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Cyclooxygenase-2 Inhibitor from *Evodia rutaecarpa*

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Abstract – By bioassay guided fractionation followed by chromatographic separation of the MeOH extract from the fruit of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), a new cyclooxygenase-2 inhibitor was isolated and identified as an alkaloid, rutaecarpine. Other alkaloids such as evodiamine and dehydroevodiamine together with limonoids were also isolated and characterized.
Key words – *Evodia rutaecarpa*, Rutaceae, alkaloid, rutaecarpine, cyclooxygenase-2 inhibitory activity.

Introduction

The Evodia fruit has been used in traditional Chinese prescriptions and there were many previous studies on the Evodia fruit (Tang and Eisenbrand, 1992). A number of indolopyridoquinazoline alkaloids such as evodiamine, rutaecarpine and dehydroevodiamine have been isolated from the fruits of *E. rutaecarpa* (Tang *et al.*, 1997; Shoji *et al.*, 1988; Danieli *et al.*, 1979). In addition, some quinolone alkaloids (Shin *et al.*, 1998; Kamikado *et al.*, 1976; Sugimoto *et al.*, 1988a), other nitrogen-containing compounds (Shoji *et al.*, 1988; Takagi *et al.*, 1979), limonoids (Sugimoto *et al.*, 1988a; 1988b) and flavonoids (Kang *et al.*, 1997) were also isolated. And some of them were reported to possess a multifaceted biological/pharmacological activities *in vitro* and *in vivo* (Chiou *et al.*, 1997; Haji *et al.*, 1994; Itokawa *et al.*, 1990; Kano *et al.*, 1991; Kim *et al.*, 1998; King *et al.*, 1980; Matsuda *et al.*, 1997; 1998a,b; Shoji *et al.*, 1986; Yang *et al.*, 1990; Yamahara *et al.*, 1989). Recently we also reported that dehydroevodiamine showed anticholinesterase and antiamnetic activities (Park *et al.*, 1996). During the screening programme of Korean medicinal plants for anti-inflammatory activity we have found considerable inhibitory activity of cyclooxygenase-2 (COX-2) in MeOH extract of *E. rutaecarpa* (Moon *et al.*, 1998). From this extract, bioassay guided fractionation followed by chromatographic separation of the extract led to the isolation of a new cyclooxygenase-2 inhibitor, rutaecarpine.

This paper describes the isolation and structure elucidation of the alkaloids and limonoids from *E. rutaecarpa* and the inhibitory activity of cyclooxygenase-2 by rutaecarpine.

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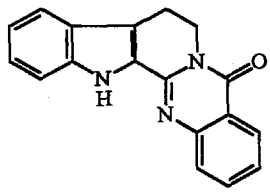
Experimental

General experimental procedures and Plant material – General experimental procedures and plant materials were described in a previous paper of Kang *et al.*(1997).

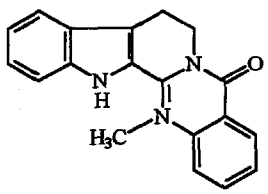
Extraction and isolation – The fruits of *E. rutaecarpa* (10 kg) were extracted three times with 80% MeOH under reflux. The MeOH extract was evaporated under reduced pressure to dryness. The dried extract was partitioned between H_2O and CH_2Cl_2 and gave 240 g of the CH_2Cl_2 extract. The CH_2Cl_2 fraction was subjected to silica gel (Merck, No. 7734, 3 kg) column chromatography eluting with $CHCl_3$, $CHCl_3$-acetone (30 : 1, 20 : 1, 10 : 1) and then $CHCl_3$-MeOH (10 : 1, 1 : 1) to give 20 subfractions. Subfraction 6 was recrystallized from MeOH to yield compound **1** (0.9 g) as pale yellowish needles. Subfractions 9 and 17 were treated with the same manner as subfraction 6 to give compound **2** (1.2 g) and **3** (0.63 g) as pale yellowish plate and as pale yellowish powder, respectively. Subfraction 11 was rechromatographed on silica gel (Merck, No. 7729) with hexane-EtOAc (5:8) to afford compound **4** (30 mg) as an amorphous powder. Subfraction 12 was recrystallized from MeOH to give compound **5** (150 mg) as needles.

Compound 1 – mp 261~262°; UV, λ_{max} (MeOH) nm 240 (sh), 288, 330, 343, 360; IR, ν_{max} (KBr) 3345, 1655, 1599, 1471, 1327, 1229, 731 cm^{-1}; MS, *m/z* (rel. int.) 287 $[M]^+$(100), 259 $[M\text{-}CO]^+$(13.3), 258 $[M\text{-}CHO]^+$(14.8), 144 (25.1), 141 (10.2), 130 (26.5), 77 (23.0); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 3.17 (2H, t, *J*=6.9Hz, H-6), 4.45 (2H, t, *J*=6.9Hz, H-5), 7.09 (1H, brt, *J*=7.7Hz, H-10), 7.26 (1H, dt, *J*=0.9, 7.0 Hz, H-11), 7.46 (1H, dt, *J*=0.9, 7.5Hz, H-12), 7.49 (1H, brd, *J*=7.9 Hz, H-18), 7.64 (1H, brd, *J*=7.9 Hz, H-9), 7.68 (1H, brd, *J*=7.9Hz, H-16), 7.80 (1H, dt, *J*=1.3, 7.9 Hz, H-17), 8.16 (1H, dd, *J*=1.3, 7.9Hz, H-19), 11.83 (1H, s, NH); ^{13}C-NMR (75.5MHz, DMSO-d_6) δ: 18.88, 40.76, 112.51, 117.80, 119.67, 119.86, 120.66, 124.66, 124.85, 125.89, 126.38, 126.52, 127.04, 134.32, 138.63, 145.22, 147.32, 160.54.



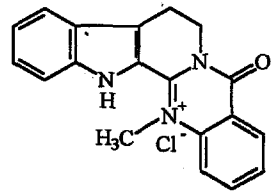
Rutaecarpine (1)

Compound 2 – mp 274 ~8°; UV, λ_{max} (MeOH) nm 225, 268, 282, 291; IR, ν_{max} (KBr) 3234, 1630, 1607, 1512, 1310, 1281, 1233, 1167, 747, 725cm^{-1}; MS, *m/z* (rel. int.) 303 $[M]^+$(35.9), 302 $[M\text{-}H]^+$(25.1), 288 $[M\text{-}CH_3]^+$(8.8), 274 $[M\text{-}CHO]^+$(19.2), 170 (30.9), 169 (100), 134 (81.8), 133 (13.1); ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 2.80 (1H, dd, *J*=5.1, 15.5Hz, H-6a), 2.88 (3H, s, CH_3), 2.90 (1H, ddd, *J*=1.6, 5.1, 15.5Hz, H-6b), 3.20 (1H, ddd, *J*=1.6, 5.5, 13.0Hz, H-5a), 4.65 (1H, ddd, *J*=1.6, 5.5, 13.0Hz, H-5b), 6.11 (1H, s, H-3), 6.97 (1H, dt, *J*=1.3, 7.8Hz, H-18), 7.01 (1H, brt, *J*=7.8Hz, H-11), 7.04 (1H, brt, *J*=8.1Hz, H-12), 7.10 (1H, dd, *J*=1.1, 8.1Hz, H-10), 7.37 (1H, brd, *J*=8.1Hz, H-9), 7.46 (1H, dd, *J*=1.7, 8.0Hz, H-16), 7.48 (1H, dd, *J*=1.8, 7.8Hz, H-17), 7.82 (1H, dd, *J*=1.5, 7.8Hz, H-19), 11.00 (1H, s, NH); ^{13}C-NMR (75.5MHz, DMSO-d_6) δ: 19.43 (CH_2), 36.37 (CH_3), 40.77 (CH_2), 69.68 (CH), 111.45 (C), 111.58 (CH), 117.42 (CH), 118.13 (CH), 118.82 (CH), 119.26 (C), 120.21 (CH), 121.78 (CH), 125.90 (C), 127.92 (CH), 130.50 (C), 133.33 (CH), 136.44 (C), 148.74 (C), 164.14 (C).



Evodiamine (2)

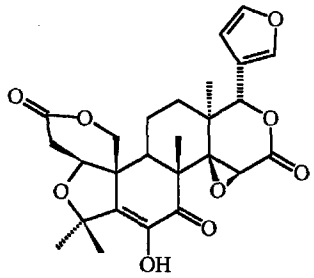
Compound 3 – mp 249~251°; UV, λ_{max} (MeOH) nm (log ε) 246 (4.08), 305 (sh, 3.64), 314 (3.71), 365 (4.36); IR, ν_{max} (KBr) 3437, 3235, 1709, 1611, 1557, 1501, 1350, 1339, 1285, 1219, 1103, 766, 721, 687 cm^{-1}; MS, *m/z* (rel. int.) 302 $[M\text{-}Cl]^+$(100), 287 $[302\text{-}CH_3]^+$(83.6), 273 $[302\text{-}CHO]^+$(43.7), 258 (23.8), 246 (35.3), 156 (9.7), 77 (45.2); ^{1}H-NMR (300MHz, DMSO-d_6) δ: 3.35 (2H, t, *J*=6.9Hz, H-6), 4.47 (2H, t, *J*=6.9Hz, H-5), 4.37 (3H, s, CH_3), 7.29 (1H, brt, *J*=7.6Hz, H-10), 7.54 (1H, brt, *J*=7.6Hz, H-11), 7.69 (1H, brd, *J*=8.6Hz, H-12), 7.81 (1H, dt, *J*=1.2, 8.5Hz, H-18), 7.89 (1H, brt, *J*=8.2Hz, H-9), 8.14 (1H, dt, *J*=1.1, 8.6Hz, H-17), 8.20 (1H, brd, *J*=8.3Hz, H-16), 8.36 (1H, brd, *J*=7.8Hz, H-19), 12.33 (1H, s, NH); ^{13}C-NMR (75.5MHz, DMSO-d_6) δ: 18.44 (C-6), 40.64 (CH_3), 42.02 (C-5), 113.37 (C-12), 118.44 (C-16), 118.65 (C-20), 120.05 (C-2), 121.50 (C-10), 121.56 (C-9), 123.26 (C-8), 127.60 (C-19), 128.55 (C-18), 128.75 (C-11), 130.38 (C-7), 136.57 (C-17), 139.62 (C-15), 141.30 (C-13), 149.94 (C-3), 158.09 (CO).



Dehydroevodiamine (3)

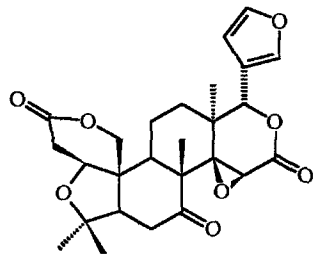
Compound 4 – UV, λ_{max} (MeOH) nm (log ε) 276 (3.59); IR, ν_{max} (KBr) 3437, 1748, 1686, 1655, 1358, 1287, 1032, 914, 876 cm^{-1}; MS, *m/z* 484 $[M]^+$; ^{1}H-NMR (300MHz, $CDCl_3$) δ: 1.04 (3H, s, H-18), 1.15 (3H, s, H-30), 1.49 (3H, s, H-28), 1.54 (3H, s, H-29), 2.67 (1H, dd, *J*=1.7, 12.9Hz, H-9), 2.83 (1H, dd, *J*=4.4, 18.1Hz, H-2a), 2.97 (1H, dd, *J*=1.6, 18.1Hz, H-2b), 4.07 (1H, brt, *J*=3.2Hz, H-1), 4.12 (1H, s, H-15), 4.63 (2H, brs, H-19), 5.43 (1H, s, H-17), 6.33 (1H, d, *J*=1.6Hz, H-22), 7.33 (1H, d, *J*=3.0Hz, H-23), 7.34 (1H, d, *J*=3.0Hz, H-21); ^{13}C-NMR (75.5MHz, $CDCl_3$) δ: 79.12 (C-1), 34.77 (C-2), 169.07 (C-3), 81.79 (C-4), 139.45 (C-5), 140.68 (C-6), 195.19 (C-7), 48.34 (C-8), 46.31 (C-9), 46.82 (C-10), 20.41 (C-11), 31.63 (C-12), 37.33 (C-13), 65.25 (C-14), 52.11 (C-15), 166.43 (C-16), 77.68 (C-17), 68.55 (C-19),

119.73 (C-20), 141.06 (C-21), 109.60 (C-22), 143.29 (C-23), 18.10, 20.18, 25.19, 25.63 (CH_3).



Evodol (**4**)

Compound 5 – mp 255-9°; UV, λ_{max} (MeOH) nm (log ε) 206 (4.09), 2.37 (sh, 3.33), 334 (2.78); IR, ν_{max} (KBr) 3437, 1757, 1709, 1287, 1263, 1165, 1030, 916, 876, 762, 602 cm^{-1}; MS, *m/z* 470 $[M]^+$; ^{1}H-NMR (300MHz, $CDCl_3$) δ: 1.08 (3H, s, 30-CH_3), 1.18 (6H, s, 18, 29-CH_3), 1.29 (3H, s, 28-CH_3), 2.24 (1H, dd, *J*=3.3, 15.9Hz, H-5), 2.46 (1H, dd, *J*=3.2, 14.4Hz, H-6), 2.69 (1H, dd, *J*=1.7, 16.6Hz, H-2), 2.87 (1H, t, *J*=15.1Hz, H-6), 2.97 (1H, dd, *J*=3.6, 16.6Hz, H-2), 4.05 (2H, s, H-1, 15), 4.47 (1H, d, *J*=13.2Hz, H-19), 4.77 (1H, d, *J*=13.2Hz, H-19), 5.47 (1H, s, H-17), 6.35 (1H, brs, H-22), 7.40 (1H, d, *J*=2.6Hz, H-23), 7.41 (1H, d, *J*=2.6Hz, H-21); ^{13}C-NMR (75.5MHz, $CDCl_3$) δ : 79.11 (C-1), 35.63 (C-2), 169.20 (C-3), 80.29 (C-4), 60.45 (C-5), 36.36 (C-6), 206.16 (C-7), 51.29 (C-8), 48.06 (C-9), 45.91 (C-10), 18.86 (C-11), 30.74 (C-12), 37.95 (C-13), 65.73 (C-14), 53.85 (C-15), 166.68 (C-16), 77.81 (C-17), 65.34 (C-19), 119.97 (C-20), 141.10 (C-21), 109.66 (C-22), 143.20 (C-23), 17.60, 20.63, 21.35, 30.12 (CH_3).



Limonin (**5**)

COX-2 inhibition test – The inhibitory activity of the isolated compounds on COX-2 was measured using aspirin-treated mouse bone marrow derived mast cells (BMMC) as described previously (Moon *et al.*, 1998; Son *et al.*, 1998). In brief, BMMC from male BALB/cJ mice were cultured for up to 10 weeks in 50% enrichment medium (RPMI 1640 containing antibiotics, 2 mM L-glutamine, 0.1 mM nonessential amino acid and 10% FBS) and 50% WEHI-3 cell conditioned medium as a source of IL-3. After 3 weeks, BMMC were suspended in enrichment medium and preincubated with 10 /ml aspirin for 2 hr in order to inactivate preexisting COX-1. The cells were activated with KL (100 ng/ml), IL-10 (100 U/ml) and LPS (100 ng/ml) in the presence/absence of plant extract or the isolated compounds for 8 hr. Media were collected and PGD_2 concentrations were measured using a PGD_2 assay kit (Amersham, Buckinghamshire, UK).

Results and Discussion

Since methanol extract of *E. rutaecarpa* showed strong inhibitory activity of PGD_2 formation (Table 1), further fractionation and chromatographic separation were carried out leading to 3 alkaloids and 2 limonoids. These compounds were identified as rutaecarpine (**1**), evodiamine (**2**), dehydroevodiamine (**3**), evodol (**4**) and limonin (**5**). The spectroscopic measurements of all isolates were consistent with the data obtained in literatures (Haji *et al.*, 1994; Itokawa *et al.*, 1990; Sugimoto *et al.*, 1988a). Although the spectroscopic data of these alkaloids from this plant were well documented, the ^{1}H signals of these compounds were not fully assigned. The ^{1}H-^{1}H COSY experiments allowed unambiguous assignment of all proton signals for **1**, **2** and **3** as indicated in Experimental. Among these isolates, rutaecarpine showed strong COX-2 inhibitory activity with an IC_{50} value of 80 ng/ml, while two other alkaloids also possessed inhibitory activity of PGD_2 formation by COX-2 (Table 1). The alkaloids from *E. rutaecarpa* showed a wide variety of biological properties. Recent investigations have found that some alkaloids from *E. rutaecarpa* showed inhibition of nitric oxide production

Table 1. Inhibition of PGD_2 formation of the activated BMMC by the extract and isolates from *E. rutaecarpa*

Extract/isolates	% Inhibition of PGD_2 formation
MeOH extract	64.5
CH_2Cl_2 fraction	76.2
Evodiamine (**2**)	66.3
Evodol (**4**)	28.7
Rutaecarpine (**1**)	100.0

All compounds and the extract were tested at 2.5 μg/ml.

and antinociceptive actions (Chiou *et al.*, 1997; Matsuda *et al.*, 1998a). These biological activities along with our findings from this study may contribute to the anti-inflammatory activity of *E. rutaecarpa* used in Chinese medicine. The results of further biological evaluation including *in vivo* study of rutaecarpine will be the subject of a separate report.

Acknowledgments

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