BIOACTIVE FLAVONOIDS FROM RUELLIA TUBEROSA

Chwan-Fwu Lin[1], Yu-Ling Huang[2], Lee-Ying Cheng[3], Shuenn-Jyi Sheu[1] and Chien-Chih Chen[2]

[1]*Department of Chemistry, National Taiwan Normal University*
[2]*National Research Institute of Chinese Medicine*
Taipei, Taiwan
[3]*Seed Improvement and Propagation Station*
Taichung, Taiwan
(Received 11st July 2006, accepted 29th August 2006)

Five flavonoids cirsimaritin (**1**), cirsimarin (**2**), cirsiliol 4′-glucoside (**3**), sorbifolin (**4**), and pedalitin (**5**) along with betulin, vanillic acid, and indole-3-carboxaldehyde were isolated from the EtOAc extracts of *Ruellia tuberosa*. The structures of all eight compounds were elucidated by IR, MS, and NMR spectral data. Compounds **1** and **3** showed cytotoxicity against KB cell line with the IC_{50} values of 30.05 and 17.91 μg/mL, respectively, while compound **2** was cytotoxic against HepG2 cell line with an IC_{50} value of 38.83 μg/mL.

Key words: *Ruellia tuberosa*, Flavonoid, Cytotoxicity.

INTRODUCTION

Ruellia tuberosa L., is a tropical plant and widely distributed in Southeast Asia. In folk medicine, it has been used as diuretic, antidiabetic, antipyretic, analgesic, antihypertensive, thirst-quenching, and antidotal agent[1,2]. It has also recently been incorporated as a component in a herbal drink in Taiwan[2]. However, very few chemical constituents and pharmacolological activities have been reported for this species[1-3]. We have now carried out a systemic chemical investigation which led to the isolation and characterization of five flavonoids along with three other compounds. In this paper, we wish to describe the isolation and the structural elucidation of flavonoids **1**-**3** and their antiproliferative activity against HepG2 and KB cancer cell lines.

MATERIALS AND METHODS

General Experimental Procedures

Melting points were determined on a Yanaco MP-I3 micro melting point apparatus and are uncorrected. IR

Correspondence to: Chien-Chih Chen, Tel: 02-2820-1999 ext. 6701; Fax: 02-2826-4276; E-mail: ccchen@nricm.edu.tw

spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Unity INOVA 500 or a Bruker AMX-400 NMR spectrometer. UV spectra were obtained on a JASCO model 7800 UV/vis spectrophotometer. LC/MS and EIMS spectra were obtained using Finnigan LCQ and Finnigan MAT GCQ spectrophotometers, respectively. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. Reversed-phase HPLC was performed using a Hewlett-Packard series 1100 pump system equipped with Hewlett-Packard UV/vis and RI detectors. Cosmosil 5C18-AR columns were used; 4.0 mm (i.d.) × 250 mm for analytical work and 10.0 mm (i.d.) × 250 mm for semipreparative isolations.

Plant Material

The aerial parts of *Ruellia tuberosa* were collected at the Seed Improvement and Propagation Station, Hsinshe, Taichung, Taiwan. A voucher specimen has already been deposited at the Herbarium of the National Research Institute of Chinese Medicine, R.O.C.

Extraction and Isolation

Dried aerial parts of *Ruellia tuberosa* (12.5 Kg) were extracted with MeOH at 50 °C (120 L × 3) for 72 h. The MeOH extract was partitioned between EtOAc and H_2O, and the EtOAc extract (970 g) was applied to a silica gel column and eluted with gradient solvent systems of *n*-hexane/EtOAc and EtOAc/MeOH to yield 8 fractions (Fr-1~Fr-8). Betulin (487 mg) was obtained from fraction Fr-1 (eluate of *n*-hexane/EtOAc = 4:1). Fraction Fr-3, the eluate of *n*-hexane/EtOAc = 1:1, was repeatedly chromatographed on silica gel, Sephadex LH-20 to give indole-3-carboxaldehyde (0.8 mg), vanillic acid (4.2 mg), and cirsimaritin (**1**, 54.2 mg). Fraction Fr-5, the eluate of EtOAc, was repeatedly chromatographed on Sephadex LH-20, silica gel, and preparative HPLC to obtain cirsimarin (**2**, 195 mg), cirsiliol 4′-glucoside (**3**, 252 mg), sorbifolin (**4**, 0.7 mg), and pedalitin (**5**, 0.5 mg).

Cirsimaritin (**1**)

Yellow prisms; mp 255-256 °C; UV λ_{max}^{MeOH} nm (log ε): 334 (4.73), 275 (4.59); IR ν_{max} (KBr) cm^{-1}: 3200-3400 (br), 1656, 1598, 1566, and 1498; ^{1}H NMR (400 MHz, acetone-d_6): δ 3.78 (3H, s, 6-OCH_3), 3.97 (3H, s, 7-OCH_3), 6.67 (1H, s, H-3), 6.85 (1H, s, H-8), 7.02 (2H, dd, J = 2.0, 6.8 Hz, H-3′, H-5′), 7.96 (2H, dd, J = 2.0, 6.8 Hz, H-2′, H-6′), 9.38 (1H, s, 4-OH), 12.92 (1H, s, 5-OH); ^{13}C NMR (400 MHz, acetone-d_6) δ 56.7 (7-OCH_3), 60.4 (6-OCH_3), 91.8 (C-8), 103.8 (C-3), 106.2 (C-10), 116.8 (C-3′ and C-5′), 123.1 (C-1′), 129.2 (C-2′ and C-6′), 133.5 (C-6), 154.0 (C-5), 154.0 (C-9), 160.0 (C-7), 162.0 (C-4′), 165.2 (C-2), 183.5 (C-4); LCMS *m/z* (%): 313 ($[M-H]^-$, 100), 150 (15).

Cirsimarin (**2**)

White prisms; mp 158-160 °C; UV λ_{max}^{MeOH} nm (log ε): 325 (4.45), 277 (4.44); IR ν_{max} (KBr) cm^{-1}: 3200-3400 (br), 1661, 1603, 1566, 1519, and 1461; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.0-3.5 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″), 3.74 (3H, s, 6-OCH_3), 3.93 (3H, s, 7-OCH_3), 5.04 (1H, d, J = 7.5 Hz, H-1″), 6.97 (1H, s, H-3), 6.99 (1H, s, H-8),

7.20 (2H, d, J = 9.0 Hz, H-3′, H-5′), 8.08 (2H, d, J = 9.0 Hz, H-2′, H-6′), 12.85 (1H, s, 5-OH); ^{13}C NMR (500 MHz, DMSO-d_6) δ 56.5 (7-OCH_3), 60.0 (6-OCH_3), 60.6 (C-6″), 69.7 (C-4″), 73.2 (C-2″), 76.5 (C-5″), 77.2 (C-3″), 91.7 (C-8), 99.8 (C-1″), 103.7 (C-3), 105.2 (C-10), 116.6 (C-3′ and C-5′), 123.9 (C-1′), 128.2 (C-2′ and C-6′), 131.9 (C-6), 152.0 (C-5), 152.7 (C-9), 158.7 (C-7), 160.4 (C-4′), 163.4 (C-2), 182.3 (C-4); LCMS *m/z* (%): 477 ([M+H]$^+$, 10), 315 (100).

Cirsiliol 4′-glucoside (**3**)

Yellow prisms; mp 214-216 °C; $[\alpha]_D^{25}$ -3.7° (c = 2.7, MeOH); UV λ_{max}^{MeOH} nm (log ε): 334 (4.52), 276 (4.49); IR ν_{max} (KBr) cm^{-1}: 3200-3400 (br), 1656, 1603, 1587, 1456, 1367, 1277, 1214, 1093, 1035; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.0-3.5 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″), 3.74 (3H, s, 6-OCH_3), 3.93 (3H, s, 7-OCH_3), 4.89 (1H, d, J = 7.5 Hz, H-1″), 6.90 (1H, s, H-3), 6.99 (1H, s, H-8), 7.25 (1H, d, J = 5.5 Hz, H-5′), 7.56 (1H, d, J = 2.0 Hz, H-2′), 7.57 (1H, dd, J = 2.0, 5.5 Hz, H-6′), 12.86 (1H, s, 5-OH); ^{13}C NMR (500 MHz, DMSO-d_6) δ 56.5 (7-OCH_3), 60.0 (6-OCH_3), 60.7 (C-6″), 69.8 (C-4″), 73.2 (C-2″), 75.8 (C-5″), 77.3 (C-3″), 91.7 (C-8), 101.2 (C-1″), 103.7 (C-3), 105.2 (C-10), 113.7 (C-2′) , 116.0 (C-5′), 118.5 (C-6′), 124.6 (C-1′), 147.0 (C-3′), 148.6 (C-4′), 131.9 (C-6), 152.0 (C-5), 152.7 (C-9), 158.7 (C-7), 163.5 (C-2), 182.3 (C-4); LCMS *m/z* (%): 491 ([M-H]$^-$, 17), 329 (100).

Sorbifolin (**4**)

Yellow solid; mp 274-275 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.91 (3H, s, 7-OCH_3), 6.80 (1H, s, H-3), 6.91 (1H, s, H-8), 6.93 (2H, d, J = 9.0 Hz, H-3′, H-5′), 7.95 (2H, d, J = 9.0 Hz, H-2′, H-6′); EIMS *m/z* (%): 299 ([M-H]$^-$, 21.7), 285 (19), 256 (50), 169 (100).

Pedalitin (**5**)

Yellow solid; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.92 (3H, s, 7-OCH_3), 6.68 (1H, s, H-3), 6.86 (1H, s, H-8), 6.88 (1H, d, J = 8.0 Hz, H-5′), 7.41 (1H, d, J = 2.0 Hz, H-2′), 7.43 (1H, dd, J = 8.0, 2.0 Hz, H-6′).

Betulin

White prisms; mp 255-256 °C; IR ν_{max} (KBr) cm^{-1}: 3300-3600 (br), 2943, 1456, 1372, and 1035; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (3H, s, H-24), 0.80 (3H, s, H-25), 0.94 (3H, s, H-23), 0.96 (3H, s, H-26), 1.00 (3H, s, H-27), 1.65 (1H, s, H-30), 2.36 (1H, ddd, J = 11.0, 11.0, 5.5 Hz, H-19), 3.16 (1H, dd, J = 11.2, 5.0 Hz, H-3α), 3.31 (1H, d, J = 10.0 Hz, H-28a), 3.77 (1H, d, J = 10.0 Hz, H-28b), 4.55 (1H, s, H-29a), 4.65 (1H, s, H-29b); EIMS *m/z* (%): 442 ([M]$^+$, 17), 427 (10), 411 (26), 393 (11), 234 (60), 220 (22), 203 (100), 189 (88), 175 (34), 161 (30), 147 (32), 133 (31), 119 (29), 107 (26), 95 (27).

Vanillic acid

Colorless solid; mp 212-213 °C; UV λ_{max}^{MeOH} nm (log ε): 290 (3.12), 258 (3.63), 217 (3.56), 208 (3.43); IR ν_{max} (KBr) cm^{-1}: 3479, 3200-2400, 1676, 1597, 1524, 1439, 1298, 1234, 1029, 761; ^{1}H NMR (500 MHz, CD_3OD): δ 3.90 (3H, s, 3-OCH_3), 4.89 (1H, s, 4-OH), 6.84 (1H, d, J = 8.0 Hz, H-5), 7.56 (2H, m, H-2, H-6); ^{13}C NMR (500 MHz,

DMSO-d_6) δ 55.2 (3-OCH_3), 112.6 (C-2), 114.6 (C-5), 121.9 (C-1), 124.0 (C-6), 147.4 (C-3), 151.4 (C-4), 168.8 (COOH); EIMS *m/z* (%): 168 ([M]$^+$, 100), 153 (36), 146 (33), 125 (11), 97 (11).

Indole-3-carboxaldehyde

Yellow solid; ^{1}H NMR (500 MHz, acetone-d_6): δ 7.27 (2H, m, H-6, H-7), 7.56 (1H, d, *J* = 8.0 Hz, H-8), 8.21 (1H, s, H-2), 8.24(1H, d, *J* = 7.0 Hz, H-5), 10.05 (1H,s, H-10); EIMS *m/z* (%): 145 ([M]$^+$, 96), 144 (100), 116 (20), 89 (14).

Cytotoxicity Testing Against Tumor Cells[4]

The hepatoma cell lines (HepG2) used in this study were provided by the Cell Bank of the Veterans General Hospital, Taipei, Taiwan. An epidermoid carcinoma cell line (KB; CCRC 60017) was purchased from the Food Industry Research and Development Institute (FIRDI, Taiwan).

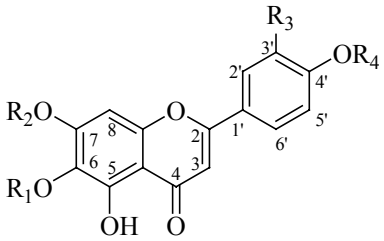
The cytotoxicity of the isolates on KB and HepG2 cell lines were tested as described previously[4].

RESULTS AND DISCUSSION

The MeOH extract of the aerial parts of *R. tuberosa* (12.5 Kg) was partitioned between EtOAc and H_2O, and the EtOAc portion was repeatedly chromatographed on silica gel, Sephadex LH-20, and by reversed-phase HPLC to afford five flavonoids cirsimaritin (**1**)[5-10], cirsimarin (**2**)[10], cirsiliol 4′-glucoside (**3**)[11], sorbifolin (**4**)[12,13], Fig. 1 and pedalitin (**5**)[13,14] along with betulin[15], vanillic acid[16], and indole-3-carboxaldehyde[17]. These compounds were identified by comparison of their spectroscopic data with literature values.

Compound **1** was recrystallized from MeOH as yellow prisms. The LC/MS spectrum of **1** showed a [M-H]$^-$ ion at *m/z* 313, indicating a molecular weight of 314. The ^{1}H NMR spectrum showed two methoxy singlets at δ 3.78 (6-OCH_3) and 3.97 (7-OCH_3), two proton singlets at δ 6.67 (H-3) and 6.85 (H-8), a pair of double doublets at δ 7.02 (dd, *J* = 6.8, 2.0 Hz, H-3′, 5′) and 7.96 (dd, *J* = 6.8, 2.0 Hz, H-2′, 6′) attributable to protons of the AA′BB′-type ring B, and two low field signals at δ 9.38 and 12.95 assignable to 4′-OH and 5-OH protons. The structure of **1** was further established by the HMBC correlations. The 5-OH signal showed correlations with C-5 (δ 154.0), C-10 (δ 106.2), and C-6 (δ 133.5). Moreover, the methoxy groups at δ 3.78 (6-OCH_3) and 3.97 (7-OCH_3) exhibited a correlation with C-6 (δ 133.5) and C-7 (δ 160.0) signals, respectively. In the NOE experiment, irradiation of the 7-OCH_3 signal caused an enhancement in the H-8 (δ 6.85) signal, but no proton signal enhancement was observed on irradiation of the 6-OCH_3 signal. Thus, the above data indicated that the methoxy groups were located at positions C-6 (δ 3.78) and C-7 (δ 3.97), and the proton signals at δ 6.85 and 6.67 were assigned to H-8 and H-3, respectively. Therefore, structure of **1** was determined to be cirsimaritin[5-10].

Compound **2** was recrystallized from MeOH as white prisms. The LC/MS spectrum of **1** showed a [M+H]$^+$ ion at *m/z* 477, indicating a molecular weight of 476, and a fragment ion peak at *m/z* 315 corresponding to the loss of one hexose unit, suggesting that compound **2** is a *O*-glycoside[18]. Analysis of the ^{1}H and ^{13}C NMR spectra indicated that **2**



	R_1	R_2	R_3	R_4
1	CH_3	CH_3	H	H
2	CH_3	CH_3	H	Glc
3	CH_3	CH_3	OH	Glc
4	H	CH_3	H	H
5	H	CH_3	OH	H

Fig. 1. Structures of compounds 1-5.

is a glucoside of **1**. In the HMBC spectrum of **2**, the correlation of the anomeric proton signal at δ 5.04 (H-1″) with the signal of C-4′ at δ 160.4 suggested that the D-glucosyl moiety was linked to C-4′. Therefore, structure **2** was identified as cirsimarin[10].

Compound **3** was recrystallized from MeOH as yellow prisms. The LC/MS spectrum of **3** showed a $[M-H]^-$ ion at *m/z* 491, indicating a molecular weight of 492, and a fragment ion peak at *m/z* 329 corresponding to the loss of one hexose unit, suggesting that compound **3** is also a *O*-glycoside[18]. Three signals for protons of the ABX-type ring B at δ 7.25 (1H, d, *J* = 5.5 Hz, H-5′), δ 7.57 (1H, dd, *J* = 2.0, 5.5 Hz, H-6′) and δ 7.56 (1H, d, *J* = 2.0 Hz, H-2′) were correlated with the carbon signals at δ 116.0, 118.5 and 113.7 in the HMQC spectrum, respectively. In HMBC spectrum of **3**, a proton signal at δ 7.57 (H-6′), was correlated with C-2, C-2′, and C-4′. The long range HMBC correlation of the anomeric proton signal at δ 4.89 (H-1″) to C-4′ suggested that the D-glucosyl moiety was linked to C-4′. Thus, the structure of **3** was identified as cirsiliol 4′-glucoside[11].

Hydrolysis of **2** and **3** with 10% HCl yielded D-glucose and the corresponding aglycones. D-Glucose was identified by HPLC using an authentic sample.

These compounds have been isolated previously from several species[5-11]. To the best of our knowledge, this is the first report of compounds **1-5**, vanillic acid, and indole-3-carboxaldehyde from the titled species.

Compounds **1-3** were tested for cytotoxicity *in vitro* against KB and HepG2 cell lines. Compounds **1** and **3** showed cytotoxicity against the KB cell line with IC_{50} values of 30.05 and 17.91 μg/mL, respectively, while compound **2** was cytotoxic against HepG2 cell line with an IC_{50} value of 38.83 μg/mL.

REFERENCES

1. Chiu NY, Chang KH. The illustrated medicinal plants of Taiwan (2). Mingtong Medical J. 226: 1, 1995.
2. Chen FA, Wu AB, Shieh P, Kuo DH, Hsieh CY. Evaluation of the antioxidant activity of *Ruellia tuberosa*. Food Chem. 94: 14-18, 2006.

3. Wagner H, Danninger H, Iyengar MA, Seligmann O, Farkas L, Subramanian SS, Nair AGR. Synthesis of glucuronides in the flavonoid-series 3. Isolation of apigenin-7-D-glucuronide from *Ruellia tuberosa* L. and its synthesis. Chem. Ber. 104: 2681-2687, 1971.
4. Teng WY, Huang YL, Shen CC, Huang RL, Chung RS, Chen CC. Cytotoxic acridone alkaloids from the stem bark of *Citrus maxima*. J. Chin. Chem. Soc. 52: 1253-1255, 2005.
5. Youssef D, Frahm AW. Constituents of the Egyptian *Centaurea scoparia*; III. Phenolic constituents of the aerial parts. Planta Med. 61: 570-573, 1995.
6. Nakasugi T, Komai K. Antimutagens in the Brazilian folk medicinal plant carqueja (*Baccharis trimera* Less.). J. Agric. Food Chem. 46: 2560-2564, 1998.
7. Liu YL, Ho DK, Cassady JM. Isolation of potential cancer chemopreventive agents from *Eriodictyon californicum*. J. Nat. Prod. 55: 357-363, 1992.
8. Mesquita AAL, Corrêa DdeB, dePádua. AP, Guedes MLO, Gottlieb OR. Flavonoids from four compositae species. Phytochemistry. 25: 1255-1256, 1986.
9. Grayer RJ, Veitch NC, Kite GC, Price AM, Kokubun T. Distribution of 8-oxygenated leaf-surface flavones in the genus *Ocimum*. Phytochemistry. 56: 559-567, 2001.
10. Hasrat JA, Pieters L, Claeys M, Vlietinck A. Adenosine-1 active ligands: cirsimarin, a flavone glycoside from *Microtea debilis*. J. Nat. Prod. 60: 638-641, 1997.
11. Morita N, Shimizu M, Arisawa M. Two new flavone glycosides from *Cirsium lineare*. Phytochemistry. 12: 421-423, 1973.
12. Silva M, Wiesenfeld A, Sammes PG, Tyler TW. New sesquiterpenes from *Pleocarphus revolutus*. Phytochemistry. 16: 379-385, 1977.
13. Zaidi F, Voirin B, Jay M, Viricel MR. Free flavonoid aglycones from leaves of *Mentha pulegium* and *Mentha Suaveolens* (Labiatae). Phytochemistry. 48: 991-994, 1998.
14. Horie T, Tominaga H, Kawamura Y, Yamada T. Studies of the selective O-alkylation and dealkylation of flavonoids. 13. An improved method for synthesizing 5,6,7-trihydroxyflavones from 6-hydroxy-5,7-dimethoxyflavones. J. Org. Chem. 57, 3343-3347, 1992.
15. Siddiqui S, Hafeez F, Begum S, Siddiqui BS. Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleander*. J. Nat. Prod. 51: 229-233, 1988.
16. Lee CK, Lee PH, Kuo YH. The chemical constituents from the aril of *Cassia fistula* L. J. Chin. Chem. Soc. 48: 1053-1058, 2001.
17. Yue Q, Miller CJ, White JFJ, Richardson MD. Isolation and Characterization of fungal inhibitors from *Epichloë festucae*. J. Agric. Food Chem. 48: 4687-4692, 2000.
18. Li QM, Claeys M. Characterization and differentiation of diglycosyl flavonoids by positive ion fast atom bombardment and tandem mass spectrometry. Biol. Mass Spectrom. 23: 406-416, 1994.

J Chin Med 17(3): 103-109, 2006

三消草中具細胞毒活性之類黃酮成分

林傳福[1]　黃鈺玲[2]　鄭梨櫻[3]　許順吉[1]　陳建志[2]

[1]國立臺灣師範大學化學研究所

[2]國立中國醫藥研究所

台北，台灣

[3]行政院農業委員會種苗改良繁殖場

台中，台灣

(2006 年 7 月 11 日受理，2006 年 8 月 29 日接受刊載)

三消草 *Ruellia tuberosa* L.為爵床科（Acanthaceae）植物，因具有治療消渴症功效以取名消渴草。經甲醇萃取濃縮後，藉由一系列分離得到五個類黃酮成分 cirsimaritin (**1**)、cirsimarin (**2**)、cirsiliol 4′-glucoside (**3**)、sorbifolin (**4**) 及 pedalitin (**5**)，和三個化合物 betulin, vanillic acid, indole-3-carboxaldehyde。各成分之結構係由紅外線、質譜及核磁共振等光譜的分析而確認。其中化合物 **1** 和 **3** 對 KB 腫瘤細胞有抑制效果，其 IC_{50} 分別為 30.05 及 17.91 μg/mL；而化合物 **2** 則對 HepG2 cell 有抑制效果，其 IC_{50} 為 38.83 μg/mL。

關鍵詞：三消草，類黃酮，細胞毒活性。

聯絡人：陳建志，國立中國醫藥研究所，電話：02-2820-1999 轉 6701，傳真：02-2826-4276，E-mail: ccchen@nricm.edu.tw