SHORT COMMUNICATION

Phenolic constituents from *Rhopalocnemis phalloides* with DPPH radical scavenging activity

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Abstract

The 80% aqueous acetone extract of the whole plant of *Rhopalocnemis phalloides* Jungh. (Balanophoraceae) showed obvious radical scavenging activity (SC₅₀ = 32.1 μg/mL) on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay. Further chemical investigation of the extract led to the isolation of 12 phenolic compounds (1–12). Among these, *bis*-6,8’-catechinylmethane (2), a flavan-3-ol dimer, was first isolated as a natural product from *Rhopalocnemis phalloides*. The full NMR assignments of *bis*-catechinylmethanes (1 and 2) are reported for the first time, on the basis of detailed spectroscopic analysis. The DPPH assay showed that flavan-3-ol dimers (1–3) had remarkable free radical scavenging activity, while flavonoid aglycones (4 and 9) presented stronger activities than those of their glycosides (5–8 and 10).

Keywords: *Balanophoraceae*; *Rhopalocnemis phalloides*; flavan-3-ol dimer; DPPH radical

Introduction

Oxidative stress is one of the main factors causing many human diseases, and antioxidants have been recognized as a useful strategy to prevent and treat the diseases (Giugliano et al., 1996; Inagi, 2006; Nunomura et al., 2006; Wood-Kaczmar et al., 2006). To find new antioxidants from natural resources has become an important approach to the development of new health care products (Slemmer et al., 2008).

*Rhopalocnemis phalloides* Jungh., a monotypic genus of the family Balanophoraceae, is distributed in the tropic and subtropic areas of southeast Asia. It is normally parasitized on the roots of evergreen broad-leaved trees from Moraceae, Theaceae, Euphorbiaceae, Araliaceae, Fagaceae, and Caesalpiniaaceae families. The whole plant is used for treatment of the common cold and injuries from falls, and as a tonic remedy in the folk medicine of Yunnan Province, China (Wu et al., 2003).

So far, the phytochemical investigation of Balanophoraceous plants has mainly focused on the genus *Balanophora*. A series of polyphenols, especially hydrolyzable tannins, were reported from *Balanophora polyandra* Griff. (Wang et al., 2006a), *Balanophora involucrata* Hook. f. (Shen et al., 1996; Xia et al., 2001), *Balanophora harlandii* Hook. f. (Teng et al., 2000), *Balanophora japonica* Makino (Jiang et al., 2001), *Balanophora abbreviata* Blume, and *Balanophora tobracola* Makino (Ito et al., 1980; Tanaka et al., 2005). However, no chemical study has been reported on *Rhopalocnemis phalloides*.

In our continuing research on natural antioxidants from medicinal plants (Wang et al., 2005, 2006a, 2006b), the acetone extract of the whole plant of *Rhopalocnemis phalloides* exhibited considerable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (SC₅₀ = 32.1 μg/mL). This led us to further detailed phytochemical investigation of this plant, from which 12 phenolic compounds (1–12) were identified. The DPPH radical scavenging activity of the isolated compounds was also tested. This article presents the details of the study.
Materials and methods

General procedures

One-dimensional (1D) and 2D nuclear magnetic resonance (NMR) spectra were run on Bruker AM-400 and DRX-500 instruments with tetramethylsilane (TMS) as internal standard. Negative-ion fast atom bombardment mass spectroscopy (FABMS) was recorded on an AutoSpec 3000 spectrometer with glycerol as the matrix. The DPPH (Aldrich Chemical Co.) radical scavenging assay was performed using an Emax precision microplate reader. Column chromatography was performed using Sephadex LH-20 (Pharmacia Fine Chemical Co. Ltd), and Toyopearl HW-40F (27–70 μm; Tosoh Co.). Thin layer chromatography (TLC) was carried on silica gel G precoated plates (Qingdao Haiyang Chemical Co.) with benzene-ethyl formate-formic acid (1:7:1) or CHCl3-MeOH-H2O (8:2:0.2 or 7:3:0.5). The ingredients were detected by spraying with 2% ethanol FeCl3 or 10% H2SO4 ethanol solution followed by heating.

Plant material

The whole plant of Rhopalocnemis phalloides was collected at Wenshan County, Yunnan Province of China, in September 2004 and identified by Prof. X. W. Li, Laboratory for Plant Biodiversity and Biogeography, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No: 0772775) is deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The fresh whole plant of Rhopalocnemis phalloides (300 g) was extracted three times with 80% aqueous acetone at room temperature (21 L × 3, each time for 1 week). After removal of the organic solvent under reduced pressure, the concentrated water solution was extracted with EtOAc (0.8 L × 3, each time for about 2 h). The organic extract (8 g) was applied to Sephadex LH-20 column chromatography (CC), eluting with H2O-MeOH (1:0–0:1) and finally with 50% aqueous acetone, to afford six fractions (frs. 1–6). Fr. 3 (2.2 g) was subjected repeatedly to MCI-gel CHP20P, Toyopearl HW-40F, and Sephadex LH-20 CC, eluting with H2O-MeOH (1:0–0:1) to yield compounds 1 (8 mg), 2 (9 mg), 5 (14 mg), 6 (9 mg), 7 (23 mg), 11 (26 mg), 10 (15 mg), and 12 (8 mg). Fr. 4 (1.0 g) was applied to CC on Sephadex LH-20 and MCI-gel CHP20P, eluting with H2O-MeOH (1:0–0:1) to afford compounds 3 (6 mg) and 9 (15 mg). Repeated CC over Sephadex LH-20 and MCI-gel CHP20P, eluting with H2O-MeOH (1:0–0:1), gave compounds 4 (4 mg) and 8 (4 mg) from fr. 5 (0.4 g) and fr. 6 (0.12 g), respectively. The acetone, EtOAc, and MeOH used in the above were chemically pure reagents, and water was purified with a Milli-Q apparatus (Millipore, USA).

DPPH radical scavenging assay

The DPPH assay was performed as described in our previous article (Wang et al., 2005), and ascorbic acid was used as positive control. Scavenging activity was determined by the following equation: % scavenging activity = 100 × (Acontrol − A sample)/ Acontrol. The SC50 value was obtained through extrapolation from linear regression analysis and denoted as the concentration of sample required to scavenge 50% of DPPH radical.

Results and discussion

Repeated CC over Sephadex LH-20, MCI-gel CHP20P, and Toyopearl HW-40F led to the isolation of 12 phenolic compounds (1–12) from the aqueous acetone extract of the fresh whole plant of Rhopalocnemis phalloides (Figure 1). These phenolics were determined to be bis-8,8'-catechylmethane (1) (Kiatgrajai et al., 1982), bis-6,8'-catechylmethane (2) (Boyer & Ducrot, 2005), procyanidin B2 (3) (Chien et al., 1979), luteolin (4) (Shen et al., 2004), galuteolin (5) (Veit et al., 1990), glucoluteolin (6) (Li & Yu, 1998), prunin (7) (Ke & Jiang, 1999), naringenin (8) (Guan et al., 2000), eriodictyol (9) (Waterman & Crichton, 1980), eriodictyol-3-O-β-D-glucopyranoside (10) (Rawat et al., 1995), 6,8-dihydroxychromone (11), and 5,7-dihydroxychromone (12), respectively, by comparison of their spectroscopic data with reported literature values and authentic samples. Both compounds 1 and 2, as flavan-3-ol dimers linked with a methylene group, have been synthesized previously (Kiatgrajai et al., 1982; Boyer & Ducrot, 2005). Later, compound 1 was reported from cacao liquor (Hatano et al., 2002) and Baccaurea ramiflora Lour. (Euphorbiaceae) (Yang et al., 2007). This is the first time that 2 has been obtained as a natural product. Since the detailed assignments of NMR data of these two compounds have not been reported before, we achieved the assignment of all carbon and proton signals by 2D NMR experiments, as shown in Table 1.

The isolated compounds (1–12) consist of three flavan-3-ol dimers (1–3), three flavones (4–6), four dihydroflavones (7–10), and two chromone derivatives (11 and 12). Previous reports showed that hydrolyzable tannins as major constituents existed in the species of genus Balanophora. The occurrence of condensed tannins (1–3) in Rhopalocnemis phalloides gives rise to the chemical difference between these two genera, which may depend mainly on their different hosts.
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Figure 1. Structures of compounds 1–12 from Rhopalocnemis phalloides.

Table 1. $^{13}$C and $^1$H NMR spectral data (in acetone-$d_6$ + D$_2$O; $\delta$ in ppm, $J$ in Hz) of compounds 1 and 2.

<table>
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<tr>
<th>Position</th>
<th>$\delta_{C}$</th>
<th>$\delta_{H}$</th>
<th>$\delta_{C,1}$</th>
<th>$\delta_{H,1}$</th>
<th>$\delta_{C,II}$</th>
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<td>2</td>
<td>83.3</td>
<td>4.51 (d, $J$ = 7.5)</td>
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<td>3</td>
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<td>68.5</td>
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<td>4</td>
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<td>2.80 (d, $J = 5.4, 16.2, H-4a$)</td>
<td>28.8</td>
<td>2.78 (d, $J = 5.6, 16.0, H-4a$)</td>
<td>28.7</td>
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<td>2.50 (d, $J = 8.8, 16.2, H-4b$)</td>
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Compounds 1-12 were evaluated for their radical scavenging activity with the DPPH assay, with ascorbic acid as positive control (Table 2). It is noted that most of the phenolic compounds showed obvious DPPH radical scavenging activity. Of them, flavan-3-ol dimers (1-3) showed remarkable free radical scavenging activity compared with the other kinds of compounds. In the case of flavonoids, the aglycones (4 and 9) showed stronger activities than those of their glycosides (5-8 and 10). In addition, the chromone derivatives (11 and 12) did not show activity in this assay.

The above results indicate R. phaloides possesses a potent radical scavenging property. Further research is needed to promote its utilization in health care.

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Declaration of interest: The authors report no conflicts of interest.

References


