SYNTHESIS AND ANTI-INFLAMMATORY EFFECT OF FOUR RUTAECARPINE METABOLITES

Tai-Hon Chen[1], Hsi-Jung Yu[2], Tzu-Chun Lai[1], Wen-Fei Chiou[1], Ming-Jaw Don[1,2,*]

[1]*National Research Institute of Chinese Medicine, Taipei, Taiwan*
[2]*Department of Chemistry, Chinese Culture University, Taipei, Taiwan*

(Received 18th March 2011, accepted 04th May 2011)

Rutaecarpine is a main quinazolinocarboline alkaloid isolated from *Evodia rutaecarpa*. In our previous studies, rutaecarpine was metabolized by liver microsomal enzymes to yield four rutaecarpine metabolites, 3-, 10-, 11-, and 12-hydroxyrutaecarpine. In this report, we described the detailed synthesis of these four metabolites and evaluated their anti-inflammatory effect on lipopolysaccheride-induced NO production in RAW264.7 macrophages. Among them, 3-hyroxy-rutaecarpine showed the most potent anti-inflammatory activity with EC_{50} value 27.1 μM.

Key words: *Evodia rutaecarpa*, rutaecarpine, hydroxyrutaecarpine, anti-inflammatory activity

Introduction

The dried fruit of *Evodia rutaecarpa*, called Wu-Chu-Yu in Chinese, is a commonly used traditional Chinese medicine (TCM) in the treatment of headache, gastrointestinal disorders, postpartum haemorrhage, amenorrhea, and hypertension[1,2]. Rutaecarpine (Fig. 1) is a main quinazolinocarboline alkaloid isolated from *Evodia rutaecarpa*, which has shown a variety of pharmacological effects such as anti-inflammatory[3], antiplatelet aggregatory[4], anti-cancer[5] and vasodilator effects[6].

Our previous report demonstrated that rutaecarpine was metabolized by liver microsomal enzymes and yielded four rutaecarpine metabolites, 3-, 10-, 11-, and 12-hydroxyrutaecarpine (Fig. 1) which were identified by using high-performance liquid chromatography (HPLC) with UV and ^{1}H NMR comparison[7]. In this report, the detailed synthesis of these four metabolites was described, and their anti-inflammatory activity to repress lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW264.7 macrophages was also evaluated.

Materials and Methods

I. General experimental procedures

Melting points were determined on a Yanaco MP-13 micro-melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet Avatar

*Correspondence to: Ming-Jaw Don, National Research Institute of Chinese Medicine, No. 155-1, Li-Nung Street, Sec. 2, Taipei 112, Taiwan, Tel: +886-2-28201999 ext. 8411, Fax: +886-2-28264276, E-mail: mjdon@nricm.edu.tw

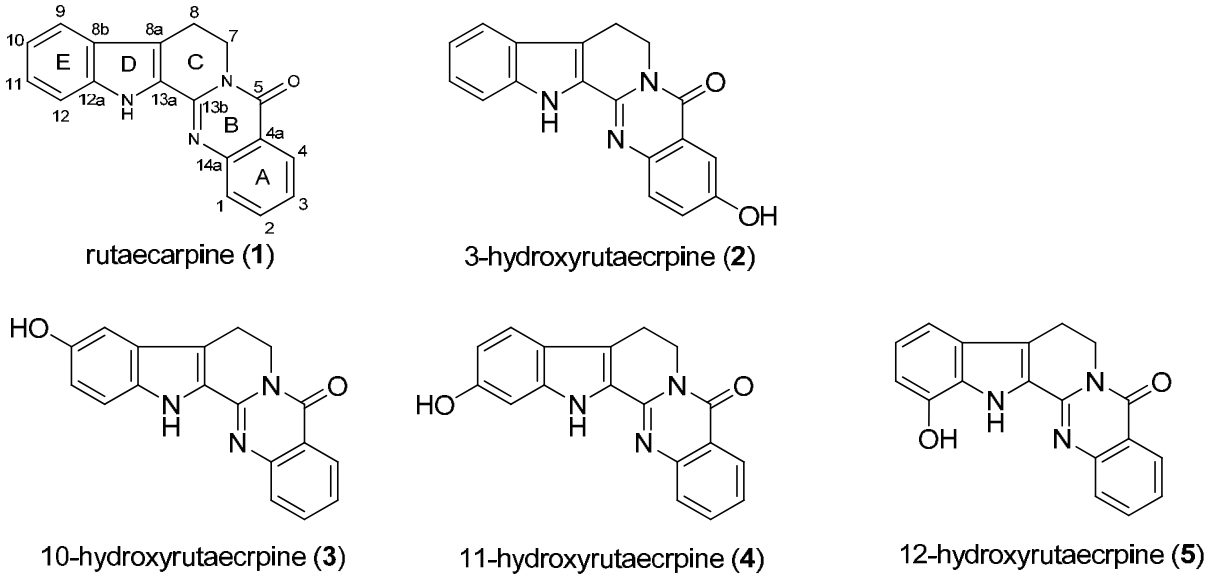


Fig. 1. The chemical structures of rutaecarpine and four metabolites.

320 FTIR spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Unity Inova 500 MHz FT-NMR spectrometers. Chemical shifts are reported in parts per million (δ) units relative to internal tetramethylsilane. The EI-MS spectra were measured with direct insertion probe on a Finnigan DSQ II mass spectrometer at 70 eV. The ESI-MS spectra were measured on a Finnigan LCQ mass spectrometer. Column chromatography was performed with E. Merck 230–400 mesh silica gel.

II. Synthesis

(Ⅰ) 3-Methoxyrutaecarpine (10)

To a mixture of **6** (372 mg, 2.0 mmol) in dry benzene (30 mL) was added $POCl_3$ (6.0 mL), and the reaction mixture was refluxed for 30 min under nitrogen. After cooling, 5-methoxyanthranilic acid (368 mg, 2.2 mmol) was added and the reaction mixture was refluxed for additional 5 hr. After cooling, the reaction mixture was poured into ice water and extracted with $CHCl_3$ three times. The combined organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography eluting with $CHCl_3$ to give **10** (292 mg, 46%) as a pale yellow solid: mp 278–280°C; IR (KBr) ν_{max} 3073, 2497, 1702, 1581, 1493, 1359, 1250, 1092, 1017, 752 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.16 (t, J = 7.0 Hz, 2H, H-8), 3.88 (s, 3H, OCH_3), 4.45 (t, J = 7.0 Hz, 2H, H-7), 7.08 (t, J = 8.0 Hz, 1H, H-10), 7.24 (t, J = 8.0 Hz, 1H, H-11), 7.42 (dd, J = 9.0, 3.0 Hz, 1H, H-2), 7.46 (d, J = 8.0 Hz, 1H, H-12), 7.55 (d, J = 3.0 Hz, 1H, H-4), 7.63 (d, J = 9.0 Hz, 1H, H-1), 7.63 (d, J = 8.0 Hz, 1H, H-9), 11.7 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.9 (C-8), 40.9 (C-7), 55.6 (OCH_3), 106.8 (C-4), 112.5 (C-12), 117.0 (C-8a), 119.6 (C-10), 119.8 (C-9), 121,5 (C-4a), 123.8 (C-2), 124.4 (C-11), 125.0 (C-8b), 127.2 (C-13a), 128.2 (C-1), 138.5 (C-12a), 141.8 (C-14a), 143.4 (C-13b), 157.4 (C-3), 160.3 (C-5); EI-MS m/z (%) 317 (100) [M^+], 302 (21).

(Ⅱ) 11-Methoxyrutaecarpine (11)

Compound **11** was prepared from **7** and anthra-

nilic acid using the same procedure as for **10**, and was obtained in 81% yield as a white solid: mp 282–284°C; IR (KBr) ν_{max} 3337, 1650, 1595, 1372, 1255, 1229, 763 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.13 (t, *J* = 7.0 Hz, 2H, H-8), 3.79 (s, 3H, OCH_3), 4.42 (t, *J* = 7.0 Hz, 2H, H-7), 6.74 (dd, *J* = 9.0, 2.0 Hz, 1H, H-10), 6.92 (d, *J* = 2.0 Hz, 1H, H-12), 7.43 (t, *J* = 8.0 Hz, 1H, H-3), 7.52 (d, *J* = 9.0 Hz, 1H, H-9), 7.64 (d, *J* = 8.0 Hz, 1H, H-1), 7.78 (t, *J* = 8.0 Hz, 1H, H-2), 8.13 (d, *J* = 8.0 Hz, 1H, H-4), 11.7 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (C-8), 40.7 (C-7), 55.2 (OCH_3), 94.4 (C-12), 110.9 (C-10), 118.5 (C-8a), 120.5 (C-4a), 120.9 (C-9), 119.3 (C-8b), 125.6 (C-3), 125.9 (C-13a), 126.3 (C-1), 126.6 (C-4), 134.4 (C-2), 139.9 (C-12a), 145.2 (C-13b), 147.5 (C-14a), 158.1 (C-11), 160.6 (C-5); EI-MS m/z (%) 317 (100) [M^+], 302 (23), 274 (15).

(Ⅲ) 3-[2-(5-Methoxy-3-indolyl)ethyl]-2-(trifluoromethyl)-4-(3*H*)-quinazolinone (14)

To a mixture of isatoic anhydride (0.9 g, 5.5 mmol) in pyridine (16 mL) was added trifluoroacetic anhydride (0.8 mL). After the reaction mixture was refluxed for 30 min, 5-methoxytryptamine (1.70 g, 8.9 mmol) was added and the reflux was continued for 1 hr. After cooling, the reaction mixture was poured into ice water, and then extracted with $CHCl_3$ three times. The combined organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography eluting with 10% EtOAc in *n*-Hexane to give **14** (0.7 g, 38%) as a white solid: mp 206–208°C; IR (KBr) ν_{max} 3379, 1686, 1610, 1203, 1146 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.08 (t, *J* = 8.0 Hz, 2H), 3.74 (s, 3H, OCH_3), 4.25 (t, *J* = 8.0 Hz, 2H), 6.73 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.12 (d, *J* = 2.0 Hz, 1H), 7.19 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 8.5 Hz, 1H), 7.74 (t, *J* = 7.5 Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.95 (t, *J* = 7.5 Hz, 1H), 8.29 (d, *J* = 7.5 Hz, 1H), 10.8 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.1, 45.7, 55.2, 99.9, 109.8, 111.2, 112.3, 118.2 (q, *J* = 275 Hz), 121.7, 123.9, 126.6, 127.3, 128.2, 129.6, 131.5, 135.3, 141.7 (q, *J* = 35.4 Hz), 144.6, 153.2, 160.9; ESI-MS m/z 388 $(M+H)^+$.

(Ⅳ) 3-[2-(7-Methoxy-3-indolyl)ethyl]-2-(trifluoromethyl)-4-(3*H*)-quinazolinone (15)

Compound **15** was prepared from isatoic anhydride and 7-methoxytryptamine using the same procedure as for **14**, and was obtained in 39% yield as a white solid: mp 230–231°C; ^1H NMR (500 MHz, DMSO-d_6) δ 3.08 (t, *J* = 8.5 Hz, 2H), 3.90 (s, 3H, OCH_3), 4.24 (t, *J* = 8.5 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 1H), 6.94 (t, *J* = 8.0 Hz, 1H), 7.13 (d, *J* = 2.0 Hz, 1H), 7.25 (d, *J* = 8.0 Hz, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.85 (d, *J* = 7.5 Hz, 1H), 7.97 (t, *J* = 7.5 Hz, 1H), 8.29 (d, *J* = 7.5 Hz, 1H), 11.0 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.1, 45.8, 55.1, 101.7, 110.6, 110.9, 119.2, 118.2 (q, *J* = 275 Hz), 121.7, 122.8, 126.4, 126.6, 128.2, 128.5, 129.6, 135.2, 141.6 (q, *J* = 34.4 Hz), 144.5, 146.3, 160.8; ESI-MS m/z 388 $(M+H)^+$.

(Ⅴ) 13b-(Trifluoromethyl)-13b,14-dihydro-10-methoxyrutaecarpine (16)

The mixture of **14** (0.50 g, 1.33 mmol), acetic acid (1.4 mL), and 37% HCl (0.3 mL) was stirred under reflux for 30 min. After cooling, the reaction mixture was poured into ice water and the white precipitate was collected by filtration and recrystallized from MeOH to give **16** as a white solid (0.14 g, 37%): mp 248–250°C; IR (KBr) ν_{max} 3349, 1647, 1492, 1164 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 2.71-2.77 (m, 1H), 2.90-2.94 (m, 1H), 3.20–3.26 (m, 1H), 3.77 (s, 3H, OCH_3), 5.09–5.13 (m, 1H), 6.83–6.87

(m, 3H), 7.05 (d, *J* = 2.5 Hz, 1H), 7.36 (t, *J* = 8.0 Hz, 1H), 7.41 (d, *J* = 9.0 Hz, 1H), 7.71 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 10.8 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.9, 37.2, 55.4, 70.5 (q, *J* = 29.6 Hz,), 100.4, 111.9, 113.1, 113.6, 114.6, 114.8, 119.0, 125.2 (C x 2), 125.5 (q, *J* = 298 Hz), 127.7, 131.9, 133.9, 143.8, 153.7, 161.4; ESI-MS m/z : 388 $(M+H)^+$.

㈥ 13b-(Trifluoromethyl)-13b,14-dihydro-12-methoxyrutaecarpine (17)

Compound **17** was prepared from **15** using the same procedure as for **14**, and was obtained in 87% yield as a white solid: mp 281–282°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.75–2.82 (m, 1H), 2.92 (dd, *J* = 16.0, 4.5 Hz, 1H), 3.24–3.30 (m, 1H), 3.97 (s, 3H, OCH3), 5.12 (dd, *J* = 14.0, 6.0 Hz, 1H), 6.76 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 6.82 (t, *J* = 8.0 Hz, 1H), 7.03 (t, *J* = 8.0 Hz, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 7.39 (t, *J* = 8.0 Hz, 1H), 7.81 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 1H), 10.8 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.6, 36.8, 55.2, 70.9 (q, *J* = 29.6 Hz), 103.5, 111.7, 112.3, 113.3, 113.8, 118.8, 120.3, 124.8, 125.5 (q, *J* = 300 Hz), 126.3, 126.9, 127.8, 134.2, 143.9, 146.1, 161.2 (C=O); ESI-MS m/z 388 $(M+H)^+$.

㈦ 10-Methoxyrutaecarpine (18)

To a hot well-stirred solution of KOH (0.13 g) in ethanol (1.0 mL) and water (0.3 mL) was added **16** (0.10 g, 0.26 mmol), and the reaction mixture was refluxed for 2.5 hr. After cooling, the crude product was collected by filtration and recrystallized from $CHCl_3$/MeOH to give **18** (61 mg g, 75%) as a white solid: mp 262–263°C; IR (KBr) ν_{max} 3337, 1655, 1603, 1497, 1473, 1400, 1221, 814, 762, 689 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.14 (t, *J* = 7.0 Hz, 2H, H-8), 3.78 (s, 3H, OCH_3), 4.43 (t, *J* = 7.0 Hz, 2H, H-7), 6.91 (dd, *J* = 9.0, 2.0 Hz, 1H, H-11), 7.10 (d, *J* = 2.0 Hz, 1H, H-9), 7.37 (d, *J* = 9.0 Hz, 1H, H-12), 7.45 (t, *J* = 8.0 Hz, 1H, H-3), 7.65 (d, *J* = 8.0 Hz, 1H, H-1), 7.79 (t, *J* = 8.0 Hz, 1H, H-2), 8.14 (d, *J* = 8.0 Hz, 1H, H-4), 11.7 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (C-8), 40.9 (C-7), 55.3 (OCH_3), 100.5 (C-9), 113.4 (C-12), 115.9 (C-11), 117.4 (C-8a), 120.6 (C-4a), 125.1 (C-8b), 125.9 (C-3), 126.4 (C-1), 126.6 (C-4), 127.4 (C-13a), 134.0 (C-12a), 134.4 (C-2), 145.3 (C-13b), 147.4 (C-14a), 153.9 (C-10), 160.6 (C-5); EI-MS m/z (%) 317 (100) $[M^+]$, 302 (50), 274 (23).

㈧ 12-Methoxyrutaecarpine (19)

Compound **19** was prepared from **17** using the same procedure as for **18**, and was obtained in 84% yield as a white solid: mp 262–263°C; IR (KBr) ν_{max} 3456, 1681, 1599, 1546, 1473, 1330, 1260, 774, 696 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.14 (t, *J* = 7.0 Hz, 2H, H-8), 3.92 (s, 3H, OCH_3), 4.43 (t, *J* = 7.0 Hz, 2H, H-7), 6.81 (d, *J* = 8.0 Hz, 1H, H-11), 7.02 (t, *J* = 8.0 Hz, 1H, H-10), 7.22 (d, *J* = 8.0 Hz, 1H, H-9), 7.46 (t, *J* = 8.0 Hz, 1H, H-3), 7.67 (d, *J* = 8.0 Hz, 1H, H-1), 7.79 (t, *J* = 8.0 Hz, 1H, H-2), 8.14 (d, *J* = 8.0 Hz, 1H, H-4), 11.7 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.1 (C-8), 40.7 (C-7), 55.5 (OCH_3), 105.0 (C-11), 112.3 (C-9), 118.6 (C-8a), 120.5 (C-10), 120.6 (C-4a), 125.9 (C-3), 126.4 (C-8b), 126.5 (C-4), 126.7 (C-1), 127.1 (C-13a), 129.2 (C-12a), 134.3 (C-2), 145.1 (C-13b), 146.7 (C-12), 147.4 (C-14a), 160.6 (C-5); EI-MS m/z (%) 317 (100) $[M^+]$, 303 (13), 288 (23), 274 (11).

㈨ 3-Hydroxyrutaecarpine (2)

To a mixture of **10** (100 mg, 0.32 mmol) in dry CH_2Cl_2 (80 mL) was added BBr_3 (2.0 mL), and the reaction mixture was reflux for 5 hr under nitrogen. After cooling, the reaction mixture was poured into

ice water, basified with 1N NaOH solution, and then partitioned with $CHCl_3$. The H_2O layer was collected, acidified with 1N HCl solution, and then extracted with $CHCl_3$ three times. The combined organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by column chromatography eluting with $CHCl_3$ to give **2** (70 mg, 73%) as a light yellow solid: mp 270–271°C; ^{1}H NMR (DMSO-d_6, 500 Mz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1 and 2; IR (KBr) ν_{max} 3134, 1696, 1641, 1585, 1499, 1352, 1302, 1224, 745 cm^{-1}; EI-MS m/z (%) 303 (100) [M$^+$].

(X) 10-Hydroxyrutaecarpine (3)

Compound **3** was prepared from **18** and BBr_3 using the same procedure as for **2**, and was obtained in 52% yield as a yellow solid: mp 295–297°C; ^{1}H NMR (DMSO-d_6, 500 Mz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1 and 2; IR (KBr) ν_{max} 3421, 1676, 1591, 1496, 1474, 1211, 772 cm^{-1}; EI-MS m/z (%) 303 (100) [M$^+$].

(XI) 11-Hydroxyrutaecarpine (4)

Compound **4** was prepared from **11** and BBr_3 using the same procedure as for **2**, and was obtained in 80% yield as a light yellow solid: mp 295–297°C; ^{1}H NMR (DMSO-d_6, 500 Mz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1 and 2; IR (KBr) ν_{max} 3301, 1661, 1579, 1471, 1374, 1248, 1202, 767 cm-1; EI-MS m/z (%) 303 (100) [M$^+$].

(XII) 12-Hydroxyrutaecarpine (5)

Compound **5** was prepared from **19** and BBr_3 using the same procedure as for **2**, and was obtained in 86% yield as a light yellow solid: mp 292–293°C; IR (KBr) ν_{max} 3119, 1685, 1634, 1578, 1484, 1423, 1312, 1247, 1143, 740 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 Mz) and ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 1 and 2; EI-MS m/z (%) 303 (100) [M$^+$].

III. Biological activity

(I) Cell culture and nitrite assay

The murine macrophage cell line RAW 264.7 was obtained from ATCC (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum, 100 U/mL penicillin and 100 μg/mL streptomycin

Table 1. The ^{1}H-NMR spectral data of 2, 3, 4, and 5 in DMSO-d_6.[a]

position	2	3	4	5
1	7.57 (d, 9.0)	7.65 (d, 8.0)	7.63 (d, 8.0)	7.68 (d, 8.0)
2	7.27 (dd, 9.0, 3.0)	7.79 (t, 8.0)	7.77 (t, 8.0)	7.82 (t, 8.0)
3		7.45 (t, 8.0)	7.42 (t, 8.0)	7.48 (t, 8.0)
4	7.47 (d, 3.0)	8.14 (d, 8.0)	8.13 (d, 8.0)	8.16 (d, 8.0)
7	4.42 (t, 7.0)	4.42 (t, 7.0)	4.40 (t, 7.0)	4.44 (t, 7.0)
8	3.14 (t, 7.0)	3.08 (t, 7.0)	3.10 (t, 7.0)	3.15 (t, 7.0)
9	7.62 (d, 8.0)	6.88 (d, 2.0)	7.43 (d, 9.0)	7.11 (d, 8.0)
10	7.07 (t, 8.0)		6.62 (dd, 9.0, 2.0)	6.93 (t, 8.0)
11	7.23 (t, 8.0)	6.80 (dd, 8.5, 2.0)		6.67 (d, 8.0)
12	7.45 (d, 8.0)	7.28 (d, 8.5)	6.83 (d, 2.0)	
NH	11.8 (s)	11.6 (s)	11.4 (s)	11.3 (s)
OH	10.1 (s)	8.96 (s)	9.40 (s)	

[a](Multiplicity, *J* in Hz) in ppm.

Table 2. The ^{13}C-NMR spectral data of 2, 3, 4, and 5 in DMSO-d_6.

position	2	3	4	5
1	128.2	126.4	126.2	125.8
2	123.8	134.4	134.3	134.6
3	155.8	125.8	125.5	126.2
4	109.7	126.6	126.6	126.7
4a	121.7	120.6	120.4	120.5
5	160.3	160.7	160.6	160.4
7	40.8	40.8	40.7	40.9
8	19.0	19.0	19.1	19.0
8a	116.6	116.8	118.7*	119.2
8b	125.0	125.5	118.6*	126.9
9	119.7*	102.7	120.7	111.0
10	119.6*	151.2	111.3	121.1
11	124.3	116.0	156.1	109.4
12	112.4	113.1	96.8	143.7
12a	138.4	133.5	140.4	128.8
13a	127.4	127.3	125.3	126.0
13b	142.6	145.4	145.3	145.3
14a	140.5	147.5	147.6	146.4

*interchangeable

and grown at 37°C with 5% CO_2 in fully humidified air. Cells were plated at a density of 2×10^5 cells/well in 96-well culture plate and stimulated with 100 ng/mL of LPS (*Escherichia coli* serotype 0128:B12) in the presence or absence of different concentrations of tested compounds (1–30 μM) for 18 hr simultaneously. All compounds were dissolved in dimethyl sulfoxide (DMSO) and further diluted with sterile phosphate buffer saline (PBS) and sterilized via a 0.2 μm filter. The final concentration of DMSO in the culture medium (less than 0.3%) had no detectable effect on NO production. Nitrite (NO_2^-) accumulation in the medium was used as an indicator of NO production which was measured by adding Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 5% phosphoric acid). $NaNO_2$ was used to generate a standard curve, and nitrite production was determined by measuring optical density at 550 nm. All experiments were performed in triplicate.

(Ⅱ) Cell viability

Cell respiration, an indicator of cell viability, was determined by the mitochondrial-dependent reduction of 3-(3,4-dimehylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. In brief, after cultured with LPS and tested compounds for 18 hr, 20 μl of 5 mg/mL MTT solution was added to each well and incubated for 4 h. The supernatants were aspirated, the formazan crystals in each well were dissolved in 200 μl of DMSO for 30 min at 37°C, and optical density at 550 nm was read on a Microplate Reader (Bio-Rad, Hercules, CA). The

mean absorbance for the duplicate cultures of each drug was calculated and the mean blank value was subtracted from these.

(Ⅲ) Statistical analysis

Results were expressed as mean±S.E. Nitrite production is indicated as absolute concentrations in μM. Computation of 50% effective concentration (EC_{50}) to inhibit LPS-induced NO production and 50% toxic concentration (TC_{50}) to reduce the cell viability were computer-assisted (PHARM/PCS v.4.2).

Results and Discussion

I. Synthesis

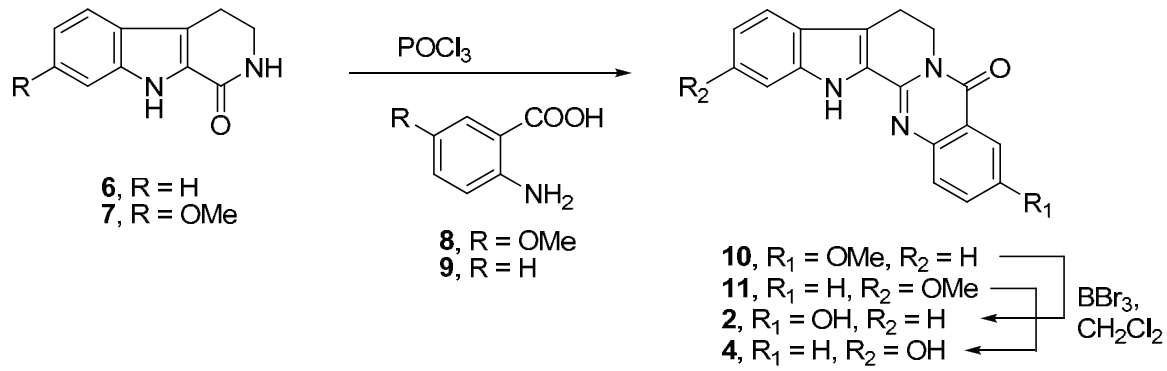
The synthetic procedure of 3-hydroxyrutaecarpine (**2**) and 11-hydroxyrutaecarpine (**4**) was shown in Scheme 1. The starting materials 1,2,3,4-tetrahydro-β-carboline-1-one (**6**) and 7-methoxy-1,2,3,4-tetrahydro-β-carboline-1-one (**7**) were prepared according to the literature method[8]. The 1,2,3,4-tetrahydro-β-carboline-1-ones (**6**, **7**) were reacted with $POCl_3$ and anthranilic acids (**8**, **9**) to yield 3-methoxyrutaecarpine (**10**) and 11-methoxyrutaecarpine (**11**), respectively[9]. Demethylation of methoxyrutaecarpines (**10**, **11**) with BBr_3 in dichloromethane produced 3-hydroxyrutaecarpine (**2**) and 11-hydroxyrutaecarpine (**4**), respectively.

The synthetic procedure of 10-hydroxyrutaecarpine (**3**) and 12-hydroxyrutaecarpine (**5**) was shown in Scheme 2. The starting material 5-methoxytryptamine (**12**) was commercial available and 7-methoxytryptamine (**13**) was prepared according to the literature method[10]. Following the Bergman's method[11], 2-(trifluoromethyl)-4*H*-benzo[*d*][1,3]oxazin-4-one was in situ prepared from isatoic anhydride and trifluoroactic acid in pyridine, and then reacted with tryptamines (**12**, **13**) to yield **14** and **15**, respectively. The acid-catalyzed ring closure of **14** and **15** yielded pentacyclic intermediates (**16**, **17**), which converted to 10-methoxyrutaecarpine (**18**) and 12-methoxyrutaecarpine (**19**) in basic condition, respectively. Demethylation of methoxyrutaecarpines (**18**, **19**) with BBr_3 in dichloromethane produced 10-hydroxyrutaecarpine (**3**) and 12-hydroxyrutaecarpine (**5**), respectively.

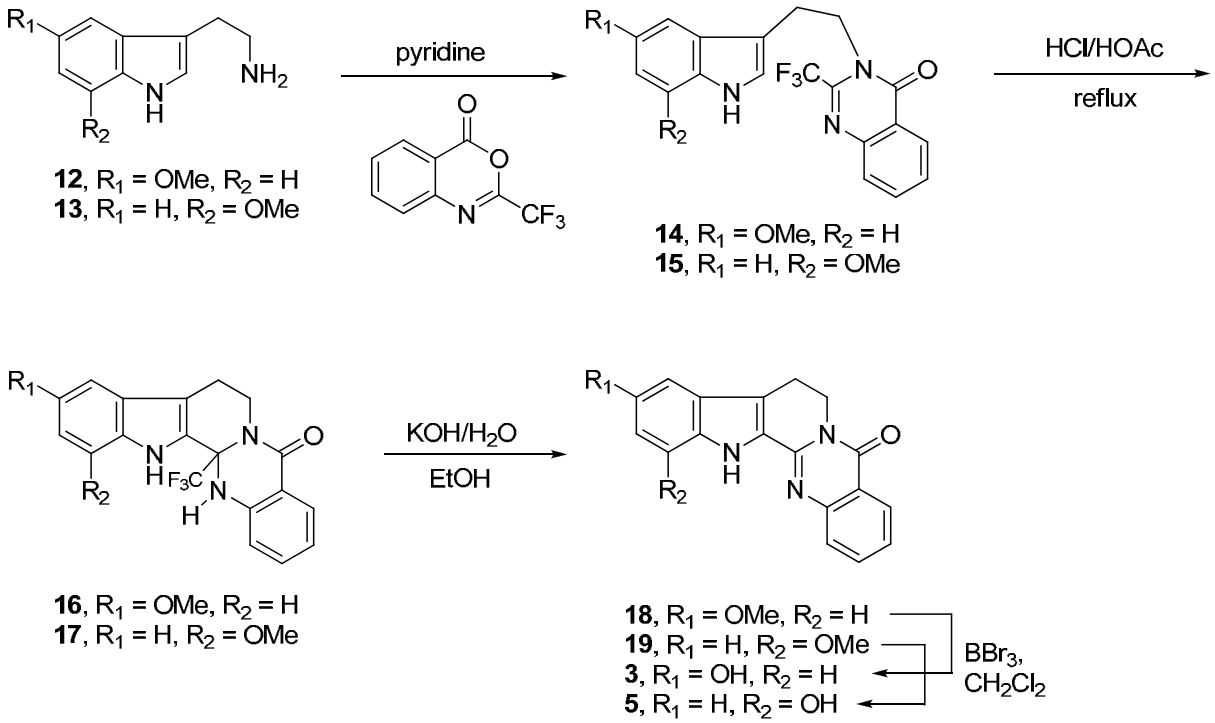
The structures of four rutaecarpine metabolites (**2**–**5**) were confirmed by detailed 1D and 2D NMR (COSY, HMQC, HMBC) and MS analyses. The ^{1}H and ^{13}C NMR assignments of these compounds are shown in Table 1 and 2.

II. Biological activity

The anti-inflammatory effects of compounds



Scheme 1. Synthesis of 3-hydroxyrutaecarpine and 11-hydroxyrutaecarpine.



Scheme 2. Synthesis of 10-hydroxyrutaecarpine and 12-hydroxyrutaecarpine.

Table 3. The inhibitory effects of selected compounds and resveratrol on lipopolysaccharide-induced nitric oxide (NO) production in RAW264.7 macrophages.[a]

compound	EC_{50} (μM)	TC_{50} (μM)
rutaecarpine (**1**)	110.2 ± 1.5	> 1000
3-hydroxyrutaecarpine (**2**)	27.1 ± 0.2	> 1000
10-hydroxyrutaecarpine (**3**)	181.7 ± 3.9	> 1000
11-hydroxyrutaecarpine (**4**)	169.3 ± 1.4	> 1000
12-hydroxyrutaecarpine (**5**)	90.4 ± 0.9	> 1000
resveratrol	28.3 ± 3.5	> 1000
aminoguanidine	21.6 ± 0.3	> 1000

[a]Results were expressed as the mean ± SE of triplicate tests. EC_{50} represented the 50% effective concentration to inhibit NO production. TC_{50} represented the 50% toxic concentration to reduce the cell viability.

1–**5** on LPS-induced NO production in RAW264.7 macrophages were shown in Table 3. Compounds **1**, **3**, **4,** and **5** slightly inhibited LPS-induced NO production with EC_{50} values ranged from 90 to 182 μM. Results showed that compound **2** was more potent than **1**, **3**, **4,** and **5** to repress NO production (EC_{50}: 27 μM) and was comparable to the well-known inducible NO synthase inhibitor aminogianidine (EC_{50}: 22 μM) and a reference anti-inflammatory natural product resveratrol (EC_{50}: 28 μM). Cell viability under this condition was still greater than 95% as measured by MTT assay, indicating that the inhibition of NO production by these compounds was not due to cell death. These results suggested that the additional

hydroxyl group in ring A of compound **2** might be important for the anti-inflammatory activity as compared to compounds **1**, **3**, **4**, and **5**. In conclusion, four rutaecarpine metabolites were synthesized in a few steps and 3-hyroxyrutaecarpine (**2**) displayed the most potent inhibition on LPS-induced NO production in RAW264.7 macrophages.

Acknowledgements

This study was financial supported by grant NRICM-97-DMC-06 from NRICM. We thank the anti-inflammation core laboratory of NRICM for the activity screening of our compounds.

References

1. Chang HM, But PP. Pharmacology and Application of Chinese Materia Medica; World Scientific: Singapore, Vol 1, pp. 605–609, 1986.
2. Tang W, Eisenbrand G. Chinese Drugs of Plant Origin. Springer-Verlag, Berlin, pp. 509–514, 1992.
3. Woo HG., Lee CH, Noh MS, Lee JJ, Jung YS, Baik EJ, Moon CH, Lee SH. Rutaecarpine, a quinazolinocarboline alkaloid, inhibits prostaglandin production in RAW264.7 macrophages. *Planta Med*., 67:505–509, 2001.
4. Sheu JR, Hung WC, Lee YM, Yen MH. Mechanism of inhibition of platelet aggregation by rutaecarpine, an alkaloid isolated from *Evodia rutaecarpa*. *Eur. J. Pharm*., 318:469–475, 1996.
5. Yang LM, Chen CF, Lee KH. Synthesis of rutaecarpine and cytotoxic analogues. *Bioorg. Med. Chem. Lett*., 5:465–468, 1995.
6. Chiou WF, Chou CJ, Liao JF, Sham AYC, Chen CF. The mechanism of the vasodilator effect of rutaecarpine, an alkaloid isolated from *Evodia rutaecarpa*. *Eur. J. Pharm*., 257:59–66, 1994.
7. Ueng YF, Yu HJ, Lee CH, Peng C, Jan WC, Ho LK, Chen CF, Don MJ. Identification of the microsomal oxidation metabolites of rutaecarpine, a main active alkaloid of the medicinal herb *Evodia rutaecarpa*. *J. Chromatogr. A*, 1076:103–109, 2005.
8. Gungor T., Malabre P, Teulon JM, Camborde F, Meignen J, Hertz F, Virone-Oddos A., Caussade F, Cloarec A. N^6-Substituted adenosine receptor agonists. Synthesis and pharmacological activity as potent antinociceptive agents. *J. Med. Chem*., 37:4307–4316, 1994.
9. Danieli B, Palmisano G, Rainoldi G, Russo G. 1-Hydroxyrytaecarpine from *Euxylophora paraensis*. *Phytochemistry*, 13:1603–1606, 1974.
10. Repke DB, Grotjahn DB, Shulgin AT. Psychotomimetic *N*-methyl-*N*-isopropyltryptamines. Effects of variation of aromatic oxygen substituents. *J. Med. Chem*., 28:892–896, 1985.
11. Bergman J, Bergman S. Studies of rutaecarpine and related quinazolinocarboline alkaloids. *J. Org. Chem*., 50:1246–1255, 1985.

吳茱萸次鹼代謝物之合成與抗發炎活性評估

陳泰宏[1]、游錫榕[2]、來姿君[1]、邱文慧[1]、董明兆[1,2,*]

[1] 國立中國醫藥研究所，台北，台灣

[2] 中國文化大學化學系，台北，台灣

（100年03月18日受理，100年05月04日接受刊載）

吳茱萸次鹼是中藥吳茱萸中主要的成份，其結構屬於 quinazolinocarboline，在先前的研究我們發現吳茱萸次鹼被肝微粒體酵素代謝成四個代謝產物，分別為 3- 羥基吳茱萸次鹼、10- 羥基吳茱萸次鹼、11- 羥基吳茱萸次鹼及 12- 羥基吳茱萸次鹼，本研究以簡易的合成方式合成出這四個吳茱萸次鹼的代謝產物，並探討這些化合物對脂多醣誘導之 RAW264.7 巨噬細胞生成一氧化氮之抑制效果，結果顯示 3- 羥基吳茱萸次鹼具有最好的抗發炎活性，其 EC_{50} 值為 27.1 μM。

關鍵字：吳茱萸、吳茱萸次鹼、羥基吳茱萸次鹼、抗發炎活性

*聯絡人：董明兆，國立中國醫藥研究所，112 台北市北投區立農街二段 155-1 號，電話：02-28201999 分機 8411，傳真：02-28264276，電子郵件信箱：mjdon@nricm.edu.tw