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Contributed Paper

Two Phenylethanoid Glycosides, Parvifloroside A and B, Isolated from *Barleria strigosa*

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ABSTRACT

Two phenylethanoid glycosides, parvifloroside A (**1**) and parvifloroside B (**2**) have been isolated for the first time from the MeOH extract of *Barleria strigosa* growing in Thailand. The structures of these compounds were elucidated from their 1D and 2D NMR spectroscopic data and ESI-MS.

Keywords: *Barleria strigosa*, Parvifloroside A, Parvifloroside B, Phenylethanoid glycosides

1. INTRODUCTION

Barleria strigosa Willd. belongs to the Acanthaceae family (Thai name: Sang ko ra ni, Ya hua nak). It is a tall shrub with blade-like leaves and blue flowers. It is native to tropical regions of Asia and has been used as a Thai traditional medicine. The leaves have been claimed to be useful to treat influenza and nosebleed. The whole plant was boiled and used as a restorative, antipyretic, and antidote for poison detoxification [1]. In addition, there are a few articles dealing with the active constituents of *B. strigosa*. The whole plant was found to contain β- and γ-sitosterol [2,3]. The leaves yield apiginin, vanillic acid, *p*-hydroxybenzoic acid and *p*-coumaric acid [3]. It was also found to contain verbascoside, isoverbascoside, decaffeoyl verbascoside, strigoside (4-hydroxyphenylethyl-4-*O*-β-D-glucopyranosyl-(1→3)-*O*-*a*-L-rhamnopyranoside), 10-*O*-*trans*-coumaryl-eranthemoside, (+)-lyoniresinol-3α-*O*-β-D-glucoside, apigenin-7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside, 7-*O*-acetyl-8-epi-loganic acid, and (3*R*)-1-octen-3-ol-3-*O*-β-D-xylosyl-(1→6)-β-D-glucoside [1]. The present constituents, parvifloroside A (**1**) and B (**2**), have not been previously reported from *B. strigosa*.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *B. strigosa* were collected from the Medicinal Plant Garden, Faculty of Pharmacy, Chiang Mai University, Thailand in May 2011. The plant material was characterized by Mr. James F. Maxwell from the Department of Biology, Chiang Mai University. A voucher specimen (voucher Number Prapalert W.2) was deposited at the herbarium of the Department of Biology, Chiang Mai University.

2.2 Experimental

Flash column chromatography (CC) was performed over Merck silica gel (40-63 μm) with gradient elution using petroleum ether, ethyl acetate and methanol as mobile phases. Reverse phase CC was done on RP-C18 bonded to silica gel with isocratic and gradient elution using methanol and water as mobile phases. TLC analysis were performed with Merck precoated silica gel 60 GF_{254} aluminum-backed plates, and the spots were first viewed under UV light at λ 254 nm and 365 nm, then stained with basic $KMnO_4$ (3 g $KMnO_4$ +20 g K_2CO_3 + 5 mL 5% aq. NaOH + 300 mL water) or stained with cerium-ammonium-molybdate, CAM (400 mL 10% aq. H_2SO_4 + 10 g $(NH_4)_2MoO_4$ + 4 g $(NH_4)_4Ce(SO_4)_4.2H_2O$) followed by heating. The optical rotations were determined using a Jasco P-2000 polarimeter with a sodium lamp in a 10 cm path length cell at 25 °C using CH_3OH as a solvent. A VNMRS PS54 500 MHz Varian Unity NMR spectrometer was used for ^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, HMBC, and NOESY experiments in CD_3OD with TMS as the internal standard. Low resolution electrospray ionization mass spectra (LR-ESI-MS) were recorded on a Waters Platform LCZ mass spectrometer.

2.3 Extraction and Isolation

The leaves of *B. strigosa* (10.5 kg) were dried under shade for 2 days and then in 50 °C hot-air blower for 2 days then ground to fine powder. The powder (2.855 kg) was extracted with 5 L of methanol for 7 days at room temperature and then filtered. The residue was extracted again with 3 L of methanol (x3). The filtrates were dried under vacuum to give a dark green residue (262.9 g). The residue was suspended in 400 mL of water and partitioned with hexane (3 × 800 mL) to yield the crude hexane extract (dark green sticky syrup 79.3 g). The aqueous layer was then extracted with ethyl acetate (3 × 600 mL) and *n*-butanol (3 × 500 mL) to yield the crude ethyl acetate extract (dark green solid 3.3 g), the crude *n*-butanol extract (dark green solid 19.8 g), and the crude aqueous extract (dark brown solid 132.3 g), respectively. The ethyl acetate extract (3.3 g) was separated by CC on flash silica gel (160 g) using gradient elution from 50 - 100% ethyl acetate in petroleum ether to give 7 fractions, BE_1 - BE_7. Fraction BE_6 (1.5975 g) was then subjected to CC on silica gel (150 g) using isocratic elution of 5% MeOH in ethyl acetate to afford 5 fractions, BE_6_1 - BE_6_5. A portion of fraction BE_6_1 (100 mg) was separated by CC over RP-C18 bonded to silica gel (35 g) using gradient elution from 20-70% MeOH in water to yield compound **1** (15.5 mg). Fraction BE_6_2 (802 mg) was also separated by CC over RP-C18 bonded silica gel (35 g) using gradient elution from 20-50% MeOH in water to afford 6 fractions, BE_6_2_1 to BE_6_2_6. Fraction BE_6_2_3 was identified as compound **1** (172.4 mg). Fraction BE_6_2_5 (86.8 mg) was separated over RP-C18 bonded silica gel (35 g) CC using isocratic elution of 20% MeOH in water to yield pure compound **2** (33.5 mg).

2.4 Phytochemical Screening of the Methanol Extract

The crude MeOH extract was screened for phytochemical constituents by the methods described by Chhetri *et al.* [5] and Egwailhide and Gimba [6].

2.4.1 Test for flavonoids:

Ten milliliters of petroleum ether was added to 0.5 g of the extract and it was shaken well, then the liquid part was discarded (repeated 2 times). The remaining residue was dissolved with 10 mL of 50% MeOH, then equally divided into 2 tubes for the control and test samples. Three small pieces of magnesium metal was added to the test sample tube, after that 5-6 drops of conc. HCl was added. A pink-red color will be observed for flavonoids and an orange color for flavones.

2.4.2 Test for alkaloids:

Ten milliliters of 2% HCl was added to 0.2 g of the extract. After heating for 15 min and filtering, three drops of Kraut's reagent (solution A (8 g $BiO(NO_3)$ in 12 mL 30% HNO_3) + solution B (27.2 g KI in 50 mL water) + water (made up to 100 mL)) was added to the supernatant. An orange red precipitate or turbidity will be observed.

2.4.3 Test for tannins:

Twenty milliliters of water was added to 0.5 g of the extract. After boiling and filtering, the solution was equally divided into 2 tubes for the control and test samples. Three drops of 0.1% $FeCl_2$ was added into the test sample tube. A blue color will be observed for hydrolysable tannins and a green black or brownish green color for condensed tannins.

2.4.4 Test for saponins:

Ten milliliters of water was added to 0.4 g of the extract. After filtering, the solution was equally divided into 2 tubes for the froth test. The first tube was shaken strongly for 1 min and then allowed to settle for 30 min to observe the froth (appearance of creamy mist of small bubbles). The second tube was boiled with 1 M HCl, then shaken for 1 min to see a froth not in a honeycomb shape.

2.4.5 Test for terpenoids and steroids:

Two milliliters of chloroform was added to 0.5 g of the extract then three drops of acetic anhydride was added. The mixture was shaken well and then a concentrated solution of sulfuric acid was added slowly into the leaning test tube. A greenish blue color was observed for steroids and a red violet color indicated the presence of terpenoids.

2.4.6 Test for antraquinones:

Ten milliliters of 10% HCl was added to 0.5 g of the extract then boiled for five min in a water bath. After filtration and cooling, 5 mL of the filtrate was added to 5 mL of $CHCl_3$ then three drops of 10% NH_3 were added. A rose-pink color will be observed after boiling in a water bath for a few minutes.

2.5 Antioxidant Activity

The antioxidant activities of the extracts were studied by measuring the scavenging activity on DPPH, 2,2-diphenyl-1-picrylhydrazyl, stable free radicals [7]. Briefly, an ethanolic solution of DPPH (180 μL) was added to 20 μL sample of different concentrations of the extracts (75-175 mg/mL) in a 96-well microtiter

plate. The reaction mixtures were incubated in the dark at 25 °C for 30 min, then the absorbances (A_s) were measured at 520 nm The DPPH solution was used as negative control (A_c). The ethanol (180 μL) and the plant extract (20 μL) was used as the blank (A_b). Ascorbic acid and Trolox were used as reference standards. The percentage of the DPPH scavenging activity was calculated using the following equation: DPPH scavenging activity (%) = $\{[A_c-(A_s-A_b)]/A_c\} \times 100$. The concentration providing 50% inhibition (IC_{50}) values were calculated from the linear equation of the inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging activity.

3. RESULTS AND DISCUSSION

The methanol crude extract of *B. strigosa* showed antioxidant activity with an IC_{50} value of 73 μg/mL, which was slightly lower than those of the standards ascorbic acid and trolox (4 and 6 μg/mL, respectively). The crude extract was screened for phytochemical constituents and found to contain tannins, saponins and steroids. However, the extract did not show a positive test for flavonoids, alkaloids or antraquinones.

Compound **1** was a pale yellow amorphous powder. The specific rotation was $[\alpha]_D^{25}$ -94.4° (c 0.7, MeOH). The IR spectrum showed bands at ν_{max} 3355, 1689, 1602, 1520, 1447, 1369, 1267, 1158, and 1023 cm^{-1}. The negative ion ES-MS spectrum exhibited an $(M-H)^-$ ion at m/z 623 and the positive ion ES-MS displayed a $[M+Na]^+$ ion at m/z 647. This information together with the NMR data allowed its molecular formula to be assigned as $C_{29}H_{36}O_{15}$ (Figure 1).

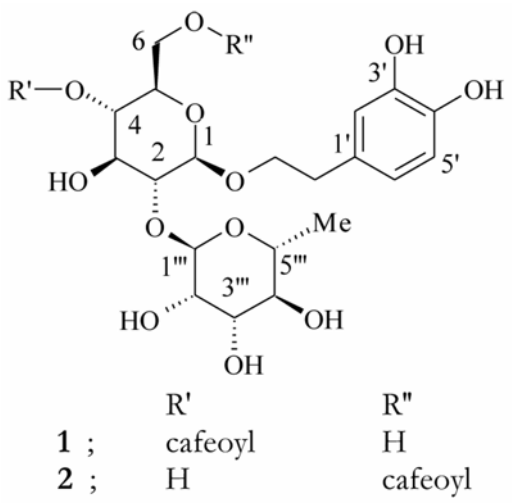

Figure 1. Structures of compounds **1** and **2**.

The ^{1}H NMR spectrum of compound **1** (Table 1) exhibited the characteristic resonances of a phenolic glycoside that contained caffeic acid and phenylethanol moieties. Two ABX-type aromatic proton signals appeared at δ 6.52 (1H, d, J = 7.8, H-6′), 6.63 (1H, d, J = 7.8 Hz, H-5′), 6.65 (1H, s, H-2′) and 6.91 (H, d, J = 7.8 Hz, H-6″), 6.73 (1H, d, J = 8.3 Hz, H-5″), and 7.01 (1H, s, H-2″). Two doublet signals at δ 6.27 (1H, d, J = 15.6 Hz, H-β″) and 7.59 (1H, d, J = 15.6 Hz, H-γ″) were assigned as a pair of *trans*-olefinic protons in the caffeoyl part. A multiplet benzylic methylene proton signal at δ 2.79 (2H, m, H-β″) and two diastereotopic protons resonances at δ 4.00 (1H, dd, J = 16.6, 7.8 Hz, H- α′) and 3.82 (1H, dd, J = 16.6, 7.8 Hz, H-α′) were assigned to the side chain of the aglycone part.

Table 1. NMR spectroscopic data of compound **1**

Position	δ_H (*J*)	δ_C	HMBC	NOESY
Glucose				
1	4.33 (*d*, *J*=7.8)	104.2	C-α′	
2	3.77 (*t*, *J*=9.3)	81.6	C-3, C-4, C-1‴	H-4, H-1‴
3	3.34 (*t*, *J*=8.3)	76.0	C-1, C-2	H-1
4	4.87 (*t*, *J*=9.3)	70.6	C-2, C-3, C-5, C-6, C-α″	H-2, H-6
5	3.49 (*m*)	76.0		
6	3.58 (*m*)	62.4	C-1, C-3	H-4
	3.47 (*m*)		4, 5	
Aglycone				
1′		131.5		
2′	6.65 (*s*)	117.1	C-6′	
3′		146.1		
4′		144.7		
5′	6.63 (*d*, *J*=7.8)	116.3	C-1′ , C-3′, C-β′	
6′	6.52 (*d*, *J*=7.8)	121.2	C-4′,C-5′, C-β′	
α′	4.00 (*dd*, ^2J=16.6, 7.8)	72.2	C-1′, C-β′, C-1	H-α′, H-β′
	3.82 (*dd*, ^2J=16.6, 7.8)			
β′	2.79 (*m*)	36.6	C-α″, C-1′, C-2′, C-6′	H-α′
Caffeic acid				
1″		127.7		
2″	7.01 (*s*)	115.2	C-3″, C-4″, C-6″, C-γ″	
3″		146.8		
4″		149.8		
5″	6.73 (*d*, *J*=8.3)	116.1	C-1″, C-3″	
6″	6.91 (*d*, *J*=7.8)	123.2	C-2″, C-4″	
γ″	7.59 (*d*, *J*=15.6)	148.0	C-2″, C-6″, C-α″	
β″	6.27 (*d*, *J*=15.6)	114.7		
α″		168.3	C-1″, C-α″	
Rhamnose				
1‴	5.14 (*s*)	103.0	C-2, C-3‴, C-5‴	H-2, H-2‴
2‴	3.87 (*br. s*)	72.3	C-4‴	H-1‴
3‴	3.54 (*m*)	72.0	C-5‴	
4‴	3.26 (*m*)	73.8	C-2‴, C-3‴	H-6‴
5‴	3.58 (*m*)	70.4	C-3‴	H-6‴
6‴	1.09 (*d*, *J*=6.0)	18.4	C-4‴, C-5‴	H-2‴, H-4‴, H-5‴

conditions: ^{1}H- and ^{13}C-NMR at 500 and 125 MHz, resp., in CD_3OD; δ in ppm, *J* in Hz.

Two sugar anomeric proton resonances were observed, one as a singlet and the other as a doublet at δ 5.14 (1H, s, H-1‴) and 4.33 (1H, d, *J* = 7.8 Hz, H-1) which correlated to δ 103.0 (C-1‴) and 104.2 (C-1), respectively in the HSQC spectrum. The ^{1}H NMR spectrum also exhibited a resonance for a secondary methyl group at δ 1.09 (d, *J*=6.0, H-6‴) which indicated the presence of a rhamnose sugar unit. In addition, all connectivities within compound **1** were supported by the HMBC experiment, where correlations were observed between H-1 (δ 4.33) of the glucose unit and the C-α′ (δ 72.2) of the aglycone moiety, H-2 (δ 3.77) of the glucose unit and the C-1‴ (δ 103.0) of the rhamnose unit, and H-4 (δ 4.87) of the glucose unit and the C-α″ (δ 168.3) of the cafeoyl moiety (Table 1). Moreover, the correlations between H-2 (δ 3.77) of the glucose unit and H-1‴ (δ 5.14) of the rhamnose unit in the NOSEY experiment confirmed the attachment of the sugar units (Figure 2).

Comparisons of this data with those in the literature indicated that the structure of compound **1** as parvifloroside A (2-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→2)-4-*O*-*E*-caffeoyl-β-D-glucopyranoside [4].

Compound **2** was isolated as a pale-yellow amorphous powder. The IR spectrum showed bands at ν_{max} 3354, 1684, 1603, 1520, 1450, 1370, 1264, 1162, 1115, and 1033 cm^{-1}. The specific rotation was $[\alpha]_D^{25}$ -50.5 (c 1.075, MeOH). The negative ion ES-MS spectrum exhibited a (M-H)$^-$ ion at *m/z* 623 and the positive ion ES-MS displayed a [M+Na]$^+$ ion at *m/z* 647.

The ^{1}H NMR spectrum of compound **2** (Table 2) had similar features to those of compound **1** except the downfield shift of the H-6 protons at δ 4.50 (1H, dd, *J* = 11.8, 1.8 Hz, H-6) and 4.36 (1H, dd, *J* = 12.0, 5.8 Hz, H-6), which showed HSQC correlations to C-6 at δ 64.6. The data from the HMBC experiment exhibited the correlation of H-6 at δ 4.50 and 4.36 with C-α″ at δ 169.1, which suggested the attachment of a caffeoyl moiety at C-6 of the glucose unit rather than at C-4. The NOESY correlations (Table 2 and Figure 2) also supported that compound **2** was parvifloroside B (2-(3,4-dihydroxyphenyl)-ethyl-*O*-α-L-rhamnopyranosyl-(1→2)-6-*O*-*E*-caffeoyl-β-D-glucopyranoside.

Compound **1** and **2** showed high potential antioxidant capacity against the DPPH radical with IC_{50} values of 12.9 and 7.3 μg/mL, respectively.

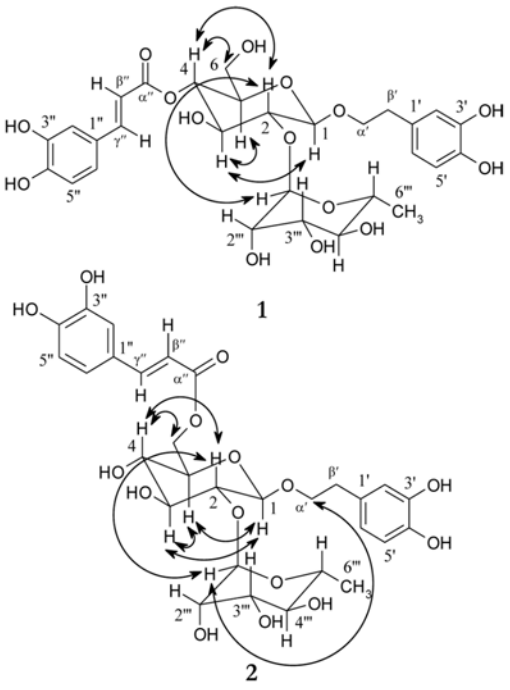

Figure 2. Selected NOESY correlations of compounds **1** and **2**.

Table 2. NMR spectroscopic data of compound **2**

Position	δ_H (*J*)	δ_C	HMBC	NOESY
Glucose				
1	4.33 (*d*, *J*=7.8)	104.4	C-α′	H-3
2	3.52 (*t*, *J*=8.9)	83.9	C-3, C-4, C-1″	H-4, H-1″
3	3.55 (*m*)	75.4	C-1, C-2	H-1, H-5
4	3.42 (*t*, *J*=9.2)	70.4	C-2	H-2
5	3.31 (*m*)	75.7	C-4	H-1, H-3, H-6
6	4.50 (*dd*, ^2J=11.8, 1.8)	64.6	C-α″, C-4, C-5	H-5, H-6
	4.36 (*dd*, ^2J=12.0, 5.8)			
Aglycone				
1′		131.4		
2′	6.67 (*d*, *J*=1.8)	117.1	C-3′, C-6′	H-β′
3′		146.1		
4′		144.7		
5′	6.64 (*d*, *J*=8.0)	116.4	C-1′, C-3′	H-β′
6′	6.53 (*dd*, ^2J=8.0, 1.8)	121.3	C-2′, C-4′	H-α′, H-β′, H-1‴
α′	3.72 (*m*)	72.4	C-1′, C-1	H-2′, H-6′, H-α′
	3.95 (*m*)			
β′	2.78 (*t*, *J*=7.1)	36.7	C- α′, C-1′, C-2′, C-6′	
Caffeic acid				
1″		127.7		
2″	7.04 (*d*, *J*=1.9)	115.1	C-4″, C-6″	
3″		146.8		
4″		149.6		
5″	6.77 (*d*, *J*=8.1)	116.5	C- γ″, C-1″, C-3″	
6″	6.89 (*dd*, ^2J=8.1, 1.9)	123.1		
γ″	7.56 (*d*, *J*=16.0)	147.2	C-2″, C-4″, C-5″	H-β″
β″	6.29 (*d*, *J*=16.0)	114.8	C-2″, C-5″, C-6″, C-α″	H- γ″
α″		169.1	C-1″, C-α″	
Rhamnose				
1‴	5.18 (*s*)	102.7	C-2‴, C-3‴	H-2, H- α′
2‴	3.96 (*m*)	72.2	C-3‴, C-4‴	H-3‴
3‴	3.70 (*m*)	72.3	C-2‴, C-4‴	H-2‴, H-5‴
4‴	3.39 (*t*, *J*=9.2)	74.0	C-2‴, C-3‴	
5‴	4.00 (*m*)	70.4	C-3‴	H-3‴
6‴	1.25 (*d*, *J*=6.2)	17.9	C-4‴, C-5‴	H-5‴

conditions: ^{1}H- and ^{13}C-NMR at 500 and 125 MHz, resp., in CD_3OD; δ in ppm, *J* in Hz.

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