Chemical Constituents from Roots of *Flemingia philippinensis*

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Abstract: **Objective** To study the chemical constituents from the roots of *Flemingia philippinensis*. **Methods** The chemical constituents were isolated and purified by combination of silica gel column, Sephadex LH-20, polyamide, and ODS column chromatography. The structures of the isolated compounds were identified by means of spectral data. **Results** Ten compounds were isolated from *F. philippinensis* and identified as isoderrone (**1**), dalparvin A (**2**), prunetin (**3**), 7,3′-dihydroxy-5,4′,5′-trimethoxyisoflavone (**4**), pratensein-7-*O*-β-*D*-glucoside (**5**), sissotrin (**6**), sophororicoside (**7**), formononetin (**8**), orobol (**9**), and biochanin A (**10**). **Conclusion** Compounds **1**－**6** are obtained from this plant for the first time.

Key words: dalparvin A; *Flemingia philippinensis*; isoderrone; isoflavones; prunetin
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Introduction

Flemingia philippinensis (Merr. Et Rolfe) Li is a shrubby herb mainly growing in tropical areas. It belongs to Leguminosae with its roots used in folk medicine for the treatment of rheumatism, arthropathy, leucorrhea, menalgia, menopausal syndrome, chronic nephritis, and improving bone mineral density (Ahn *et al*, 2003). Previous phytochemical studies have reported that the root extract of *F. philippinensis* contained various isoflavones, flavones, and isoflavanones (Chen, Luo, and Chen, 1990; Li *et al*, 2008), which exhibited anti-oxidative, anti-inflammatory, estrogenic, and anti-estrogenic activities (Ahn *et al*, 2004). Especially isoflavonoids as phytoestrogens were isolated from this plant, such as flemiphilippinin A, flemiphilippinin B, erythrinin B (Chen, Luo, and Chen, 1991), flemiphilippinin C, 5,7,3′,4′-tetrahydroxy-6,8-diprenyl-isoflavone (Chen, Luo, and Chen, 1990), flemiphilippinin E, flemiphilippinin F (Li *et al*, 2008), genistein, 3′-*O*-methylorobol, 8-(1,1-dimethylallyl) genistein, biochanin A, auriculasin, 2′-hydroxygenistein, 5,7,3′-trihydroxy-2′-(3-methylbut-2-enyl)-4′,5′-(3,3-dimethylpyrano) isoflavone, 5,7,3′,4′-tetrahydroxy-2′,5′-di (3-methylbut-2-enyl)-isoflavone (Ahn *et al*, 2003), orobol (Li *et al*, 2009), and so on. In order to investigate more bioactive compounds from this herbal medicine, during our chemical investigation, ten known compounds were isolated from the ethyl acetate part of the ethanol extract from the roots of *F. philippinensis*. These compounds were identified as isoderrone (**1**), dalparvin A (**2**), prunetin (**3**), 7,3′-dihydroxy-5,4′,5′- trimethoxy-isoflavone (**4**), pratensein-7-*O*-β-*D*-glucoside (**5**), sissotrin (**6**), sophororicoside (**7**), formononetin (**8**), orobol (**9**), and biochanin A (**10**). Among the isolated compounds, **1**－**6** were reported for the first time in this plant, and there were nine isoflavonoids except compound **2**, which suggested that isoflavones were the major constituents of *F. philippinensis*, and provided evidence for its application in folk medicine.

Materials and methods

General

TLC was performed on silica gel GF_{254} plates (Qingdao Marine Chemical Co., Ltd., Qingdao, China). TLC

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coloring agent is 5% sulphuric acid in ethanol. For column chromatography, silica gel 60 (100－200 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), polyamide (60－100 mesh), Develosil ODS (75 μm, Nomura Chemical Co., Ltd., Japan), and Sephadex LH-20 (Pharmacia) were used. ESI-MS were collected on an MDS SCIEX API 2000 LC-MS-MS Instrument. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) data were recorded on a Bruker DRX—400 Instrument using the residual solvent peak as reference.

Plant materials

The roots of *Flemingia philippinensis* (Merr. Et Rolfe) Li were purchased from the Qingping Herbal Medicine Market (Guangzhou, China) in October 2009, and were identified by Prof. DENG Yun-fei, South China Botanical Garden, Chinese Academy of Sciences. The voucher sample (200903) was deposited at the herbarium of South China Botanical Garden, Chinese Academy of Sciences (Guangzhou, China).

Extraction and isolation

The powdered dry roots of *F. philippinensis* (10 kg) were extracted for three times with 95% ethanol (50 L × 3) at room temperature, for 3 d each. After evaporation of the solvent *in vacuo*, the combined crude ethanolic extract (250 g) was suspended in water and partitioned with EtOAc (2 L × 3). The EtOAc-soluble extract (40 g) was subjected to silica gel column chromatography (CC) and eluted with $CHCl_3$-MeOH (98:2→10:90) to yield six fractions (Frs. 1－6). Fr. 2 (16.7 g) was further separated by polyamide CC with MeOH-H_2O (70:30→100:0) to afford three subfractions (Subfrs. 2a, 2b, and 2c). Subfr. 2a (2.3 g) was subjected to silica gel CC and eluted with petroleum ether-EtOAc (90:10→60:40) to afford compounds **1** (15 mg) and **9** (20 mg). Subfr. 2b (3.4 g) was subjected to recrystalization with MeOH to give compounds **2** (120 mg) and **8** (35 mg). Subfr. 2c (1.6 g) was further separated by Sephadex LH-20 using MeOH to yield compounds **3** (45 mg) and **10** (50 mg). Fr. 3 (8.2 g) was subjected to silica gel CC using petroleum ether-acetone (80:20→50:50) to afford three subfracions (Subfrs. 3a, 3b, and 3c). Subfr. 3a (0.9 g) was subjected to silica gel CC and eluted with $CHCl_3$-MeOH (90:10→50:50) to yield compound **4** (60 mg). Subfr. 3c (1.5 g) was purified by ODS CC using 60% MeOH as an eluent, followed by Sephadex LH-20 CC eluted with MeOH to obtain compounds **5** (10 mg), **6** (25 mg), and **7** (30 mg).

Results

Compound **1**: white amorphous powder. ESI-MS *m/z*: 337 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.38 (6H, s, CH_3-5″, 6″), 5.78 (1H, d, J = 10.0 Hz, H-3″), 6.21 (1H, s, J = 2.0 Hz, H-6), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.42 (1H, d, J = 10.0 Hz, H-4″), 6.78 (1H, d, J = 8.0 Hz, H-5′), 7.26 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 7.28 (1H, d, J = 2.0 Hz, H-2′), 8.35 (1H, s, H-2), 12.89 (1H, s, OH-5); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 27.8 (C-5″, 6″), 76.4 (C-2″), 93.8 (C-8), 99.1 (C-6), 104.4 (C-10), 115.7 (C-5′), 120.7 (C-3′), 121.7 (C-4″), 122.0 (C-3), 127.0 (C-2′), 129.8 (C-6′), 131.4 (C-3″), 152.4 (C-2), 154.3 (C-4′), 157.6 (C-9), 162.0 (C-5), 164.5 (C-7), 180.1 (C-4). Compound **1** was identified as isoderrone by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Maximo and Lourenco, 1998).

Compound **2**: yellow amorphous powder. ESI-MS *m/z*: 333 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.74 (3H, s, -OCH_3), 3.81(3H, s, -OCH_3), 4.23 (1H, dd, J = 10.8, 6.4 Hz, H-3), 4.32 (1H, dd, J = 10.8, 6.4 Hz, H-2a), 4.45 (1H, t, J = 10.8 Hz, H-2b), 5.87 (1H, d, J = 2.0 Hz, H-6), 5.89 (1H, d, J = 2.0 Hz, H-8), 6.54 (1H, s, H-6′), 6.74 (1H, s, H-3′), 12.26 (1H, s, 5-OH); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 46.4 (C-3), 56.2 (-OCH_3), 56.8 (-OCH_3), 69.8 (C-2), 95.2 (C-8), 96.3 (C-6), 101.3 (C-3′), 101.8 (C-10), 112.8 (C-1′), 141.5 (C-5′), 147.3 (C-4′), 151.9 (C-2′), 163.0 (C-9), 163.9 (C-5), 167.4 (C-7), 197.0 (C-4). Compound **2** was identified as dalparvin A by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Umehara *et al*, 2008).

Compound **3**: white amorphous powder. ESI-MS *m/z*: 285 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.79 (3H, s, OCH_3-7), 6.42 (1H, d, J = 2.0 Hz, H-6), 6.67 (1H, d, J = 2.0 Hz, H-8), 6.82 (2H, d, J = 8.0 Hz, H-3′, 5′), 7.38 (2H, d, J = 8.0 Hz, H-2′, 6′), 8.43 (1H, s, H-2), 9.63 (1H, brs, OH-4′), 12.94 (1H, s, OH-5); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 55.2 (OCH_3-7), 92.4 (C-8), 98.1 (C-6), 104.4 (C-10), 113.7 (C-3′, 5′), 121.0 (C-1′), 122.5 (C-3), 130.2 (C-2′, 6′), 154.3 (C-2), 157.5 (C-9), 159.2 (C-4′), 161.8 (C-5), 164.3 (C-7), 180.1 (C-4). Compound **3** was identified as prunetin by

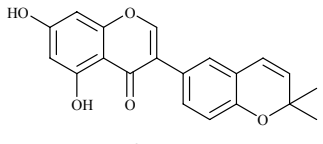
comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Huang and Tu, 2004).

Compound **4**: white amorphous powder. ESI-MS *m/z:* 345 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.63 (3H, s, -OCH_3), 3.69 (3H, s, -OCH_3), 3.76 (3H, s, -OCH_3), 6.36 (1H, d, J = 2.0 Hz, H-6), 6.38 (1H, d, J = 2.0 Hz, H-8), 6.58 (1H, d, J = 1.6 Hz, H-6′), 6.87 (1H, d, J = 1.6 Hz, H-2′), 8.33 (1H, s, H-2); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 55.8 (-OCH_3), 56.5 (-OCH_3), 59.8 (-OCH_3), 94.6 (C-8), 97.7 (C-6), 105.8 (C-6′), 106.9 (C-10), 112.4 (C-2′), 125.8 (C-3), 128.7 (C-1′), 140.4 (C-4′), 151.2 (C-3′), 151.8 (C-5′), 153.9 (C-2), 159.3 (C-9), 161.5 (C-5), 163.4 (C-7), 174.5 (C-4). Compound **4** was identified as 7,3′-dihydroxy-5,4′,5′-trimethoxyisoflavone by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Fang *et al*, 2008).

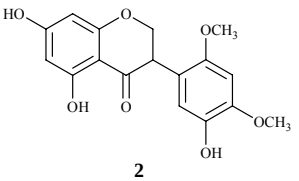
Compound **5**: yellow amorphous powder. ESI-MS *m/z*: 463 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.30 — 3.71 (6H, m, sugar protons), 3.78 (3H, s, OCH_3-4′), 4.64—5.47 (4H, m, sugar hydroxyl protons), 5.05 (1H, d, J = 7.2 Hz, Glc-H-1), 6.46 (1H, d, J = 2.0 Hz, H-6), 6.70 (1H, d, J = 2.0 Hz, H-8), 6.83 (1H, d, J = 8.0 Hz, H-5′), 6.99 (1H, dd, J = 2.0, 8.0 Hz, H-6′), 7.14 (1H, d, J = 2.0 Hz, H-2′), 8.41 (1H, s, H-2), 9.21 (1H, s, OH-3′), 12.94 (1H, s, OH-5); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 55.8 (OCH_3-4′), 60.8 (Glc-C-6), 69.7 (Glc-C-4), 73.2 (Glc-C-2), 76.5 (Glc-C-3), 77.3 (Glc-C-5), 94.7 (C-8), 99.7 (C-6), 100.0 (Glc-C-1), 106.2 (C-10), 112.3 (C-5′), 116.4 (C-2′), 121.6 (C-6′), 121.8 (C-3), 122.7 (C-1′), 146.9 (C-3′), 147.4 (C-4′), 154.9 (C-2), 157.3 (C-9), 161.8 (C-5), 163.1 (C-7), 180.5 (C-4). Compound **5** was identified as pratensein-7-*O*-β-*D*-glucoside by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Ma *et al*, 2005).

Compound **6**: yellow amorphous powder. ESI-MS *m/z*: 447 $[M + H]^+$; ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 3.15 — 3.72 (6H, m, sugar protons), 3.80 (3H, s, OCH_3-4′), 4.61—5.44 (4H, m, sugar hydroxyl protons), 5.07 (1H, d, J = 7.2 Hz, Glc-H-1), 6.48 (1H, d, J = 2.4 Hz, H-6), 6.74 (1H, d, J = 2.4 Hz, H-8), 7.02 (2H, d, J = 8.8 Hz, H-3′, 5′), 7.53 (2H, d, J = 8.8 Hz, H-2′, 6′), 8.49 (1H, s, H-2), 12.92 (1H, s, OH-5); ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 55.4 (OCH_3-4′), 60.8 (glc C-6), 69.7 (Glc-C-4), 73.2 (Glc-C-2), 76.5 (Glc-C-3), 77.3 (Glc-C-5), 94.6 (C-8), 99.6 (C-6), 99.8 (Glc-C-1), 106.3 (C-10), 113.5 (C-3′, 5′), 122.2 (C-1′), 122.8 (C-3), 130.4 (C-2′, 6′), 154.7 (C-2), 157.3 (C-9), 159.4 (C-4′), 161.7 (C-7), 163.0 (C-5), 180.6 (C-4). Compound **6** was identified as sissotrin by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data (Zhao *et al*, 2009).

Another four compounds were isolated from the roots of *F. philippinensis* and were identified as sophoroside (**7**) (Li *et al*, 2009), formononetin (**8**) (Huang and Tu, 2004), orobol (**9**) (Li *et al*, 2009), and biochanin A (**10**) (Huang and Tu, 2004) by comparison of the physical, ^{1}H-NMR, and ^{13}C-NMR data with the reported data. The structures are listed in Fig. 1.



1



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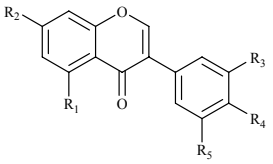

3 $R_1 = R_4 = OH$; $R_2 = OCH_3$; $R_3 = R_5 = H$
4 $R_1 = R_4 = R_5 = OCH_3$; $R_2 = R_3 = OH$
5 $R_1 = R_4 = OH$; $R_2 = OGlc$; $R_3 = OCH_3$; $R_5 = H$
6 $R_1 = OH$; $R_2 = OGlc$; $R_3 = R_5 = H$; $R_4 = OCH_3$
7 $R_1 = R_2 = OH$; $R_3 = R_5 = H$; $R_4 = OGlc$
8 $R_1 = R_3 = R_5 = H$; $R_2 = OH$; $R_4 = OCH_3$
9 $R_1 = R_2 = R_3 = R_4 = OH$; $R_5 = H$
10 $R_1 = R_2 = OH$; $R_3 = R_5 = H$; $R_4 = OCH_3$

Fig. 1 Structures of compounds 1—10

Discussion

Isoflavones as a kind of phytoestrogens are effective in preventing and treating estrogen-mediated diseases. Previous phytochemical studies have reported that the root extract of *F. philippinensis* contains various isoflavones. In the *in vitro* estrogen assay, genistein, biochanin A, 2′-hydroxygenistein, 8-(1,1-dimethylallyl) genistein, and 3′-*O*-methylgenistein exhibited significant

estrogenic activity to stimulate cell proliferation of MCF-7 (human breast cancer cell). In the yeast two-hybrid assay, genistein and 2′-hydroxy-genistein showed induction of β-galactosidase activity. On the other hand, 5,7,3′,4′-tetrahydroxy-6,8-diprenyl-isoflavone showed strong anti-estrogenic activity (Ahn *et al*, 2004). The agonistic and antagonistic activity may help to reduce the risk of estrogen-mediated cancer and be applicable to clinical trials for breast cancer in postmenopausal women who are treated with estrogen replacement therapy. In the present study, we isolated ten known compounds from the roots of *F. philippinensis* including nine isoflavonoids, suggested that isoflavones be the major constituents of *F. philippinensis*, and provided evidence for its application in folk medicine for treatment of leucorrhea, menalgia, menopausal syndrome, and chronic nephritis.

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