Phenylethanoid and Iridoid Glycosides from the Thai Medicinal Plant, *Barleria strigosa*

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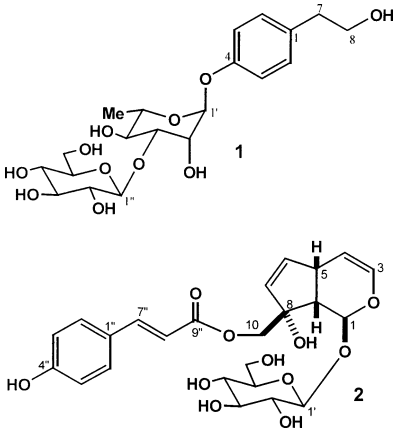
A phenylethanoid (4-hydroxyphenylethyl 4-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside) and an iridoid (10-*O*-*trans*-coumaroyl-eranthemoside) were isolated from an entire *Barleria strigosa* plant together with verbascoside, isoverbascoside, decaffeoylverbascoside, (+)-lyoniresinol 3α-*O*-β-D-glucoside, 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. The structural elucidations were based on analyses of physical and spectroscopic data.

Key words *Barleria strigosa*; Acanthaceae; phenylethanoid; iridoid; strigoside; 10-*O*-*trans*-coumaroyl-eranthemoside

Barleria strigosa WILLD. (Acanthaceae, Thai name: Sang-Ko-Ra-Ni) is a shrub native to tropical regions of Asia. The leaves are used in Thai traditional medicine as an antipyretic as well as an antidote for detoxification of poisons. In our continuing studies on Thai medicinal plants, the constituents of this plant were investigated, following plant collection from the Botanical Gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. In the preliminary study, a quaternary amine (betaine) was reported.[1] The present study deals with the isolation and structural determinations of a new phenylethanoid glycoside (**1**) and a new iridoid glucoside (**2**), together with seven known compounds (**3**—**9**) from an entire plant.

Results and Discussion

The methanolic extract was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to a column of Diaion HP-20, using H_2O, MeOH and Me_2CO, successively. The portion eluted with MeOH was repeatedly chromatographed on columns of silica gel, RP-18 and prep. HPLC-ODS to afford nine compounds. Seven were identified as known compounds: verbascoside (**3**), isoverbascoside (**4**), decaffeoylverbascoside (**5**),[2] (+)-lyoniresinol 3α-*O*-β-D-glucoside (**6**),[3] apigenin 7-*O*-α-L-rhamnosyl-(1→6)-*O*-β-D-glucoside (**7**),[4] 7-*O*-acetyl-8-*epi*-loganic acid (**8**)[5] and (3*R*)-1-octen-3-ol-3-*O*-β-D-xylosyl-(1→6)-β-D-glucoside (**9**)[6] by comparison of physical data with literature values and from spectroscopic evidence.



The molecular formula of compound **1** was determined as $C_{20}H_{30}O_{11}$ by negative high-resolution (HR)-FAB mass spectrometry. The ^{1}H-NMR spectrum revealed the presence of the signals of a *para*-disubstituted aromatic ring at δ 6.98 (2H, d, J=8.5 Hz) and δ 7.15 (2H, d, J=8.5 Hz), two sets of methylene protons at δ 2.76 (2H, t, J=6.9 Hz) and δ 3.71 (2H, t, J=6.9 Hz) in addition to two anomeric signals at δ 4.59 (1H, d, J=7.8 Hz) and δ 5.40 (1H, d, J=1.8 Hz). The ^{13}C-NMR spectrum showed 20 carbon signals (Table 1), of which eight were assignable to the aglycone moiety, and the remaining belonging to the sugar part. All protonated carbons were assigned by heteronuclear single quantum coherence (HSQC). From these spectral data, compound **1** was a glycoside of 4-hydroxyphenylethyl alcohol. Acid hydrolysis

Table 1. NMR Spectral Data of **1** (CD_3OD, ^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz)

Position	δ_C	δ_H
1	134.2	
2, 6	131.0	7.15 (2H, d, J=8.5 Hz)
3, 5	117.5	6.98 (2H, d, J=8.5 Hz)
4	156.1	
7	39.3	2.76 (2H, t, J=6.9 Hz)
8	64.3	3.71 (2H, t, J=6.9 Hz)
Rha		
1′	99.6	5.40 (1H, d, J=1.8 Hz)
2′	71.4	4.28 (1H, dd, J=3.0, 1.8 Hz)
3′	82.7	3.94 (1H, dd, J=9.3, 3.0 Hz)
4′	72.6	3.63 (1H, dd, J=9.5, 9.3 Hz)
5′	70.2	3.70 (1H, m)
6′	18.1	1.22 (3H, d, J=5.9 Hz)
Glc		
1″	105.8	4.59 (1H, d, J=7.8 Hz)
2″	75.4	3.34 (1H, dd, J=8.8, 7.8 Hz)
3″	77.7	3.40 (1H, dd, J=9.3, 8.8 Hz)
4″	71.0	3.39 (1H, dd, J=9.3, 8.1 Hz)
5″	77.6	3.32 (1H, m)
6″	62.2	3.84 (1H, dd, J=12.0, 2.0 Hz)
		3.73 (1H, dd, J=12.0, 4.4 Hz)

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Table 2. NMR Spectral Data of **2** (CD_3OD, ^{1}H-NMR 400 MHz, ^{13}C-NMR 100 MHz)

Position	δ_C	δ_H
1	94.4	5.37 (1H, d, J=3.4 Hz)
3	139.9	6.11 (1H, dd, J=6.2, 2.0 Hz)
4	105.9	4.90 (1H, dd, J=6.2, 3.4 Hz)
5	40.0	3.25 (1H, m)
6	138.2	5.89 (1H, dd, J=5.6, 2.4 Hz)
7	132.4	5.56 (1H, dd, J=5.6, 1.9 Hz)
8	85.0	
9	46.9	2.52 (1H, dd, J=8.3, 3.4 Hz)
10	70.1	4.20 (1H, d, J=11.2 Hz)
		4.09 (1H, d, J=11.2 Hz)
Glc		
1′	99.7	4.61 (1H, d, J=7.8 Hz)
2′	74.7	3.17 (1H, dd, J=8.8, 7.8 Hz)
3′	77.9	3.30 (1H, dd, J=8.8, 8.5 Hz)
4′	71.4	3.23 (1H, dd, J=8.5, 8.1 Hz)
5′	78.2	3.24 (1H, m)
6′	62.5	3.74 (1H, dd, J=12.0, 2.0 Hz)
		3.56 (1H, dd, J=12.0, 5.1 Hz)
Coumaroyl moiety		
1″	127.1	
2″, 6″	131.3	7.37 (2H, d, J=8.8 Hz)
3″, 5″	116.8	6.72 (2H, d, J=8.8 Hz)
4″	161.3	
7″	147.0	7.54 (1H, d, J=15.9 Hz)
8″	114.9	6.25 (1H, d, J=15.9 Hz)
9″	169.1	

afforded D-glucose and L-rhamnose by comparison of the optical rotation with authentic samples. The appearance of the methylene signal (C-8) at δ 64.3 suggested that this position was unsubstituted, demonstrating that the sugar moiety was located at C-4. Negative FAB-MS of compound **1** exhibited a significant fragment ion at m/z 283 $[M-Glc]^-$, indicating that glucose is the terminal sugar. This sugar was assigned to be at C-3′ of the rhamnosyl unit based on the downfield shift of C-3′ (+10.6) together with the upfield shift of C-2′ (−0.8) and C-4′ (−1.3), when compared to the rhamnosyl carbon signals of compound **5**. The assignment was supported by heteronuclear multiple bond connectivity (HMBC), in which long-range correlations were found between (i) H-1′ (δ 5.40, J=1.8 Hz) and C-4 (δ 156.1), C-5′ (δ 70.2), C-3′ (δ 82.7), and (ii) H-1″ (δ 4.59, J=7.8 Hz) and C-3′ (δ 82.7) as shown in Fig. 1. Consequently, the structure of compound **1** was concluded to be 4-hydroxyphenylethyl 4-*O*-β-D-glucopyranosyl-(1→3)-*O*-α-L-rhamnopyranoside, namely strigoside.

The molecular formula of compound **2** was determined as $C_{24}H_{28}O_{11}$ by negative HR-FAB mass spectrometry. Inspection of the ^{13}C-NMR spectral data revealed the presence of one β-glucosyl unit and one coumaroyl moiety in addition to nine carbon signals for the aglycone moiety. The distortionless enhancement by polarization transfer (DEPT) experiments indicated that compound **2** contained one methylene (δ 70.1), seven methines (δ 40.0, 46.9, 94.4, 105.9, 132.4, 138.2 and 139.9) and one quaternary carbon (δ 85.0) for an aglycone part, consistent with an iridoid skeleton. The chemical shift at δ 94.4 was characteristic of an acetal group of C-1. The methine signals at δ 139.9, 105.9, 138.2 and 132.4 were assigned to two disubstituted olefin groups, locating at C-3, C-4, C-6 and C-7, respectively. The coumaroyl moiety

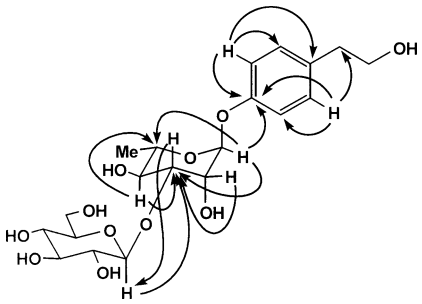

Fig. 1. Significant HMBC Correlation of Compound **1**

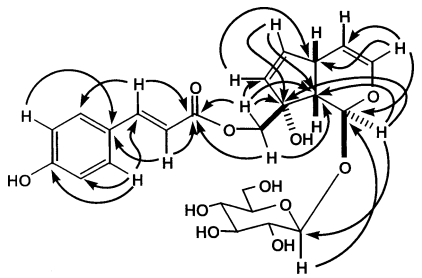

Fig. 2. Significant HMBC Correlation of Compound **2**

was assigned as *trans* by the coupling constant of the signals at δ 7.54 and 6.25 with J=15.9 Hz from the ^{1}H-NMR spectrum. The ^{1}H-NMR spectrum showed methine signals at δ 5.37 (d, J=3.4 Hz), 6.11 (dd, J=6.2, 2.0 Hz), 4.90 (dd, J=6.2, 3.4 Hz), 3.50 (m), 5.89 (dd, J=5.6, 2.4 Hz), 5.56 (dd, J=5.6, 1.9 Hz) and 2.52 (dd, J=8.3, 3.4 Hz) assignable to H-1, H-3, H-4, H-5, H-6, H-7 and H-9, respectively. Also, it showed an AB type of methylene signals at δ 4.20 and 4.09 (each d, J=11.2 Hz) attributable to H-10a and H-10b. The ^{1}H- and ^{13}C-NMR spectral data were closely related to those of eranthemoside,[7] except for lacking the coumaroyl moiety. The complete assignments were established by analyzing the 2D-NMR spectra including HSQC and HMBC in addition to the coupling constants in the ^{1}H-NMR spectrum. In the HMBC spectrum (Fig. 2), the long-range correlations were observed between H-10a, H-10b and C-9″, indicating that the coumaroyl moiety linked to C-10. The glucosyl moiety attached to C-1 from the chemical shift value of this carbon at δ 94.4, and this was confirmed by the HMBC correlations (Fig. 2). The coupling constant between H-1 and H-9 (J=3.4 Hz), H-5 and H-9 (J=8.3 Hz) led to the conclusion that the position of the protons at C-1, C-5 and C-9 were in α, β and β-orientations, respectively. Therefore, the structure of compound **2** was elucidated as 10-*O*-*trans*-coumaroyl-eranthemoside.

Experimental

General Procedures NMR spectra were recorded in CD_3OD using a JEOL JNM α-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR). MS values were obtained on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a union PM-1 digital polarimeter. For column chromatography, silica gel 60 (70—230 mesh, GE0049, Scharlau Chemie S.A.), RP-18 (50 μm, YMC) and Diaion HP-20 (Mitsubishi Chem. Ind. Co., Ltd.) were used. Preparative HPLC was carried out on an ODS column (150×20 mm i.d., YMC) with a Shimadzu RID-6A refractive index detector. The flow rate was 6 ml/min. The solvent systems were: (I) EtOAc–MeOH (9 : 1), (II) EtOAc–MeOH–H_2O (40 : 10 : 1), (III) EtOAc–MeOH–H_2O (70 : 30 : 3), (IV) 10—50% aq. MeOH. (V) 6% aq. MeCN, (VI)

10% aq. MeCN, (VII) 15% aq. MeCN and (VIII) 18% MeCN, The spraying reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

Plant Material *Barleria strigosa* WILLD. was cultivated and collected in July 2003 from the Botanical Garden, Faculty of Pharmaceutical Sciences, Khon Kaen University, Khon Kaen, Thailand. The plant was identified by Mr. Bamrung Thavinchiua, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences of Khon Kaen University. A voucher sample (KKU 0043) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences at the university.

Extraction and Isolation The dried intact *B. strigosa* (1.9 kg) was extracted with MeOH (20 l) under reflux for 15 h. The MeOH extract (246.5 g) was concentrated to dryness and partitioned between Et_2O and H_2O. The aqueous layer was applied to a column of Diaion HP-20 and eluted successively with H_2O, MeOH and Me_2CO. The fraction eluted with MeOH (39.6 g) was subjected to a silica gel column using solvent systems I, II and III. Six fractions were collected. Fraction 3 (7.2 g) was applied to a column of RP-18 using solvent system IV to provide eight fractions. Fraction 3—6 was purified by prep. HPLC-ODS with solvent system VIII to afford compounds **2** (90 mg), **3** (85 mg) and **4** (47 mg). Fraction 4 (3.7 g) was subjected to a column of RP-18 using solvent system IV, affording eleven fractions. Fraction 4-2 was purified by prep. HPLC-ODS with solvent system V to give compound **5** (116 mg). Fraction 4-4 was further purified by prep. HPLC-ODS with solvent system VII to provide compound **6** (79 mg). Compound **7** (309 mg) was crystallized from fraction 4-7. Fraction 4-9 was dried to give an amorphous powder of compound **8** (105 mg). Fraction 5 (6.3 g) was similarly separated on a column of RP-18 using solvent system IV to give seven fractions. Fraction 5-2 was purified by prep. HPLC-ODS with solvent system VI to obtain compound **1** (73 mg). Finally, fraction 5-4 was purified by prep. HPLC-ODS using solvent system VII to provide compound **9** (24 mg).

Compound **1**: Amorphous powder, $[\alpha]_D^{27}$ −25.1° (c=3.07, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) spectra: Table 1; Negative HR-FAB-MS, m/z: 445.1717 ($C_{20}H_{29}O_{11}$ required 445.1709).

Compound **2**: Amorphous powder, $[\alpha]_D^{27}$ −33.3° (c=1.35, MeOH); ^{1}H- and ^{13}C-NMR (CD_3OD) spectra: Table 2; Negative HR-FAB-MS, m/z: 491.1545 ($C_{24}H_{27}O_{11}$ required 491.1553).

Acid Hydrolysis of Compound 1 Compound **1** (34 mg) was dissolved in 5% HCl and heated at 90 °C for 2 h. After cooling, the reaction mixture was extracted with Et_2O. The aqueous layer was neutralized with saturated $NaHCO_3$ and concentrated to dryness. The residue was applied to a silica gel column using solvent system II to give D-glucose (9 mg, $[\alpha]_D^{27}$ +50.2°) and L-rhamnose (7 mg, $[\alpha]_D^{27}$ +8.3°) in comparison with authentic samples.

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