that of the control sample (topoisomerase only).

### Assay for cytotoxicity

The tetrazolium-based colorimetric assay (MTT assay) was used to determine the cytotoxicity of the test compounds towards human colon carcinoma (HT-29), human breast carcinoma (MCF-7), and human hepatoblastoma (HepG2) cell lines (Rubinstein *et al.*, 1990).

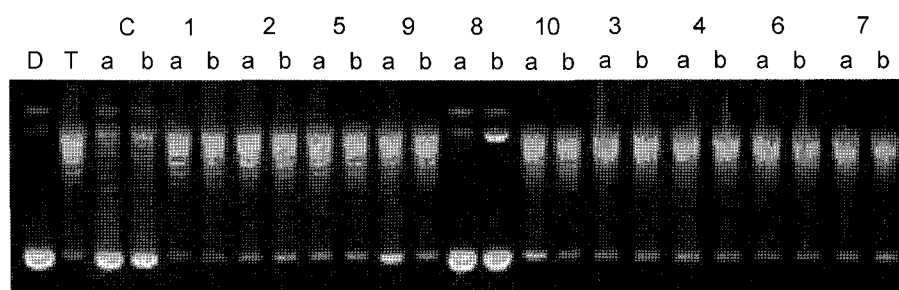
## RESULTS AND DISCUSSION

The methanol extract of the fruits of *E. officinalis* was partitioned by different solvents, giving *n*-hexane, EtOAc, *n*-BuOH and water layers. The EtOAc extract was repeatedly subjected to normal-phase silica gel and reverse-phase gel chromatography to afford four known indol-alkaloids (**1-4**) and three known quinolone alkaloids (**5-7**). The water extract was also repeatedly subjected to Diaion gel and reverse-phase gel chromatography to afford three known flavanoid glucosides (**8-10**). By comparing their

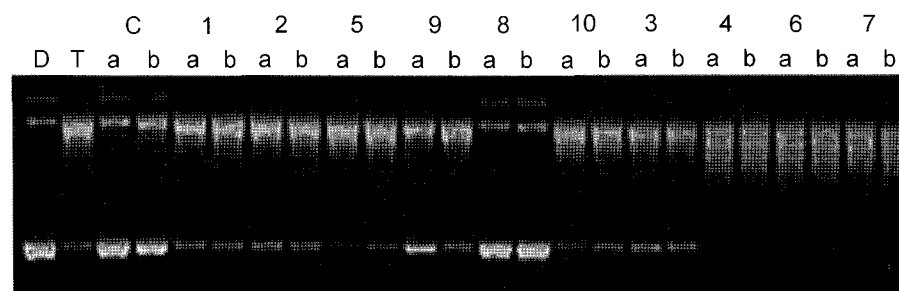
optical rotation values,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra, and MS data with published values, compounds **1-10** were characterized as rutaecarpine (**1**), evodiamine (**2**), 7-hydroxy-rutaecarpine (**3**), wuchuyamide I (**4**), dihydroevocarpine (**5**), 1-methyl-2-[(*Z*)-9-pentadecenyl]-4(1H)-quinolone (**6**), evocarpine (**7**), limocitrin 3-*O*-rutinoside (**8**), veronicafolin 3-*O*-rhamnosyl-glucoside (**9**), and isorhamnetin 3-*O*-rutinoside (**10**). Among these compounds, **3**, **8**, **9**, and **10** were isolated from this plant for the first time, and the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR data of **8** are presented for the first time here, based on the available literature.

The cytotoxic potentials of compounds **1-10** were tested on cultured human colon carcinoma (HT-29), human breast carcinoma (MCF-7), and human hepatoblastoma (HepG-2) cell lines (Rubinstein *et al.*, 1990) using the MTT assay. As shown in Table I, compounds **1**, **2**, **3**, **5**, **6**, and **7** exhibited moderate to weak cytotoxicity against tumor cell lines, with compound **2** showing the strongest activity ( $\text{IC}_{50}$ , 14 and 10  $\mu\text{M}$  against the HT-29 and HepG-2 cell lines, respectively).

To investigate whether the cytotoxicities mediated by these compounds (**1-10**) are related to intrinsic inhibitory activities against DNA topoisomerases I and II, the conversion of supercoiled pBR 322 plasmid DNA to relaxed DNA by topoisomerase I or II were examined in the presence of the compounds. As illustrated in Figs. 2 and 3, at concentrations of 100 and 20  $\mu\text{M}$ , only compound **8**



**Fig. 2.** Inhibitory activities of compounds **1-10** against DNA topoisomerase I. Lane D: supercoiled DNA alone; Lane T: supercoiled DNA + topoisomerase I (calf thymus); Lane C: supercoiled DNA + topoisomerase I (calf thymus) + camptothecin-positive control; Lanes **a** and **b**: **a**: 100  $\mu\text{M}$ , **b**: 20  $\mu\text{M}$  (test compound concentration).



**Fig. 3.** Inhibitory activities of compounds **1-10** against DNA topoisomerase II. Lane D: supercoiled DNA alone; Lane T: supercoiled DNA + topoisomerase II (calf thymus); Lane C: supercoiled DNA + topoisomerase II (human) + etoposide-positive control; Lanes **a** and **b**: **a**: 100  $\mu\text{M}$ , **b**: 20  $\mu\text{M}$  (test compound concentration).

**Table I.** Inhibitory effects of compounds **1-10** on topoisomerases I and II (% inhibition ratio of relaxation) and their IC<sub>50</sub> values against HT-29, MCF-7, and HepG-2 cell lines

Compounds	(% Inhibition ratio for topoisomerase I		(% Inhibition ratio for topoisomerase II		Cytotoxicity IC <sub>50</sub> (μg/mL)		
	100 μM	20 μM	100 μM	20 μM	HT-29 <sup>a</sup>	MCF-7 <sup>b</sup>	HepG-2 <sup>c</sup>
1	NA	NA	3	NA	42	18	>100
2	NA	NA	4	1	14	>100	10
3	NA	NA	NA	NA	38	42	41
4	NA	NA	NA	NA	>100	94	>100
5	NA	NA	NA	NA	59	56	50
6	NA	NA	NA	NA	43	52	48
7	NA	NA	NA	NA	41	33	39
8	93	70	100	97	>100	>100	>100
9	13	NA	37	6	>100	>100	>100
10	4	NA	9	5	>100	>100	>100
CPT <sup>d</sup>	89	72	NA	NA	0.96	1.0	0.86
etoposide <sup>e</sup>	NA	NA	78	58	NA	NA	NA

<sup>a</sup>HT-29: Human colon carcinoma; <sup>b</sup>MCF-7: Human breast carcinoma; <sup>c</sup>HepG-2: Human hepato blastoma; <sup>d</sup>camptothecin: positive control for topoisomerase I; <sup>e</sup>positive control for topoisomerase II; NA: not applicable.

showed potent inhibitory activity on both DNA topoisomerases I and II. These results indicate that no obvious correlation exists between the cytotoxicity of these compounds and any inherent inhibitory activity on DNA relaxation and decatenation by DNA topoisomerases I and II.

## ACKNOWLEDGEMENTS

This work was supported by Korea Research Foundation Grant (KRF-2004-005-E00004).

## REFERENCES

- Ahmed, A., Ishak, M. S., Micheal, H. N., El-ansari, M. A., and El-Sissi, H. I., Flavonoids of *Asteriscus fraveolens*, 54, 1092-1093 (1991).
- Akira, I., Hisaom, U., and Takayuki, N., A new indolopyridoquinazoline-type alkaloid from *Phellodendron amurense* Callus Tissues. *J. Nat. Prod.*, 61, 1012-1014 (1988).
- Asano, N., Kato, A., Kizu, H., and Matsui, K., 1β-amino-2α,3β,5β-trihydroxycycloheptane from *Physalis alkekengi* var. *francheti*. *Phytochemistry*, 42(3), 719-721 (1996).
- Ulubelen, A., Timmermann, N., and Mabry, T. J., Flavonoids from *Brickellia chlorolepis* and *B. Dentata*. *Phytochemistry*, 19, 905-908 (1980).
- Bergman J. and Bergamn S., Studies of rutaecarpine and related quinazolinocarboline alkaloids. *J. Org. Chem.*, 50, 1246-1255 (1985).
- Buschi, C. A. and Pomilio, A. B., Isorhamentin 3-O-robinobioside from *Gomphrena Martiana*. *J. Nat. Prod.*, 45, 557-559 (1982).
- Chen, A. Y. and Liu, L. F., DNA topoisomerase: essential enzymes and lethal targets. *Annu. Rev. Pharmacol. Toxicol.*, 34, 191-218 (1994).
- Crawford, D., Flavonoid chemistry of *Chenopodium fremontii*. Intraspecific variation and systematic implications at the interspecific level. *J. Biochem. Syst. Ecol.*, 6, 189. (1978).
- D'Arpa, P. and Liu, L. F., Topoisomerase-targeting antitumor drugs. *Biochim. Biophys. Acta.*, 989, 163-177 (1998).
- Fukuda, M., Nishio, K., Kanzawa, F., Ogasawara, H., Ishida, T., Arioka, H., Bojamowski, K., Oka, M., and Saijo, N., Synergism between cisplatin and topoisomerase I inhibitors, NB-506 and SN-38, in human small cell lung cancer cells. *Cancer Res.*, 56, 789-793 (1996).
- Harborne, J. B., Two Gossypetin methyl ethers as ultraviolet patterning guides in the Flowers of *Coronilla valentine*. *Phytochemistry*, 20, 1117-1119 (1981).
- Ikuta, A., Nakamura, T., and Urabe, H., Indolopyridoquinazoline, furoquinoline and canthinone type alkaloids from *Phellodendron amurense* Callus Tissues. *Phytochemistry*, 48, 285-291 (1998).
- Ikuta, A., Urabe, H., and Nakamura, T., A new indolopyridoquinazoline-type alkaloid from *Phellodendron amurense* Callus Tissues. *J. Nat. Prod.*, 61, 1012-1014 (1988).
- Ito, Y., Nishitani, E., Konoshima, T., Takasaki, M., Kozuka, M., and Yoshida, T., Flavonoid and benzophenone glycosides from *Coleogyne ramosissima*. *Phytochemistry*, 54, 695-700 (2000).
- Liu, L. F., DNA topoisomerase poisons as antitumor drugs. *Annu. Rev. Biochem.*, 58, 351-375 (1989).
- Pommier, Y., DNA topoisomerase I and II in cancer chemothe-

- rapy: update and perspectives. *Cancer Chemother. Pharmacol.*, 32, 103-108 (1993).
- Potmesil, M., Camptothecins: from bench research to hospital wards. *Cancer Res.*, 54, 1431-1439 (1994).
- Rubinstein, L. V., Shoemaker, R. H., Paul, K. D., Simon, R. M., Tosini, S., Skehan, P., Scudiero, D. A., Monks, A., and Boyd, M. R., Comparison of *in vitro* anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines. *J. Nat. Cancer Inst.*, 82, 1113-1118 (1990).
- Slichenmyer, W. J., Rowinsky, E. K., Donehower, R. C., and Kaufmann, S. H., The current status of camptothecin analogues as antitumor agents. *J. Natl. Cancer Inst.*, 85, 271-291 (1993).
- Sugimoto, T., Miyase, T., Kuroyanagi, M., and Ueno, A., Limonoids and quinolone alkaloids from *Evodia rutaecarpa* Benth. *Chem. Pharm. Bull.*, 36, 4453-4461 (1988).
- Ulubelen, A., Öksüz, S., Halfon, B., Aynehchi, Y., Mabry, T. J., and Matlin, S. A., Flavonoids from *Haplophyllum pedicellatum*, *H. robustum* and *H. glabrinum*. *Phytochemistry*, 23, 2941-2943 (1984).
- Ulubelen, A., Barbara, N., and Tom J. M., Flavonoids from *brickellia chlorolepis* and *B. Dentata*. *Phytochemistry*, 19, 905-908 (1980).
- Wang, J. C., DNA topoisomerases. *Annu. Rev. Biochem.*, 65, 635-692 (1996).
- Wang, Y., Hamburger, M., Gueho, J., and Hostettmann, K., Antimicrobial. Flavonoids from *Psiadia trinervia* and their methylated and acetylated derivatives. *Phytochemistry*, 28, 2323-2327 (1989).
- Wattanapiromsakul, C., Forster, P. I., and Waterman, P. G., Alkaloids and limonoids from *Bouchardatia neurococca*: systematic significance. *Phytochemistry*, 64, 609-615 (2003).
- Tang, Y. Q., Feng, X. Z., and Huang, L., Quinolone alkaloids from *Evodia rutaecarpa*. *Phytochemistry*, 43, 719-722 (1996).
- Zuo, G. Y., Yang, X. S., and Hao, X. J., Two new indole alkaloids from *Evodia rutaecarpa*. *Chinese Chem. Lett.*, 11, 127-128 (2000).