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Constituents of the Fruits and Leaves of *Euodia daniellii*

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Four flavonoid glycosides, flavaprin (**7**), evodioside B (**8**), vitexin (**11**), and hesperidin (**12**), as well as the coumarins bergapten (**1**), xanthotoxin (**2**), and isopimpinellin (**3**), the lignan simplexoside (**10**), the steroids β-sitosterol (**4**) and daucosterol (**5**), the limonoids isolimonexic acid (**6**) and limonin (**9**), and uracil (**13**) and *myo*-inositol (**14**) have been isolated from *Euodia daniellii*. The structures of these compounds were established from spectral data. Among the isolates, bergapten showed cyclooxygenase-2 inhibitory activity with an IC_{50} value of 6.2 μg/ml. Flavonoids isolated from this plant exhibited no cytotoxic activity against the human tumor cell lines, A549, SKOV-3, SKMEL-2, XF498, and HCT15.

Key words: *Euodia daniellii*, Rutaceae, Flavonoids, Coumarins, Steroids, Limonoids, Lignan, Nucleotide, Inositol, Cyclooxygenase-2, Cytotoxicity

INTRODUCTION

Euodia daniellii Hemsley is a plant belonging to Rutaceae family growing in Korea and has been used in folk medicine for gastric inflammation, extermination of noxious insects, and headache. The oil from the fruits of this plant has also been used in various diseases such as dermatitis and scabies (Lee, 1993; Chung, 1970). The plant is known to contain alkaloids, sterols, limonoids and other compounds, and only a limited number of these compounds have been reported (Chung, 1970, 1979; Chung and Ko, 1977; Mitsunaga *et al.*, 1991; Ju *et al.*, 2000). However, reports are sparse on the biological activities of this plant. Recently, we have reported the isolation of cyclooxygenase-2 (COX-2) inhibitory cerebrosides from *Phytolacca* sp. (Kang *et al.*, 2001), flavonoids from *Alpinia officinarum* (Kang *et al.*, 2000) and alkaloid from *Euodia rutaecarpa* (Moon *et al.*, 1999). In our continuing search for COX-2 inhibitory compounds from natural sources, we have isolated fourteen compounds from *Euodia daniellii*. Here, we report the isolation and identification of the constituents from this plant along with a coumarin compound with COX-2 inhibitory activity.

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MATERIALS AND METHODS

Instruments and reagents

Melting points were determined on Mitamura-Riken melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT/IR-5300 spectrometer and UV spectra were obtained with a Hitachi U-3210 UV-VIS spectrophotometer. Mass spectra were taken with a Hewlett Packard model 5989B GC/MS system. NMR spectra were taken on a Varian Gemini-2000 (300 MHz) spectrometer. Optical rotations were performed with Rudolph Research autopolarimeter. Chromatography was performed using Merck silica gel of an appropriate particle size for TLC (precoated, No. 5715), column (70-230 mesh), and MPLC (230-400 mesh).

Plant material

The leaf and fruit of *Euodia daniellii* Hemsley (Rutaceae) were collected in August 2000, at the Yeonkun Medical Campus, Seoul National University, Seoul, Korea and authenticated by Dr. Tae Jin Kim of Korea Research Institute of Bioscience and Biotechnology. A voucher

specimen (SSK0101) was deposited in the herbarium of the Natural Products Research Institute, Seoul National University.

Extraction and isolation

The dried leaves (2 kg) and fruits (2 kg) were extracted three times with MeOH at room temperature. The MeOH extracts from both parts were partitioned successively with *n*-hexane (H), CH_2Cl_2 (C), EtOAc (E) and *n*-BuOH (B), and individual extracts were obtained in yields of 28 (FH), 10 (FC), 10 (FE), and 80 g (FB) from fruits (F) and 48 (LH), 21 (LC), 22 (LE), and 180 g (LB) from leaves (L), respectively. The FH was submitted to column chromatography on silica gel eluted with EtOAc-MeOH (mixtures of gradually increasing polarity, 9 : 1→1 : 1) and 45 fractions (FH 01–45) were collected and evaporated under reduced pressure. Fractions FH-13, FH-15 and FH-17 were purified by recrystallization to afford **1** (30 mg), **2** (20 mg), and **3** (25 mg), respectively. The FC was treated in a similar manner as described above to obtain 25 fractions (FC 01-25). Recrystallization of FC-03, FC-06, and FC-21 to yield 4 (40 mg), **5** (60 mg), and **6** (30 mg), respectively. The FE was chromatographed on a silica gel column by elution with CH_2Cl_2-MeOH-H_2O (7 : 1 : 0.5) to afford 22 fractions (FE 01-22). The FE-06 (1.2 g) and FE-09 (1.5 g) were chromatographed on a silica gel column using CH_2Cl_2-MeOH-H_2O (7 : 2 : 0.5) followed by crystallization from MeOH to afford **7** (15 mg) and **8** (20 mg), respectively. The LC and LE were treated in the same manner as described above to yield 37 and 38 fractions, respectively. Compounds **9** (20 mg) and **10** (25 mg) were obtained from LC-10 and LC-36, respectively, by recrystallization from MeOH. The fraction LE-18 (1 g) was further purified by recrystallization from CH_2Cl_2-MeOH mixture to give **11** (18 mg). Similarly, **12** (20 mg) from LE-17 (1.3 g) and **8** (100 mg) from LE-21 (1.5 g) were also obtained. LE-11 and LE-19 were purified by MPLC using CH_2Cl_2-MeOH mixtures of gradually increasing polarity (99 : 1 → 1 : 5, MeOH 100%) followed by recrystallization from MeOH affording **13** (10 mg) and **14** (7 mg), respectively.

Bergapten (1) white needles, mp 174-175 °C. IR ν_{max}^{KBr} 1732, 1626 cm^{-1}, UV λ_{max} (MeOH) 248 nm (log ε 4.56), 267 (4.56), 308 (4.46). EI-MS (rel. int., %) *m/z* 216 $[M]^+$ (100), 201 $[M-CH_3]^+$ (31.3), 188 $[M-CO]^+$ (11.8), 173 $[M-CH_3-CO]^+$ (58.8), 145 $[M-CH_3-2CO]^+$ (26.5), 89 (10.8). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.27 (1H, d, *J* = 9.6 Hz, H-3), 8.15 (1H, dd, *J* = 0.6, 9.6 Hz, H-4), 7.14 (1H, t, *J* = 0.9 Hz, H-8), 7.59 (1H, d, *J* = 2.4 Hz, H-2′), 7.02 (1H, dd, *J* = 0.9, 2.4 Hz, H-3′), 4.27 (3H, s, OCH_3). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 160.6 (C-2), 111.9 (C-3), 138.7 (C-4), 104.6 (C-4a), 144.3 (C-5), 112.0 (C-6), 157.8 (C-7), 93.1 (C-8), 152.2 (C-8a), 144.3 (C-2′), 105.7 (C-3′), 59.5 (OCH_3).

Xanthotoxin (2) white powder, mp 145-146 °C. IR ν_{max}^{KBr} 1716, 1587 cm^{-1}. UV λ_{max} (MeOH) 248 nm (log ε 4.75), 300 (4.43). EI-MS (rel. int., %) *m/z* 216 $[M]^+$ (100), 201 $[M-CH_3]^+$ (27.4), 188 $[M-CO]^+$ (9.8), 173 $[M-CH_3-CO]^+$ (48), 145 $[M-CH_3-2CO]^+$ (17.6), 89 (17.8). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.38 (1H, d, *J* = 9.3 Hz, H-3), 7.77 (1H, d, *J* = 9.3 Hz, H-4), 7.36 (1H, s, H-5), 7.69 (1H, s, H-2′), 6.83 (1H, s, H-3′), 4.31 (3H, s, OCH_3). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 160.4 (C-2), 114.8 (C-3), 144.3 (C-4), 116.5 (C-4a), 112.8 (C-5), 126.1 (C-6), 147.7 (C-7), 132.8 (C-8), 143.0 (C-8a), 146.6 (C-2′), 106.7 (C-3′), 61.3 (OCH_3).

Isopimpinellin (3) golden-yellow needles, mp 128-130 °C. IR ν_{max}^{KBr} 3430, 1720, 1608, 1479, 1365, 1151, 1072 cm^{-1}. UV λ_{max} (MeOH) 268 nm (log ε 4.47), 310 (4.25). EI-MS (rel. int., %) *m/z* 246 $[M]^+$ (96), 231 $[M-CH_3]^+$ (100), 203 $[M-CH_3-CO]^+$ (15.8), 188 $[M-2CH_3-CO]^+$ (4.26), 175 $[M-CH_3-2CO]^+$ (68.8), 160 $[M-2CH_3-2CO]^+$ (47.5), 147 $[M-CH_3-3CO]^+$ (54.9), 132 $[M-2CH_3-3CO]^+$ (20.5), 119 $[M-CH_3-4CO]^+$ (19.7), 104 $[M-2CH_3-4CO]^+$ (24.6), 76 $[M-2CH_3-5CO]^+$ (49.2). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.30 (1H, d, *J* = 9.6 Hz, H-3), 8.13 (1H, d, *J* = 9.6 Hz, H-4), 7.63 (1H, d, *J* = 2.1 Hz, H-2′), 7.00 (1H, d, *J* = 2.1 Hz. H-3′), 4.17 (6H, s, 2×OCH_3). ^{13}C-NMR (75.5 MHz, $CDCl_3$) δ: 159.8 (C-2), 112.7 (C-3), 139.9 (C-4), 106.9 (C-4a), 144.5 (C-5), 114.6 (C-6), 149.6 (C-7), 127.4 (C-8), 143.3 (C-8a), 146.5 (C-2′), 105.9 (C-3′), 61.5 and 61.0 (OCH_3).

β-Sitosterol (4) white powder, mp 138-140 °C. EI-MS *m/z* 414 $[M]^+$. IR ν_{max}^{KBr} 3410, 1460, 1383, 1060 cm^{-1}.

Daucosterol (5) amorphous powder, mp 285-287 °C. IR ν_{max}^{KBr} 3416, 2934, 1637, 1460, 1383, 1078, 1024 cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.31 (1H, br s, H-6), 4.85 (1H, d, *J* = 8.1 Hz, H-1′), 4.41 (1H, t, *J* = 5.7 Hz, H-6′α), 4.21 (1H, d, *J* = 7.5 Hz, H-6′β), 0.94 (3H, s, CH_3-19), 0.64 (3H, s, CH_3-18). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 141.1 (C-5), 121.9 (C-6), 100.9 (C-1′), 78.6 (C-3), 77.1 (C-3′), 76.9 (C-5′), 73.6 (C-2′), 71.9 (C-4′), 63.0 (C-6′), 56.3 (C-14), 55.6 (C-17), 49.8 (C-9), 45.3 (C-24), 42.0 (C-13), 40.5 (C-4), 38.9 (C-12), 37.0 (C-1), 36.4 (C-10), 35.7 (C-20), 34.4 (C-22), 31.6 (C-7), 31.6 (C-8), 30.2 (C-2), 29.4 (C-25), 28.9 (C-16), 27.9 (C-23), 24.1 (C-15), 22.8 (C-28), 20.8 (C-11), 19.9 (C-27), 19.3 (C-19), 19.1 (C-26), 19.0 (C-21), 18.8 (C-18), 12.0 (C-29).

Isolimonexic acid (6) amorphous powder, mp 298-300 °C. IR ν_{max}^{KBr} 3437, 2922, 1752, 1385 cm^{-1}. EI-MS (rel. int., %) *m/z* 487 $[M-CH_3]^+$ (9.9), 445 (6.6), 415 (5), 373 (11.2), 281 (14.9), 248 (14), 235 (45.5), 207 (52.9), 121 (51.2), 109 (81), 91 (83.5), 55 (100). ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 6.63 (1H, s, H-22), 6.59 (1H, s, H-21), 5.83 (1H, s, H-17), 5.25 (1H, d, *J* = 13.2 Hz, H-19β), 4.70 (1H, d, *J* =

13.2 Hz, H-19α), 4.50 (1H, s, H-15), 4.35 (1H, br s, H-1), 3.30 (1H, t, *J* = 15 Hz, H-6β), 3.18 (1H, dd, *J* = 3.6, 16 Hz, H-2β), 3.07 (1H, br d, *J* = 16 Hz, H-2α), 2.84 (1H, dd, *J* = 3.6, 11.4 Hz, H-9), 2.66 (1H, dd, *J* = 2.7, 15.6 Hz, H-5), 2.56 (1H, dd, *J* = 3, 14.1 Hz, H-6α), 2.27 (1H, m, H-12), 2.04 (2H, m, H-11), 1.87 (1H, m, H-12), 1.37 (3H, s, 18-CH_3), 1.25 (6H, s, 29,30-CH_3), 1.17 (3H, s, 28-CH_3). ^{13}C-NMR (75.5 MHz, pyridine-d_5) δ: 207.6 (C-7), 170.1 (C-3), 169.8 (C-23), 166.4 (C-16), 164.5 (C-20), 122.8 (C-22), 99.3 (C-21), 80.4 (C-4), 79.8 (C-1), 78.8 (C-17), 66.4 (C-14), 65.8 (C-19), 60.4 (C-5), 54.1 (C-15), 52.0 (C-8), 48.2 (C-9), 46.4 (C-10), 38.8 (C-13), 36.9 (C-6), 36.5 (C-2), 30.4 (C-12), 29.8 (C-28), 21.7 (C-29), 20.8 (C-18), 18.9 (C-11), 17.0 (C-30).

Flavaprin (8-isopentenylnaringenin-7-*O*-glucoside) (7) white needles, mp 180-181 °C. $[\alpha]_D^{25}$-11.7° (c 0.1, pyridine). IR ν_{max}^{KBr} 3427, 1638, 1520, 1375, 1263, 1078 cm^{-1}. UV, λ_{max} (MeOH) 287 nm (log ε 4.07), 345 (3.42); λ_{max} (MeONa) 289 (4.12), 351 (3.43); λ_{max} (NaOAc) 285 (4.08), 347 (3.42); λ_{max} (NaOAc+H_3BO_3) 287 (4.10), 346 (3.47); λ_{max} ($AlCl_3$) 312 (4.30), 390 (3.92); λ_{max} ($AlCl_3$+HCl); 310 (4.21), 399 (3.75). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.45 (1H, dd, *J* = 3, 12.3 Hz, H-2), 3.26 (1H, m, H-3), 2.78 (1H, dd, *J* = 3, 17.1 Hz, H-3), 6.25 (1H, s, H-6), 3.40 (1H, m, H-11a), 3.05 (1H, dd, *J* = 7.2, 13.6 Hz, H-11b), 5.10 (1H, br t, *J* = 7.2 Hz, H-12), 1.54 (3H, br s, 14-CH_3), 1.57 (3H, br s, 15-CH_3), 7.31 (2H, d, *J* = 8.7 Hz, H-2′, 6′), 6.78 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 4.90 (1H, d, *J* = 7.5 Hz, H-1″), 12.07 (1H, s, 5-OH), 9.57 (1H, s, 4′-OH). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 78.5 (C-2), 42.3 (C-3), 197.8 (C-4), 161.1 (C-5), 95.3 (C-6), 163.2 (C-7), 109.1 (C-8), 157.6 (C-9), 103.3 (C-10), 21.7 (C-11), 122.8 (C-12), 129.4 (C-13), 25.7 (C-14), 17.8 (C-15), 130.4 (C-1′), 129.2 (C-2′, 6′), 115.3 (C-3′, 5′), 159.2 (C-4′), 100.4 (C-1″), 73.5 (C-2″), 76.7 (C-3″), 69.8 (C-4″), 77.4 (C-5″), 60.8 (C-6″).

Evodioside B (Flavaprenin 7,4′-diglucoside) (8) white powder, mp 243-245 °C. $[\alpha]_D^{25}$-47° (c 0.1, pyridine). IR ν_{max}^{KBr} 3393, 1638, 1514, 1375, 1230, 1074 cm^{-1}. UV, λ_{max} (MeOH) 288 nm (log ε 4.47), 345 (3.86); λ_{max} (MeONa) 289 (4.48), 358 (3.93); λ_{max} (NaOAc) 288 (4.63), 344 (4.06); λ_{max} (NaOAc+H_3BO_3) 288 (4.61), 340 (3.84); λ_{max} ($AlCl_3$) 313 (4.68), 401 (3.95); λ_{max} ($AlCl_3$+HCl) 310 (4.66), 397 (3.99). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 5.54 (1H, dd, *J* = 3, 10.3 Hz, H-2), 3.25 (1H, m, H-3), 2.80 (1H, dd, *J* = 3, 17.4 Hz, H-3), 6.26 (1H, s, H-6), 3.37 (1H, m, H-11a), 3.05 (1H, dd, *J* = 7.1, 14.1 Hz, H-11b), 5.10 (1H, br t, *J* = 7.1 Hz, H-12), 1.56 (3H, s, 14-CH_3), 1.57 (3H, s, 15-CH_3), 7.42 (2H, d, *J* = 8.7 Hz, H-2′, 6′), 7.05 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 4.91 (1H, d, *J* = 6.9 Hz, H-1″), 4.89 (1H, d, *J* = 7.2 Hz, H-1‴), 12.06 (1H, s, 5-OH). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 78.3 (C-2), 42.4 (C-3), 197.6 (C-4), 161.4 (C-5), 95.4 (C-6), 163.3 (C-7), 109.1 (C-8), 157.6 (C-9), 103.3 (C-10), 21.7 (C-11), 122.7 (C-12), 130.5 (C-13), 25.7 (C-14), 17.9 (C-15), 132.2 (C-1′), 128.0 (C-2′, 6′), 116.3 (C-3′, 5′), 159.0 (C-4′), 100.4 (C-1″/1‴) 73.4/73.5 (C-2″/2‴), 76.8 (C-3″/3‴), 69.8/69.9 (C-4″/4‴), 77.2/77.4 (C-5″/5‴), 60.9 (C-6″/6‴).

Limonin (9) amorphous powder, mp 299-300 °C. IR ν_{max}^{KBr} 3429, 2968, 1757, 1385 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.42 (1H, dd, *J* = 0.6, 1.5 Hz, H-21), 7.40 (1H, t, *J* = 1.8 Hz, H-23), 6.34 (1H, ddd, *J* = 0.6, 0.9, 1.5 Hz, H-22), 5.47 (1H, s, H-17), 4.77 (1H, d, *J* = 13.2 Hz, H-19β), 4.46 (1H, d, *J* = 13.2, H-19α), 4.04 (1H, s, H-15), 4.04 (1H, s, H-1), 2.98 (1H, dd, *J* = 3.9, 16.8 Hz, H-2β), 2.86 (1H, dd, *J* = 14.7, 15.9 Hz, H-6β), 2.68 (1H, dd, *J* = 1.8, 16.8 Hz, H-2α), 2.56 (1H, dd, *J* = 2.5, 12.0 Hz, H-9), 2.47 (1H, dd, *J* = 3.3, 14.4 Hz, H-6α), 2.22 (1H, dd, *J* = 3.3, 15.9 Hz, H-5), 1.90 (1H, m, H-11β), 1.83 (1H, m, H-11α), 1.78 (1H, m, H-12β), 1.51 (1H, m, H-12α), 1.30 (3H, s , H-28), 1.18 (3H, s, H-29), 1.17 (3H, s, H-18), 1.07 (3H, s, H-30).

Simplexoside (10) amorphous powder, mp 298-300 °C. $[\alpha]_D^{25}$-9° (c 0.1, MeOH). IR ν_{max}^{KBr} 3360, 2932, 1595, 1344 cm^{-1}. EI-MS (rel. int., %) *m/z* 356 $[M\text{-}Glc]^+$ (16.5), 203 (6.7), 161 (16.5), 151 (65.3), 149 (86), 135 (38), 131 (39.7), 77 (54.5), 60 (100). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.03 (1H, d, *J* = 8.4 Hz, H-5′), 6.94 (1H, d, *J* = 1.8 Hz, H-2′), 6.91 (1H, br s, H-2), 6.85 (3H, m, H-5, 6, 6′), 5.98 (2H, s, OCH_2O), 4.86 (1H, d, *J* = 7.2 Hz, H-1″), 4.65 (1H, d, *J* = 3.6 Hz, H-7 or H-7′), 4.64 (1H, d, *J* = 4.2 Hz, H-7 or H-7′), 4.12 (2H, dd, *J* = 6.9, 9.0 Hz, H-9/9′), 3.76 (3H, s, OCH_3), 3.75 (2H, m, H-9/9′), 3.65 (1H, br dd, *J* = 5.7, 10.5 Hz, H-6″a), 3.44 (1H, dd, *J* = 6.0, 11.7 Hz, H-6 b), 3.01 (2H, m, H-8/8′). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 149.1 (C-4′), 147.6 (C-3), 146.7 (C-4), 146.0 (C-3′), 135.7 (C-1′), 135.3 (C-1), 119.6 (C-6), 118.3 (C-6′), 115.4 (C-5′), 110.7 (C-2′), 108.1 (C-5), 106.7 (C-2), 101.1 (OCH_2O), 100.4 (C-1″), 85.0/85.1 (C-7/7′), 77.2 (C-5″), 77.0 (C-3″), 73.4 (C-2″), 71.2 (C-9/9′), 69.9 (C-4″), 60.9 (C-6″), 55.9 (OCH_3), 53.8/53.9 (C-8/8′).

Vitexin (11) yellowish powder, mp 259-260 °C. $[\alpha]_D^{25}$-9.1° (c 0.1, pyridine). IR ν_{max}^{KBr} 3387, 2920, 1655, 1570, 1508, 1363, 1092 cm^{-1}. UV, λ_{max} (MeOH) 270 nm (log ε 4.29), 334 (4.33). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 13.16 (1H, s, OH-5), 8.02 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 6.88 (2H, d, *J* = 8.7 Hz, H-3′, 5′), 6.77 (1H, s, H-3), 6.26 (1H, s, H-6), 4.66 (1H, d, *J* = 9.9 Hz, H-1″). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 182.3 (C-4), 164.1 (C-2), 162.8 (C-7), 161.4 (C-4′), 160.6 (C-5), 156.2 (C-9), 129.1 (C-2′, 6′), 121.8 (C-1′), 116.0 (C-3′, 5′), 104.8 (C-8), 104.2 (C-10), 102.6 (C-3), 98.3 (C-6), 82.0 (C-5″), 78.8 (C-3″), 73.6 (C-1″), 71.0

(C-2″) 70.7 (C-4″), 61.5 (C-6″).

Hesperidin (12) amorphous powder, mp 269-270 °C. $[\alpha]_D^{25}$-20.1° (c 0.1, pyridine). IR ν_{max}^{KBr} 3474, 2918, 1649, 1609, 1096, 1069 cm^{-1}. UV, λ_{max} (MeOH) 282 nm (log ε 3.85), 334 (3.03); λ_{max} (MeONa) 283 (3.91), 337 (3.06); λ_{max} (NaOAc) 283 (4.27), 334 (3.55); λ_{max} (NaOAc+H_3BO_3) 283 (4.28), 332 (3.56); λ_{max} ($AlCl_3$) 307 (4.05), 386 (3.42); λ_{max} ($AlCl_3$+HCl) 306 (4.02), 387 (3.42). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 12.02 (1H, s, OH-5), 9.10 (1H, br s, OH-3′), 6.94 (1H, d, *J* = 8.4 Hz, H-5′), 6.92 (1H, d, *J* = 2.4 Hz, H-2′), 6.87 (1H, dd, *J* = 1.5, 8.4 Hz, H-6′), 6.13 (1H, s, H-8), 6.11 (1H, s, H-6), 5.50 (1H, dd, *J* = 3.2, 12.0 Hz, H-2). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 197.2 (C-4), 165.3 (C-7), 163.3 (C-5), 162.7 (C-9), 148.2 (C-3′), 146.7 (C-4′), 131.1 (C-1′), 118.2 (C-6′), 114.4 (C-2′), 112.2 (C-5′), 103.5 (C-10), 100.8 (C-1‴), 99.7 (C-1″), 96.6 (C-6), 95.8 (C-8), 78.6 (C-2), 76.5 (C-3″), 75.7 (C-5″), 73.2 (C-2″), 72.3 (C-4‴), 70.9 (C-4″), 70.5 (C-2‴), 69.8 (C-3‴), 68.5 (C-5‴), 66.3 (C-3″), 55.9 (OCH_3), 42.3 (C-3), 18.1 (C-6‴).

Uracil (13) pale yellowish powder, mp 330-332 °C. EI-MS (rel. int., %) *m/z* 112 $[M]^+$ (69.4), 84 (4.1), 69 (100), 57 (18.5). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.0 (1H, s, 3-NH), 10.8 (1H, s, 1-NH), 7.36 (1H, d, *J* = 7.8 Hz, H-6), 5.43 (1H, d, *J* = 7.5 Hz, H-5). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 164.5 (C-4), 151.7 (C-2), 142.3 (C-6), 100.4 (C-5).

***myo*-Inositol (14)** amorphous, mp 210-211 °C. IR ν_{max}^{KBr} 3436, 1637, 1053 cm^{-1}. ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 75.4 (C-5), 73.0 (C-1, 3), 72.9 (C-2), 72.2 (C-4, 6).

Hydrolysis of 10 with β-glucosidase : Enzymatic hydrolysis of **10** with β-glucosidase afforded D-glucose and an aglucon. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 8.49 (1H, s, OH-4′), 6.91~6.69 (6H, m, H-2, 2′, 5, 5′, 6, 6′), 5.98 (2H, s, OCH_2O), 4.65 (1H, d, *J* = 4.5 Hz, H-7 or 7′), 4.57 (1H, d, *J* = 4.2 Hz, H-7 or 7′), 4.10 (2H, dd, *J* = 6.3, 9.0 Hz, H-9/9′), 3.74 (3H, s, OCH_3), 3.72 (2H, dd, *J* = 3.6, 9.0 Hz, H-9/9′), 3.00 (2H, m, H-8, 8′). ^{13}C-NMR (75.5 MHz, DMSO-d_6) δ: 147.8 (C-3), 147.6 (C-3′), 146.7 (C-4), 146.6 (C-4′), 135.7 (C-1), 131.9 (C-1′), 119.6 (C-6), 118.9 (C-6′), 115.4 (C-5′), 110.7 (C-2′), 108.2 (C-5), 106.8 (C-2), 101.1 (OCH_2O), 85.1 (C-7), 85.0 (C-7′), 71.2 (C-9/9′), 55.9 (OCH_3) 53.8, 53.9 (C-8/8′).

Biological activities

COX-2 inhibition test : The COX-2 inhibition activity was measured using aspirin-treated mouse bone marrow derived from mast cells (BMMC). In brief, BMMC from male BALB/cJ mice were cultured for up to 5 weeks in 50% enriched medium (RPMI 1640 containing antibiotics, 2 mM L-glutamine, 0.1 mM nonessential amino acids and 10% fetal bovine serum) and 50% WEHI-3 cell conditioned medium as a source of interleukin-3. After 3 weeks, BMMC were suspended in enriched medium and preincubated with 10 μg/ml aspirin for 2 h in order to inactivate preexisting COX-1. The cells were activated with c-*kit* ligand (100 ng/ml), IL-10 (100 U/ml) and lipopolysaccharide (100 μg/ml) in the presence/absence of plant extract or the isolated compounds previously dissolved in dimethylsulfoxide (DMSO). All reactions were stopped by centrifugation at 120 g at 4 °C for 5 min. The supernatant and cell pellet were immediately frozen in liquid N_2 and stored at -80 °C for further analysis. Concentrations of prostaglandin D_2 in the supernatant were measured using a PGD_2 assay kit (Amersham, Buckinghamshire, U.K.). Data were the arithmetic mean of triplicate determinations (Moon *et al.*, 1999; Kang *et al.*, 2001).

***In vitro* anticancer activity test :** Anticancer assay was performed using five different human tumor cell lines, A-549 (human lung), SK-OV-3 (human ovarian), SK-MEL-2 (human melanoma), HCT-15 (human colon), XF-498 (human CNS) which were purchased from the National Cancer Institute (NCI) in U.S.A.

The cells were grown at 37 °C in RPMI 1640 medium supplemented with 10% FBS and separated using PBS containing 0.25% trypsin and 3 mM EDTA. 5×10^3~2×10^4 cells were added to each well of 96 well plates and incubated at 37 °C for 24 h. Each compound was dissolved in DMSO and diluted with the above mentioned medium at five different concentrations with the range of 0.1~30 μg/ml (the DMSO concentration was set to be below 0.5 %) and filtrated using 0.22 mg filter. After removing the well medium by aspiration, a 200 ml portion of the solution was added to above well plates which were placed in 5% CO_2 incubator for 48 h. The protein stain assay was performed according to SRB method.

RESULT AND DISCUSSION

Previous phytochemical investigations on this plant have resulted in the isolation of bergapten (Chung, 1970; Ju *et al.*, 2000), alkaloids (Chung, 1970; Mitsunaga *et al.*, 1991), triglycerides (Chung and Ko, 1977), and limonin (Ju *et al.*, 2000; Mitsunaga *et al.*, 1991). More recently study of this species has resulted in the isolation of squalene and 2-oxotridecanyl acetate (Ju *et al.*, 2000). However, the constituents responsible for biological activities have not been clarified. Cyclooxygenase is an important enzyme which catalyzes the formation of mediators involved in the inflammatory process. Both the *n*-hexane and the dichloromethane extract of the fruits and the EtOAc extract of the leaves of *E. daniellii* showed inhibitory activity on COX-2 as shown in Table I. Repeated chromatography of the active fractions resulted in the isolation of fourteen compounds.

Three coumarins bergapten (**1**), xanthotoxin (**2**), and isopimpinellin (**3**) (Elgamal *et al.*, 1979; Razdan *et al.*, 1987; Macias *et al.*, 1990), and a limonoid isolimonexic acid (**6**) (Lee *et al.*, 1999; Ng *et al.*, 1987) from hexane and dichloromethane extract of the fruits were isolated and determined by means of spectroscopic methods and confirmed by direct comparison with reported data. On the other hand, three flavonoids evodioside B (**8**) (Arisawa *et al.*, 1993), vitexin (**11**), and hesperidin (**12**) (Kang and Son, 2000) from the EtOAc extract of leaves were isolated. The COX-2 inhibitory activity of the isolated compounds was determined. Among the compounds isolated from the active fractions, only bergapten (**1**) showed COX-2 inhibitory activity with an IC_{50} value of 6.2 μg/ml as indicated in Fig. 1. Other coumarins (**2**, **3**), daucosterol (**5**), isolimonexic acid (**6**), and flavonoids (**8**, **11**, and **12**) possessed no inhibitory effect on COX-2. Very recently, Liu *et al.* (1998) reported that bergapten (**1**) exhibited no inhibitory activities on COX-1 and 5-LO. In this study, we have also isolated and identified additional known components

Table I. Inhibition (%) of PGD_2 generation by extracts and fractions from *E. daniellii*

Parts	Extract/fractions	PGD_2 generation (12.5 μg/ml)
Leaf	MeOH	37.8
	n-hexane	14.6
	dichloromethane	36.3
	EtOAc	40.8
	n-BuOH	48.8
Fruit	MeOH	31.4
	n-hexane	64.5
	dichloromethane	52.4
	EtOAc	33.1
	n-BuOH	25.3

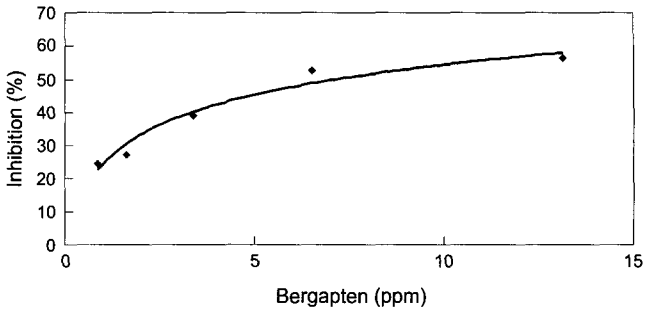
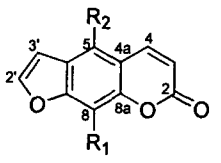


Fig . 1. Cyclooxygenase-2 inhibitory activity of bergapten (**1**)

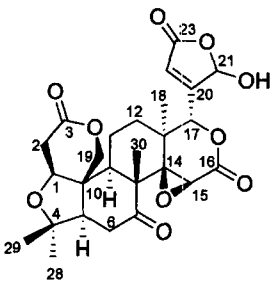
Table II. Cytotoxicity of flavonoids isolated from *E. daniellii*

Compounds	ED_{50} (μg/ml)*				
	A549	SKOV-3	SKMEL-2	XF-498	HCT-15
Doxorubicin	0.08	0.35	0.02	0.31	0.07
Vitexin (**11**)	>30	>30	>30	>30	>30
Hesperidin (**12**)	>30	>30	>30	>30	>30
Flavaprin (**7**)	>30	21.04	29.15	>30	>30
Evodioside B (**8**)	>30	>30	>30	>30	>30

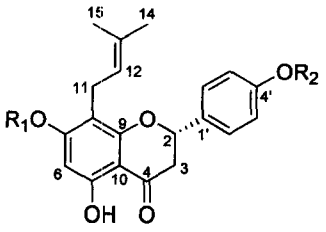
*Results are mean of triplicate.



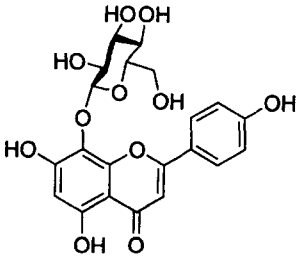
1. Bergapten R_1=H R_2=OCH_3
2. Xanthotoxin R_1=OCH_3 R_2=H
3. Isopimpinellin R_1=R_2=OCH_3



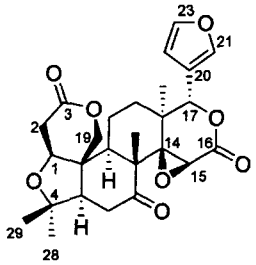
6. Isolimonexic acid



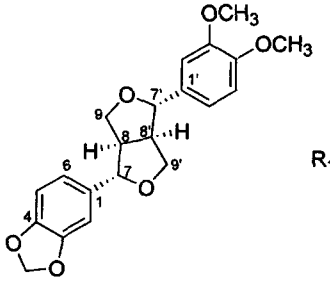
7. Flavaprin R_1=Glc R_2=H
8. Evodioside B R_1=R_2=Glc



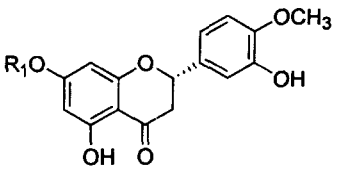
11. Vitexin



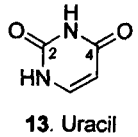
9. Limonin



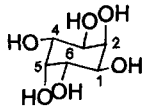
10. Simplexoside



12. Hesperidin R_1=Glc^6—Rha



13. Uracil



14. *myo*-Inositol

limonin (**9**), simplexoside (**10**) (Ghosal *et al.*, 1980), vitexin (**11**), uracil (**13**) (Pretsch *et al*, 2000; Schneider *et al.*, 1992) and *myo*-inositol (**14**) (Breitmaier and Voelter, 1987) in leaves and β-sitosterol (**4**) and daucosterol (**5**), flavaprin (**7**) (Mitchomn *et al.*, 1974), and evodioside B (**8**) in fruits. We also identified for the first time many compounds such as xanthoxin (**2**), isopimpinellin (**3**), isolimonexic acid (**6**), flavaprin (**7**), hesperidin (**12**) and *myo*-inositol (**14**) which have never been reported in this genus. This is the first report of the isolation and characterization of simplexoside (**10**), vitexin (**11**), and uracil (**13**) from the Rutaceae family.

Flavonoid C-glycosides are quite rare in the genus Rutaceae. To date, only a few species of the Rutaceae have been shown to contain this form of metabolites (Jay, 1994; Chopin and Dellamonica, 1988; Chopin *et al.*, 1982). The biosynthesis of linear furocoumarins in Rutaceae is well documented (Gray and Waterman, 1978). Limonoids and furocoumarins have been found in other *Euodia* species. The finding of both limonoids and furocoumarins in *Euodia daniellii* characterizes this species as being chemically in accordance with other *Euodia* genus and the Rutaceae family.

On the other hand, flavonoids have been reported to have several pharmacological activities, such as antitumor activities anticarcinogenesis, and DNA-repair deficiencies (Konoshima and Takasaki, 2000; Middleton Jr. and Kandaswami, 1994). However, flavonoids isolated from this plant exhibited no cytotoxicity against the human tumor cell lines, A549, SKOV-3, SKMEL-2, XF498, and HCT15 as summarized in Table II.

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REFERENCES

Arisawa, M., Horiuchi, T., Hayashi, T., Tezuka, Y., Kikuchi, T. and Morita, N., Studies on Constituents of *Evodia rutaecarpa* (Rutaceae). I. Constituents of the Leaves. *Chem. Pharm. Bull.*, 41, 1472-1474 (1993).

Breitmaier, E. and Voelter, W., *Carbon-13 NMR spectroscopy* (3rd Ed.). VCH, New York, (1987).

Chopin, J., Bouillant, M. L., and Besson, E., C-Glycosylflavonoids, In Harborne, J. B. and Mabry, T. J. (Eds.). *The Flavonoids, Advances in Research.* Chapman & Hall, London, pp. 449-503, (1982).

Chopin, J. and Dellamonica, G., C-Glycosylflavonoids, In Harborne, J. B. (Ed.). *The Flavonoids, Advances in Research since 1980.* Chapman & Hall, London, pp. 63-97 (1988).

Chung, B. S., Studies on the components of *Evodia daniellii* Hemsley in Korea. *Kor. J. Pharmacogn.*, 1, 119-124 (1970).

Chung, B. S., Studies on the sterol components of *Evodia daniellii* Hemsley in Korea (II). *Kor. J. Pharmacogn.*, 10, 9-12 (1979).

Chung, B. S. and Ko, Y. S., Studies on the Triglyceride Composition of *Evodia daniellii* Hemsley in Korea (III). *Kor. J. Pharmacogn.*, 8, 149-153 (1977).

Elgamal, M. H. A., Elewa, N. H., Elkhrisy, E. A. M., and Duddeck, H., ^{13}C NMR chemical shifts and carbon-proton coupling constants of some furocoumarins and furochromones. *Phytochemistry,* 18, 139-143 (1979).

Ghosal, S., Banerjee, S. and Jaiswal, D., New Furofurano Lignans from *Justicia simplex*. *Phytochemistry,* 19, 332-334 (1980).

Gray, A. I. and Waterman, P. G., Coumarins in the Rutaceae. *Phytochemistry,* 17, 845-864 (1978).

Grimshaw, J. and Lamer-Zarawska, E., An isopentenylflavanone from *Evodia rutaecarpa*. *Phytochemistry,* 14, 838-839 (1975).

Jay, M., C-Glycosylflavonoids, In Harborne, J. B. (Ed.). *The Flavonoids, Advances in Research since 1986.* Chapman & Hall, London, pp. 57-93, (1994).

Ju, H. K., Hwang, B.Y., Ahn, B. T., Kim, M. J., Choi, W. H., Cho, B. J., Ro, J. S. and Lee, K. S., Chemical Components of *Evodia daniellii*. *Kor. J. Pharmacogn.*, 31, 300-305 (2000).

Kang, S. S. and Son, K. H., *Structure Elucidation of Natural Products by Spectroscopy.* Seoul National University Press, Seoul, (2000).

Kang, S. S., Kim, J. S., Son, K. H., Kim, H. P., and Chang, H. W., Isolation of Cyclooxygenase-2 Inhibitors from *Alpinia officinarum*. *Kor. J. Pharmacogn.*, 31, 57-62 (2000).

Kang, S. S., Kim, J. S., Son, K. H., Kim, H. P., and Chang, H. W., Cyclooxygenase-2 Inhibitory Cerebrosides from Phytolaccae Radix. *Chem. Pharm. Bull.*, 49, 321-323 (2001).

Konoshima, T. and Takasaki, M., Anti-Tumor-Promoting Activities (Cancer Chemopreventive Activities) of Natural Products. *Stud. Nat. Prod. Chem.*, 24, 215-267 (2000).

Lee, T. B., *Illustrated Flora of Korea.* HyangMun Press, Seoul, p. 503, (1993).

Lee, S.-Y., Morita, H., Takeya, K., Itokawa, H. and Fukaya, H., Limonoids from *Citrus nippokoreana*. *Nat. Med.*, 53, 255-258 (1999).

Liu, J.H., Zschocke, S. and Bauer, R., Inhibitory Effects of *Angelica pubescens* f. *biserrata* on 5-Lipoxygenase and Cyclooxygenase. *Planta Med.*, 64, 525-529 (1998).

Macias, F. A., Massanet, G. M., Rodriguez-Luis, F. and Salva, J., ^{13}C NMR of Coumarins. IV-Furanocoumarins. *Magn. Reson. Chem.*, 28, 219-222 (1990).

Middleton Jr, E., and Kandaswami, C., The Impact of Plant Flavonoids on Mammalian Biology: Implications for Immunity, Inflammation and Cancer, In Harborne, J. B. (Ed.). *The Flavonoids, Advances in Research since 1986.* Chapman &

Hall, London, pp. 619-652, (1994).

Mirhomn, Y. W., Reisch, J., Gellert, M., Csedo, K., Novak, I. and Szendrei, K., Flavaprine. A new flavonone glycoside from *Evodia hupehensis. Acta Pharm. Hung.*, Suppl. 32-35 (1974).

Mitsunaga, K., Koike, K. and Ohmoto, T., Constituents of the Bark of *Evodia daniellii. Shoyakugaku Zasshi,* 45, 263-265 (1991).

Moon, T. C., Murakami. M., Kudo, I., Son, K. H., Kim, H. P., Kang, S. S. and Chang, H. W., A new Class of COX-2 Inhibitor, Rutaecarpine from *Evodia rutaecarpa. Inflammation Res.*, 48, 621-625 (1999).

Ng, K. M., Gray, A. I. and Waterman, P. G., Limonoids, Alkaloids, and a Coumarin from the Root and Stem Barks of *Tetradium glabrifolium. J. Nat. Prod.*, 50, 1160-1163 (1987).

Pretsch, E., Buhlmann, P. and Affolter, C. *Structure Determination of Organic Compounds.* Springer, Berlin, (2000).

Radzan, T. K., Qadri, B., Harkar, S. and Waight, E. S., Chromones and Coumarins from *Skimmia laureola. Phytochemistry*, 26, 2063-2069 (1987).

Schneider, H.-J., Blatter, T., Palm, B., Pfingstag, U., Rüdiger, V. and Theis, I., Complexation of Nucleosides, Nucleotides, and Analogs in an Azoniacyclophane. Van der Waals and Electrostatic Binding Increments and NMR Shielding Effects. *J. Am. Chem. Soc.*, 114, 7704-7708 (1992).