Phenolic Compounds from Roots of *Imperata cylindrica* var. *major*

LIU Rong-hua[1, 2*], CHEN Shi-sheng[1, 3], REN Gang[1], SHAO Feng[1], HUANG Hui-lian[1]

1. Key Laboratory of Modern Preparation of Traditional Chinese Medicine, Ministry of Education, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China
2. College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, Nanchang 330004, China
3. Zhejiang Research Institute of Traditional Chinese Medicine Co., Ltd., Hangzhou 310023, China

Abstract: **Objective** To study the chemical constituents from the roots of *Imperata cylindrica* var. *major*. **Methods** The chemical constituents were isolated and purified by combination of silica gel, Sephadex LH-20, polyamide, and ODS column chromatography. The structures of the isolated compounds were identified by means of spectral data and physicochemical property. **Results** Twelve phenolic compounds were isolated from *I. cylindrica* var. *major* and identified as 4,7-dimethoxy-5-methylcoumarin (**1**), 7-hydroxy-4-methoxy-5-methylcoumarin (**2**), 7-*O*-β-*D*-glucopyranosyl-4-methoxy-5-methylcoumarin (**3**), 6-hydroxy-5-methoxyflavone (**4**), 5-methoxyflavone (**5**), 5,7-dihydroxy-8-methoxyflavone (**6**), 4-hydroxybenzaldehyde (**7**), 4-hydroxy-cinnamic acid (**8**), 4-hydroxy-3-methoxybenzoic acid (**9**), 3,4-dimethoxyphenyl-*O*-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside (**10**), impecyloside (**11**), and graminone A (**12**). **Conclusion** Compounds **2**—**4**, **6**, and **10** are obtained from this plant for the first time.

Key words: flavonoids; Gramineae; *Imperata cylindrica* var. *major*; phenylpropanoids; phenols
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Introduction

Imperata cylindrica Beauv. var. *major* (Nees) C. E. Hubb. (Gramineae) is a popular herbal medicine in China with the name "*Baimaogen*", and has been used as a diuretic and anti-inflammatory agents in traditional Chinese medicine (Pharmacopeia Committee of P. R. China, 2010). Previous phytochemical studies on the roots of *I. cylindrica* var. *major* resulted in the isolation of coumarins, flavones, chromones, and other phenolics (Liu *et al*, 2010), which exhibited diverse pharmacological activities, such as cytotoxic, neuroprotective, and vasodilative activities (Matsunaga, Shibuya, and Ohizumi, 1994; Yoon *et al*, 2006; Abdel-Lateff *et al*, 2009). In previous studies, we have already isolated and purified 10 compounds from the EtOAc fraction, and among them, three phenolic compounds can inhibited the complement system activity towards the classical pathway (Fu *et al*, 2010). To further study the bioactive compounds from this herbal medicine, twelve phenolic compounds were isolated and identified as 4,7-dimethoxy-5-methylcoumarin (**1**), 7-hydroxy-4-methoxy-5-methylcoumarin (**2**), 7-*O*-β-*D*-glucopyranosyl-4-methoxy-5-methylcoumarin (**3**), 6-hydroxy-5-methoxyflavone (**4**), 5-methoxyflavone (**5**), 5,7-dihydroxy-8-methoxyflavone (**6**), 4-hydroxybenzaldehyde (**7**), 4-hydroxy-cinnamic acid (**8**), 4-hydroxy-3-methoxybenzoic acid (**9**), 3,4-dimethoxyphenyl-*O*-α-*L*-rhamnopyranosyl-(1→6)-β-*D*-glucopyranoside (**10**), impecyloside (**11**), and graminone A (**12**). Among the isolated compounds, compounds **2**—**4**, **6**, and **10** are isolated from this plant for the first time, which suggested that phenolics were the major constituents of *I. cylindrica* var. *major*, and will provide evidence for its application in folk medicine.

Materials and methods

General

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance III 600 M Spectrometer with TMS as the internal standard. MS data were obtained on a VG Auto Spec—3000 Spectrometer (Manchester, UK). Melting

* Corresponding author: Liu RH Address: College of Pharmacy, Jiangxi University of Traditional Chinese Medicine, 18 Yunwan Road, Wanli District, Nanchang 330004, China
Tel: +86-791-8711 8992 Fax: +86-791-8711 8658 E-mail: rhliu@163.com
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points were determined on a Chinese X—5 Melting Point Apparatus. Silica gel GF254 prepared for TLC and silica gel (200—300 meshes and H) for column chromatography were obtained from Qingdao Marine Chemical Factory (China). Diaion HP-20 resin and MCI GEL CHP20P (75—150 μm) were products of Mitsubishi Chemical Corporation (Tokyo, Japan). Sephadex LH-20 (20—150 μm) was a product of Pharmacia. Octadecyl silica gel was purchased from YMC Company.

Plant materials

The roots of *Imperata cylindrica* Beauv. var. *major* (Nees) C. E. Hubb. were purchased from Jiangxi Huiren Pharmaceutical Co., Ltd. (Nanchang, China) in October 2009, and identified by Prof. LIU Rong-hua, College of Pharmacy, Jiangxi University of Traditional Chinese Medicine. A voucher specimen (0906003) has been deposited in B501 Laboratory of Key Laboratory of Modern Preparation of TCM, Ministry of Education, Jiangxi University of Traditional Chinese Medicine.

Extraction and isolation

The roots of *I. cylindrica* var. *major* (9.5 kg) were successively extracted with 70% EtOH (25 L) for three times under reflux for 2, 1.5, and 1 h. The combined EtOH solutions were concentrated under vacuum to remove EtOH, then suspended in water and partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively.

The combined petroleum ether and EtOAc extracts (90 g) were subjected to a silica gel column chromatography (CC) eluted with petroleum ether-EtOAc (1:0→1:1) to yield seventeen fractions (Frs. 1—17). Fr. 13 was subjected to a silica gel CC eluted with petroleum ether-EtOAc (20:1→8:1), then subjected to Sephadex LH-20 CC eluted with $CHCl_3$-MeOH (1:1) to yield compounds **1** (117.6 mg) and **6** (16.5 mg). Fr. 15 was subjected to silica gel CC eluted with petroleum ether-acetone (10:1→1:1) to yield 10 subfractions (Frs. 15-1—15-10). Fr. 15-9 was subjected to an ODS CC eluted with MeOH-H_2O (45:55→65:35) to yield compounds **2** (10.0 mg) and **9** (29.1 mg). Fr. 17 was subjected to a silica gel CC eluted with petroleum ether-acetone (5:1→1:1) to yield fifteen subfractions (Frs. 17-1—17-15). Fr. 17-7 was subjected to silica gel CC eluted with $CHCl_3$-EtOAc (80:1) to yield compound **5** (21.0 mg). Fr. 17-13 was recrystallized with $CHCl_3$ to yield compound **12** (13 mg).

The *n*-BuOH extract (120.0 g) was subjected to Diaion HP-20 CC washed with EtOH-H_2O (10:90→95:5) to yield six fractions (Frs. A—F). Fr. A (26.5 g) was applied to silica gel CC eluted with EtOAc-MeOH (20:1→1:1) to give five fractions (Frs. A1—A5). Fr. A5 was subjected to a polyamide CC with MeOH-H_2O (20:80→100:0) and recrystallization to yield compound **10** (29.3 mg). Fr. B (20.1 g) was applied to polyamide CC eluted with $CHCl_3$-MeOH (8:1→1:1) to give two fractions (Frs. B1 and B2). Fr. B1 was subjected to silica gel CC eluted with $CHCl_3$-MeOH (15:1→5:1) to yield Frs. B1A — B1C. Fr. B1A was repeatedly subjected to silica gel CC eluted with $CHCl_3$-acetone (8:1) to yield compound **7** (8 mg). Fr. C (11.4 g) was applied to silica gel CC eluted with $CHCl_3$-MeOH (10:1→1:1) to give two fractions (Frs. C1 and C2). C1 was subjected to silica gel CC to yield five subfractions (Frs. C1A—C1E). C1D was subjected to pre-TLC ($CHCl_3$-EtOAc 5:1) to yield compound **8** (7.2 mg). Fr. C2 was repeatedly subjected to Sephadex LH-20 CC eluted with MeOH and recrystallization or preparation TLC with $CHCl_3$-MeOH (3:1) to yield compounds **3** (30 mg, Rf = 0.6) and **11** (5 mg, Rf = 0.3). Fr. F (5.0 g) was applied to MCI column eluted with EtOH-H_2O (50:50→100: 0) to give eight fractions (Frs. F1—F8). Fr. F6 was subjected to Sephadex LH-20 CC with $CHCl_3$-MeOH (1:1) to yield three subfractions (Frs. F6A—F6C). Fr. F6C was subjected to a silica gel CC with $CHCl_3$-MeOH (10:1) to yield compound **4** (5.9 mg).

Results

The structures of the twelve compounds were established by comparison of their spectral data, physical, and chemical characteristics.

Compounds **1**—**3** were identified as coumarins. Compound **1** was 4,7-dimethoxy-5-methylcoumarin (Qi *et al*, 2003). Compound **2** was 7-hydroxy-4-methoxy-5-methylcoumarin (Kimura *et al*, 1983).

Compound **3**: colorless needles; mp 246—248 ℃. ESI-MS *m/z*: 369 $[M + H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 6.91 (1H, d, J = 2.5 Hz, H-6), 6.88 (1H, dd, J = 2.5, 0.7 Hz, H-8), 5.67 (1H, s, H-3), 4.00 (3H, s, -OCH_3), 2.65 (3H, s, -CH_3). The data were in accordance with the skeleton of compounds **1** and **2**. In addition, the ^{1}H-NMR data [δ: 5.02 (1H, d, J = 7.8 Hz, H-1′), 3.91 (1H, dd, J = 12.1, 2.2 Hz, H-6′), 3.70 (1H, dd, J = 12.2, 5.9 Hz, H-6′), 3.50 (1H, ddd, J = 9.5, 5.7,

2.1 Hz, H-5′), 3.47 (1H, m, H-3′), 3.48 (1H, m, H-2′), and 3.39 (1H, m, H-4′)] indicated the existence of a sugar moiety. ^{13}C-NMR (150 MHz, CD_3OD) δ: 101.51 (C-1′), 76.92 (C-5′), 76.42 (C-3′), 73.32 (C-2′), 69.82 (C-4′), 60.97 (C-6′) further confirmed a sugar moiety. ^{13}C-NMR (150 MHz, MeOD) δ: 170.39 (C-4), 164.08 (C-2), 159.97 (C-7), 156.02 (C-9), 138.80 (C-5), 116.75 (C-6), 108.77 (C-10), 100.25 (C-8), 87.06 (C-3), 55.58 (-OCH_3), 22.25 (-CH_3) supported above analysis. Compound **3** was identified as 7-*O*-β-*D*-glucopyranosyl-4-methoxy-5-methylcoumarin by comparison of ^{1}H-NMR and ^{13}C-NMR data with those of compounds **1** and **2**, as well as reported data (Hernández-Carlos, Burgueño-Tapia, and Joseph-Nathan, 2003; Zhang *et al*, 2009).

Compounds **4**—**6** were identified as flavonoids. They were 6-hydroxy-5-methoxyflavone (Awaad *et al*, 2006), 5-methoxyflavone (Lee *et al*, 2008b), and 5,7-dihydroxy-8-methoxyflavone (Li *et al*, 2007), respectively.

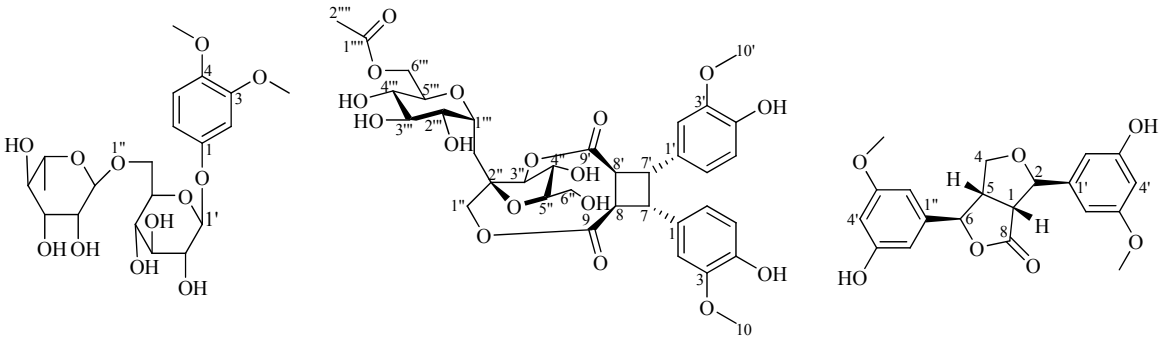
Compounds **7**—**9** were identified as phenolics. They were 4-hydroxybenzaldehyde (Feng *et al*, 2008), 4-hydroxy-cinnamic acid (Zhou and Li, 2006), and 4-hydroxy-3-methoxybenzoic acid (Cheng *et al*, 2011), respectively.

Compound **10**: white powder, mp 110—112 ℃. ESI-MS *m/z*: 463 $[M + H]^+$. ^{1}H-NMR (600 MHz, CD_3OD) δ: 6.88 (1H, d, J = 9.0 Hz, H-5), 6.77 (1H, d, J = 2.4 Hz, H-2), 6.68 (1H, d, J = 9.0, 2.4 Hz, H-6), 4.77 (1H, d, J = 7.4 Hz, H-1′), 4.72 (1H, d, J = 1.4 Hz, H-1″), 4.03 (1H, dd, J = 11.0, 1.7 Hz, H-6′), 3.94—3.83 (m, 1H), 3.82 (m, 1H), 3.79 (3H, s, -OCH_3), 3.75 (3H, s, -OCH_3), 3.73—3.50 (m, 2H), 3.50—3.35 (m, 4H), 3.38 (m, 1H), 1.22 (3H, d, J = 6.2 Hz, -CH_3). ^{13}C-NMR (150 MHz, CD_3OD) δ: 152.39 (C-1), 149.67 (C-3), 144.75 (C-4), 112.53 (C-5), 107.93 (C-6), 102.87 (C-2), 101.96 (C-1′), 100.76 (C-1″), 76.58 (C-3′), 75.51 (C-5′), 73.54 (C-2′), 72.64 (C-4″), 71.00 (C-5″), 70.77 (C-2″), 70.19 (C-4′), 68.46 (C-3″), 66.58 (C-6′), 55.75 (3-OCH_3), 55.15 (4-OCH_3), 16.58 (C-6″). Compound **10** was identified as 3,4-dimethoxyphenyl-(6-*O*-α-*L*-rhamnopyranosyl)-β-*D*-glucopyranoside by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Graikou *et al*, 2005) (Fig. 1).

Compound **11**: yellow powder. ESI-MS *m/z*: 737 $[M + H]^+$. In the ^{1}H-NMR (600 MHz, acetone-d_6), two methoxy proton signals were observed at δ 3.61 and 3.73, along with six aromatic protons [δ 6.76 (1H, brs, H-2), 7.03 (1H, d, J = 8.0 Hz, H-5), 6.94 (1H, brd, J = 8.0 Hz, H-6), 6.88 (1H, brs, H-2′), 6.97 (1H, d, J = 8.0 Hz, H-5′), 6.71 (1H, brd, J = 8.0 Hz, H-6′)] suggesting the presence of a dual 1,3,4-trisubstituted phenyl moiety. There were some oxygenated methine and methylene signals of carbohydrate moieties in the region from δ 4.22 to 6.18 including one anomeric proton doublet at δ 6.18 with J of 3.2 Hz, an indicating a-glucosyl form. In the aliphatic region, methine proton signals at δ 4.80 (dd, J = 5.2, 9.6 Hz, H-7′), 4.57 (dd, J = 5.2, 9.6 Hz, H-7), 4.49 (dd, J = 5.2, 11.2 Hz, H-8), and 4.37 (dd, J = 5.2, 11.2 Hz, H-8′) from the cyclobutane ring and acetyl-CH_3 proton signal at δ 1.88 were observed. ^{13}C-NMR (150 MHz, acetone-d_6) δ: 174.80 (C-9), 171.95 (C-9′), 171.29 (C-1‴), 147.99 (C-3), 147.80 (C-3′), 145.90 (C-4), 145.78 (C-4′), 131.41 (C-1), 131.18 (C-1′), 121.66 (C-6), 121.35 (C-6′), 115.29 (C-5), 115.06 (C-5′), 112.74 (C-2), 112.63 (C-2′), 109.47 (C-2″), 93.67 (C-1‴), 88.94 (C-4″), 79.18 (C-3″), 75.55 (C-3‴), 74.95 (C-5″), 73.39 (C-2‴), 71.66 (C-4‴), 71.39 (C-5‴), 65.34 (C-1″), 64.62 (C-6‴), 63.18 (C-6″), 56.21 (10-OCH_3), 56.13 (10′-OCH_3), 45.72 (C-7), 44.83 (C-7′), 44.63 (C-8′), 44.12 (C-8), 20.79 (C-2‴). Compound **11** was identified as impecyloside by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Lee *et al*, 2008a) (Fig. 1).

Compound **12**: ESI-MS *m/z*: 373 $[M + H]^+$. In the ^{1}H-NMR spectrum (600 MHz, $CDCl_3$), two methoxy proton signals [δ 3.89 (6H, s)] and two hydroxyl signals [δ 5.83 (lH, s), 5.75 (lH, s)] were observed, along with six aromatic protons signals [δ 6.90 (2H, brs, Ar-H), 6.88 (2H, brs, Ar-H), 6.79 (2H, brs, Ar-H)] suggesting the presence of a dual 1,3,5-trisubstituted phenyl moiety. There were some oxygenated methine and methylene signals of carbohydrate moieties in the region from δ 4.05—5.35 [4.05 (1H, dd, J = 10.0, 5.4 Hz, H-4), 4.33 (1H, dd, J = 10.0, 6.2 Hz, H-4), 5.33 (1H, d, J = 4.6 Hz, H-6), 5.35 (1H, d, J = 4.6 Hz, H-2)], along with methane signals [δ 3.25 (1H, m, H-5), 3.49 (1H, dd, J = 9.2, 4.6 Hz, H-l)] suggesting the compound had a furanofuran lignan lactone. ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 177.10 (C-8), 146.96 (C-5′), 146.75 (C-3′), 146.04 (C-3″), 145.30 (C-5″), 132.24 (C-1″), 131.05 (C-1′), 118.40 (C-2″), 118.00 (C-6″), 114.74 (C-6′), 114.44 (C-2′), 108.14 (C-4″), 107.85 (C-4′), 84.72 (C-6), 83.35 (C-2), 72.68 (C-4), 56.06 (-OCH_3), 56.00 (-OCH_3), 53.30 (C-1), 49.91 (C-5). Com-

pound **12** was identified as graminone A by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Matsunaga, Shibuya, and Ohizumi, 1994) (Fig. 1).



10 **11** **12**

Fig. 1 Structures of compounds 10—12

Discussion

Phenolic compounds are a major class of widely distributed and chemically diverse secondary metabolites in plants, which have diverse pharmacological activities, such as anti-oxidative (Sabeena, Farvin, and Jacobsen, 2013), antimicrobial (Pereira *et al*, 2007), and antitumor activities (Huang, Cai, and Zhang, 2009). In our chemical investigation, twelve phenolic compounds were isolated, such as flavonoids, simple phenols, phenolic acids, coumarins, and lignans, suggesting that phenolics are the major constituents of *I. cylindrical* var. *major*, which would provide the evidence for its application in folk medicine.

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