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Original Article

Iridoid and phenylethanoid glycosides from the aerial part of *Barleria lupulina*

Seoung Rak Lee[a], Jon Clardy[b], Donald Robert Senger[c], Shugeng Cao[d,*], Ki Hyun Kim[a,*]

[a] School of Pharmacy, Sungkyunkwan University, Suwon, Gyeonggi-do, Republic of Korea
[b] Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA
[c] Department of Pathology and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[d] Department of Pharmaceutical Sciences, Daniel K Inouye College of Pharmacy, University of Hawaii at Hilo, Hilo, HI, USA

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ABSTRACT

A new iridoid glycoside, barlupulin C methyl ester (**1**), together with two known phenylethanoid glycosides (**2** and **3**) and three known simple phenolic glycosides (**4–6**) were isolated from the aerial parts of *Barleria lupulina* Lindl., Acanthaceae. The structure of the new compound (**1**) was elucidated through 1D and 2D NMR spectroscopic data, and HR-ESIMS. Interestingly, compound (**1**) has a formate group attached to the C-6 hydroxy group of the glucose unit. Compounds **2–6** were identified as poliumoside (**2**), decaffeoylacteoside (**3**), protocatechuic acid 4-*O*-β-glucoside (**4**), vanillic acid 4-*O*-β-glucoside (**5**), and leonuriside A (**6**) on the basis of NMR spectroscopic data analyses and comparison with those reported in the literature. Compounds **3–6** were isolated from *B. lupulina* for the first time.

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Introduction

The genus *Barleria* L., a member of the Acanthaceae family, is a large and widespread genus of herbs and shrubs comprising approximately 300 species, growing mainly in Africa and Asia. The plants of the genus *Barleria* have been long used for boils, bee bites, and tooth-ache (Abd El-Mawla et al., 2005). *Barleria lupulina* Lindl. is a tiny bush widely distributed and domesticated in the South-east Asia region. In Thai traditional medicine, this plant has long been used as a primary anti-inflammatory agent for insect bites and as a remedy for herpes simplex and varicella zoster lesions (Kanchanapoom et al., 2001; Kim et al., 2015a). Previous phyto-chemical investigations on the aerial parts and leaves of *B. lupulina* have led to the isolation of a variety of compounds including iridoid glycosides, phenylpropanoid glycosides, lignan glucosides, aliphatic glycosides, and benzyl alcohol glycosides (Byrne et al., 1987; Tuntiwachwuttikul et al., 1998; Kanchanapoom et al., 2001; Suksamrarn et al., 2003).

During our ongoing search for new bioactive metabolites from medicinal plants, recently we reported the isolation of four new iridoid glycosides with fourteen known analogs and 4,8,8-trimethylcyclooct-2-enone derivatives with six known lignans from the water extracts of *B. lupulina* (Kim et al., 2015a,b). Our continued interest in discovering new compounds from this plant led us to isolate a new iridoid glycoside, barlupulin C methyl ester (**1**), together with two known phenylethanoid glycosides (**2** and **3**) and three known simple phenolic glycosides (**4–6**). The structure of the new compound (**1**) was elucidated through 1D and 2D NMR spectroscopic data, and HR-ESIMS. To the best of our knowledge, this is the first report on the isolation of compounds **3–6** from *B. lupulina*.

Materials and methods

General experimental procedures

Optical rotations were obtained using a Jasco P-1010 polarimeter. UV spectra were recorded on an Amersham Biosciences Ultrospec 5300 Pro spectrophotometer, and IR spectra were measured on a Bruker Alpha-P spectrometer. All NMR experiments were carried out on a Varian INOVA 600 NMR spectrometer. ESIMS spectra were obtained by LC/MS analysis which was performed on an Agilent 1200 Series HPLC/6130 Series mass spectrometer. High resolution mass spectra were obtained on a Waters Micromass Q-Tof Ultima ESI-TOF mass spectrometer. All the compounds were purified on an Agilent 1100 series HPLC (Agilent Technologies) using a Phenomenex Luna phenyl-hexyl column (250 mm × 10 mm, 5 μm particle size), a Phenomenex Luna phenyl-hexyl column (250 mm × 21.2 mm, 10 μm particle size) and a Phenomenex Luna

* Corresponding authors.
E-mails: scao@hawaii.edu (S. Cao), khkim83@skku.edu (K.H. Kim).

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C_{18} column (250 mm × 21.2 mm, 5 μm particle size). Merck precoated silica gel F_{254} plates and RP-18 F_{254s} plates were used for thin layer chromatography (TLC). Spots were detected on TLC under UV light or by heating after spraying with anisaldehyde–sulfuric acid.

Plant material

The aerial part of *Barleria lupulina* Lindl., Acanthaceae, was purchased at Vung Tau Vietnam, in March, 2012. A voucher specimen (No. 101) was deposited at BIDMC, Harvard Medical School.

Extraction and isolation

The air-dried aerial parts (200 g) of *B. lupulina* were sliced and boiled in water (1.2 l) for 4–5 h to 100 ml. This solution was then centrifuged at 10,000 × *g* for 30 min and filtered/sterilized. The combined extracts (200 ml) were suspended in H_2O and then successively partitioned with EtOAc and *n*-BuOH, yielding 0.52 g and 9 g of residues, respectively. The EtOAc-soluble fraction (0.52 g) was fractionated by preparative HPLC (C_{18} column, Phenomenex Luna, 250 mm × 21.2 mm, 5 μm) using 23% aqueous MeCN (0.1% formic acid) for 20 min, then to 100% MeCN (0.1% formic acid) in the next 10 min, and 100% MeCN (0.1% formic acid) for the following 10 min (flow rate: 10 ml/min) to give eight fractions (A–H) according to HPLC chromatography analysis. Fraction B was separated by preparative HPLC (C_{18} column, Phenomenex Luna) using 10% aqueous MeCN for 32 min, then to 100% MeCN in the next 10 min, and 100% MeCN for the following 10 min (flow rate: 10 ml/min) to yield twelve fractions (B1–B12) according to HPLC chromatography analysis. Fraction B4 was purified using a semi-preparative Phenomenex Luna phenyl-hexyl column (6% MeCN with 0.1% formic acid, flow rate: 2 ml/min) to yield compounds **4** (0.8 mg, t_R 29.6 min) and **5** (0.6 mg, t_R 25.6 min). Fraction B6 was separated using a semi-preparative Phenomenex Luna phenyl-hexyl column (7% MeCN with 0.1% formic acid, flow rate: 2 ml/min) to afford compound **6** (0.8 mg, t_R 19.7 min). Fraction B7 was separated by semi-preparative Phenomenex Luna phenyl-hexyl column (10% MeCN with 0.1% formic acid, flow rate: 2 ml/min) to afford compound **3** (0.9 mg, t_R 15.0 min). Fraction B12 was separated by semi-preparative Phenomenex Luna phenyl-hexyl column (13% MeCN with 0.1% formic acid, flow rate: 2 ml/min) to afford compound **1** (0.9 mg, t_R 20.3 min). Fraction H was further separated by preparative HPLC (C_{18} column, Phenomenex Luna) using 40% aqueous MeCN (0.1% formic acid) for 20 min, then to 60% MeCN (0.1% formic acid) in the next 10 min, and 100% MeCN (0.1% formic acid) for the following 10 min (flow rate: 10 ml/min) to yield 39 fractions (H1–H39) according to HPLC chromatography analysis. The combined mixture of fractions from H5 to H9 (assigned as K) was further separated using a preparative Phenomenex Luna phenyl-hexyl column (250 mm × 21.2 mm, 10 μm particle size) using 10% aqueous MeCN (0.1% formic acid) for 30 min, then to 100% MeCN (0.1% formic acid) in the next 5 min, and 100% MeCN (0.1% formic acid) for the following 10 min (flow rate: 10 ml/min) to yield 39 subfractions (K1–K39). The consolidated mixture of fractions from K36 to K38 was separated using a preparative Phenomenex Luna phenyl-hexyl column (21% MeCN with 0.1% formic acid, flow rate: 10 ml/min) to give compound **2** (1.8 mg, t_R 13.5 min).

*Barlupulin C methyl ester (**1**)*

Amorphous powder. $[\alpha]_D^{25}$ −35.8 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3375, 2924, 1657, 1597, 1452, 1352, 1276, 1170, 1025 cm^{-1}; UV (MeOH) λ_{max} (log ε) 236 (3.56) nm; ^1H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) data, see Table 1; positive HR-ESIMS *m/z* 457.1317 $[M+Na]^+$ (calcd. for $C_{18}H_{26}O_{12}Na$, 457.1322).

*Acid hydrolysis of **1***

Compound **1** (0.5 mg) was refluxed in 6% HCl (1 ml) at 80 °C for 2 h. The reaction mixture was extracted with $CHCl_3$ (3 × 6 ml), and the H_2O phase was dried using a speedvac concentrator. The dried water-soluble residue was separately subjected to column chromatography over silica gel with EtOAc–EtOH–H_2O (7:4:1) as an eluent, to yield glucose (0.1 mg), which showed the optical rotation, $[\alpha]_D^{25}$ +42.5 (*c* 0.01, H_2O). TLC identification of glucose was analyzed by silica gel co-TLC with an authentic sample [solvent system ($CHCl_3$–MeOH–H_2O, 8:5:1), R_f of glucose, 0.30] (Kim et al., 2011).

Results and discussion

The present study reports the isolation and identification of an iridoid glycoside (**1**), two phenylethanoid glycosides (**2** and **3**), and three simple phenolic glycosides (**4**–**6**) from the aerial parts of *B. lupulina*. The iridoid glycoside (**1**) was characterized as a new compound. Compounds **4**–**6** were isolated from the genus *Barleria* for the first time, and compound **3** was isolated from *B. lupulina* for the first time.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D^{25}$ −35.8 (*c* 0.05, MeOH). The molecular formula was determined to be $C_{18}H_{26}O_{12}$, by the molecular ion peak at *m/z* 457.1317 $[M+Na]^+$ (calcd. for $C_{18}H_{26}O_{12}Na$, 457.1322) in the positive-ion HR-ESIMS and ^{13}C NMR data. The IR spectrum displayed the presence of hydroxy (3375 cm^{-1}) and carbonyl (1657 cm^{-1}) groups and an enol ether system (1597 cm^{-1}). The ^1H NMR spectrum of **1** (Table 1) showed signals for one methyl group at δ_H 1.24 (3H, s), one methoxy group at δ_H 3.72 (3H, s), one anomeric proton at δ_H 4.65 (1H, d, *J* = 8.5 Hz), one olefinic proton at δ_H 7.39 (1H, s), and one aldehyde proton at δ_H 8.14 (1H, s). The ^{13}C NMR and HSQC spectra for **1** showed 18 carbon signals classified as two methyls (including one methoxy group), one methylene, five methines (including three oxygenated), three quaternary carbons (including one oxygenated), one aldehyde group, and six carbon signals (including one oxygenated methylene and five oxygenated methines), indicating a hexose residue.

The comparison of the NMR data of **1** with those reported for iridoid glycosides revealed that compound **1** has a similar structure

Table 1
^1H (600 MHz) and ^{13}C NMR (150 MHz) data of compound **1** in CD_3OD.[a]

Position	1	
	δ_C	δ_H (*J* in Hz)
1	95.4 d	5.40, d (3.5)
3	153.0 d	7.39, s
4	111.5 s	
5	42.3 d	3.01, dd (10.0, 4.0)
6	78.1 d	4.03, m
7α	49.5 t	2.00, dd (13.0, 6.0)
7β		1.82, dd (14.0, 6.0)
8	79.4 s	
9	52.1 d	2.57, dd (10.0, 3.5)
10	24.8 q	1.24, s
11	169.4 s	
OCH_3	52.2 q	3.72, s
1′	100.6 d	4.65, d (8.5)
2′	74.9 d	3.17, m
3′	78.0 d	3.34, m
4′	71.8 d	3.30, m
5′	75.8 d	3.50, m
6′a	64.2 t	4.51, dd (12.0, 2.0)
6′b		4.28, dd (12.0, 6.0)
6′-COH	163.4 s	8.14, s

[a] The assignments were based on ^1H–^1H COSY, HSQC, TOCSY, and HMBC experiments.

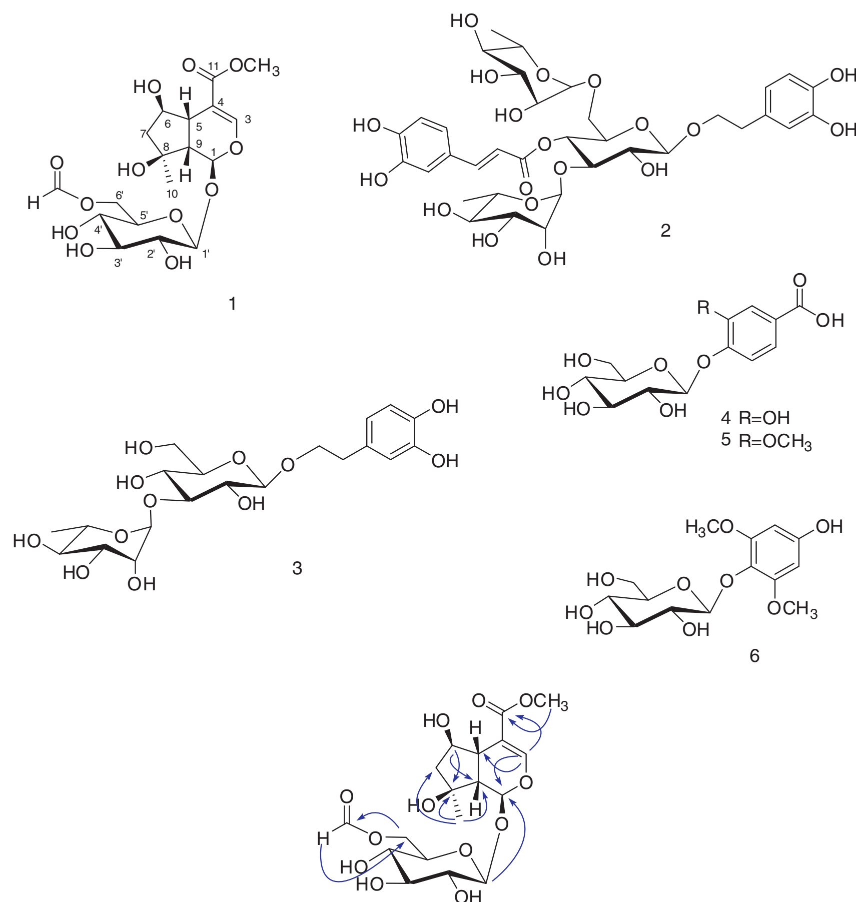

Fig. 1. Key HMBC correlations of compound **1**.

to barlupulin C isolated from this plant, with the exception of the appearance of a methoxy group (Jensen et al., 2007; Kim et al., 2015a). The position of the methoxy group was assigned to C-11 by the HMBC correlations between δ_H 3.72 and δ_C 169.4 (C-11) (Fig. 1). Meanwhile, the position of the ester group (C-11) was confirmed by HMBC correlations from δ_H 7.39 (H-3) and δ_H 3.01 (H-5) to δ_C 169.4 (C-11). Acid hydrolysis of **1** afforded D-glucose, which was identified by TLC comparison with an authentic sample (Kim et al., 2015a), and the configuration was determined by comparison of optical rotation data. The β-anomeric configuration for the glucose was determined by the coupling constant of anomeric proton (d, J = 8.5 Hz). The location of the D-glucose was determined on the basis of HMBC correlation between δ_H 4.65 (H-1′) and δ_C 95.4 (C-1). The relative configuration of **1** was confirmed by analysis of the NOESY spectrum where NOESY correlations between H-9 and H-5/H-7β indicated that H-5 and H-9 are both β-oriented, and NOESY correlations between H-10 and H-1/H-6/H-7α implied that H-1, H-6, and H-10 are all α-oriented. The ^{1}H–^{1}H COSY, TOCSY, HMBC, and NOESY spectra analysis (Fig. 1) allowed us to establish the complete structure of **1**, as shown in Fig. 1. Interestingly, compound **1** has a formate group attached to the C-6 hydroxy group of the glucose unit. Iridoid glycosides with a formate group have yet to be reported in other higher plants, however they were recently isolated from *B. lupulina* (Kim et al., 2015a). This finding suggests that the occurrence of iridoid glycosides with the formate group can serve as a chemotaxonomic marker for *B. lupulina*.

Compounds **2**–**6** were identified as poliumoside (**2**) (Akdemir et al., 2004), decaffeoylacteoside (**3**) (Kim et al., 2009), protocatechuic acid 4-*O*-β-glucoside (**4**) (Singab et al., 2011), vanillic acid 4-*O*-β-glucoside (**5**) (Cui et al., 1993), and leonuriside A (**6**) (Otsuka et al., 1989), respectively, on the basis of NMR spectroscopic data analyses and comparison with those reported in the literature.

Conclusions

The phytochemical investigation of the aerial parts of *B. lupulina* afforded a new iridoid glycoside, barlupulin C methyl ester (**1**), together with two known phenylethanoid glycosides (**2** and **3**); poliumoside (**2**) and decaffeoylacteoside (**3**), and three known simple phenolic glycosides (**4**–**6**); protocatechuic acid 4-*O*-β-glucoside (**4**), vanillic acid 4-*O*-β-glucoside (**5**), and leonuriside A (**6**). Compound **1** has a formate group attached to the C-6 hydroxy group of the glucose unit, which suggested that the structural feature of the formate group in iridoid glycosides may serve as an important chemotaxonomic marker of *B. lupulina*. Compound **3** was isolated from *B. lupulina* for the first time, and compounds **4**–**6** were isolated from the genus *Barleria* for the first time.

Authors' contribution

SRL contributed to the experiment and wrote the manuscript. JC reviewed the manuscript. DRS conducted the experiment. SC and KHK contributed to the design of the study and critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

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