PERGAMON

Phytochemistry 58 (2001) 337–341

PHYTOCHEMISTRY

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Iridoid glucosides from *Barleria lupulina*

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Received 29 December 2000; received in revised form 12 April 2001

Dedicated to Professor Vichiara Jirawongse on the occasion of his 83rd birthday

Abstract

From the aerial part of *Barleria lupulina*, 8-*O*-acetyl-6-*O*-*trans*-*p*-coumaroylshanzhiside, saletpangponosides A–C and 8-*O*-acetylmussaenoside were isolated together with 13 known compounds. The structural elucidations were based on analyses of physical and spectroscopic data.

Keywords: *Barleria lupulina*; Acanthaceae; Iridoid glucosides; Saletpangponosides A–C; Shanzhiside derivatives; 8-*O*-Acetylmussaenoside

1. Introduction

As part of our ongoing study on Thai medicinal plants, we investigated the constituents of *Barleria lupulina* Lindl. (Acanthaceae, Thai name: Sa-let-pang-pon, Chong-ra-ar) collected in the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. *B. lupulina* is a small shrub, distributed in the South-east Asia region. In Thai traditional medicine, the plant is externally used as an antiinflammatory for insect bite, herpes simplex and herpes zoster. In preliminary investigations nine iridoid glucosides (Suksamrarn 1986; Byrne et al., 1987; Tuntiwachwuttikul et al., 1998) have been isolated. In addition, some anti-HSV-2 (herpes simplex virus type 2) activity of organic extracts of the plant have been reported (Yoosook et al., 1999). The present study deals with the isolation and structure elucidation of iridoid glucosides (**1**–**12**), of which five are new (**5**–**8**, **12**), together with six other known compounds; phenylpropanoid glycosides (**13**–**15**), lignan glucoside (**16**), aliphatic glycoside (**17**) and benzyl alcohol glycoside (**18**) from the aerial part of this plant.

2. Results and discussion

The methanolic extract of the aerial part of *B. lupulina* was suspended in H_2O and defatted with Et_2O. The aqueous layer was subjected to column chromatography using a highly porous copolymer of styrene and divinylbenzene, and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH was repeatedly subjected to column chromatography using silica gel and octadecylsilyl silica gel, then by preparative HPLC-ODS to afford 18 compounds (**1**–**18**). Thirteen were known compounds; shanzhiside methyl ester (**1**), 8-*O*-acetylshanzhiside methyl ester (barlerin) (**2**), 6-*O*-acetylshanzhiside methyl ester (**3**), 6,8-*O*,*O*-diacetylshanzhiside methyl ester (acetylbarlerin) (**4**) (Damtoft et al., 1982; Byrne et al., 1987), ipolamiide (**9**) (Damtoft et al., 1984b), ipolamiidoside (**10**) (Byrne et al., 1987), phlorigidoside B (**11**) (Takeda et al., 2000), forsythoside B (**13**) (Endo et al., 1982), verbascoside (**14**) (Andary et al., 1982), poliumoside (**15**) (Andary et al., 1985), (+)-lyoniresinol 3a-*O*-β-glucopyranoside (**16**) (Achenbach et al., 1992), (3*R*)-1-octen-3-yl-β-primeveroside (**17**) (Yamamura et al., 1998) and benzyl alcohol β-(2′-*O*-β-xylopyranosyl) glucopyranoside (**18**) (Sudo et al., 2000) by physical data and spectroscopic evidences.

The molecular formula of compound **5** was determined as $C_{27}H_{32}O_{14}$ by HR–FAB mass spectrometry. Inspection of the ^{13}C NMR spectral data revealed the

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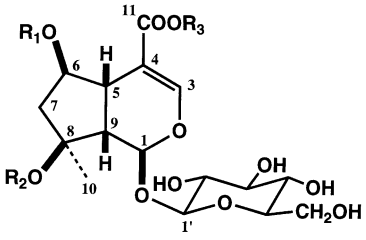
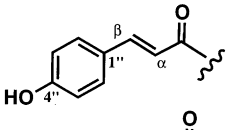
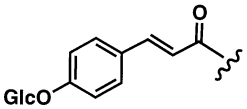
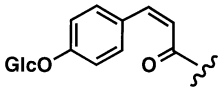
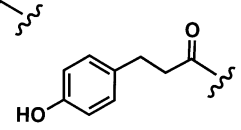
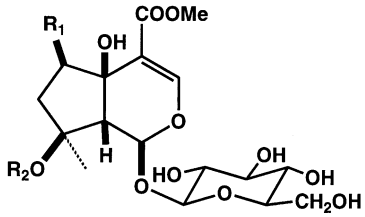
0031-9422/01/$ - see front matter
PII: S0031-9422(01)00236-9

presence of one β-glucopyranosyl unit, one coumaroyl moiety and one acetyl group in addition to eleven carbon signals for the aglycone moiety. The coumaroyl moiety was assigned as *trans* by the coupling constant of the α and β protons in the ^{1}H NMR spectrum (δ 6.28 and 7.56, $J = 15.9$ Hz). The ^{1}H and ^{13}C NMR spectral data were very similar to those of 8-*O*-acetyl-6-*O*-*trans*-*p*-coumaroylshanzhiside methyl ester (Tuntiwachwuttikul et al., 1998), except for lacking the methoxy signal due to the carbomethoxy group, which established the presence of a carboxyl group. On the basis of these spectral data, the structure of compound **5** was determined as 8-*O*-acetyl-6-*O*-*trans*-*p*-coumaroylshanzhiside.

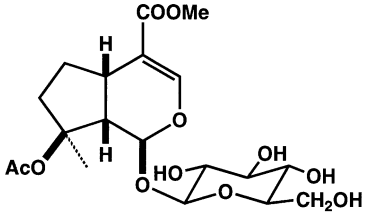


	R_1	R_2	R_3
1	H	H	Me
2	H	Ac	Me
3	Ac	H	Me
4	Ac	Ac	Me
5		Ac	H
6		Ac	Me
7		Ac	Me
8	H		Me

The molecular formula of compound **6** was determined as $C_{34}H_{44}O_{19}$ by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectral data showed the presence of two β-glucopyranosyl units, one *trans*-coumaroyl moiety, as well as the signals for an acetyl group and a carbomethoxy group. The ^{1}H and ^{13}C NMR spectra were very similar to those of 8-*O*-acetyl-6-*O*-*trans*-*p*-coumaroylshanzhiside methyl ester (Tuntiwachwuttikul et al., 1998), except for a set of additional signals arising from a β-glucopyranosyl moiety in **6**. The additional glucopyranosyl unit was assigned to be attached at C-4″ (δ 160.9) of the coumaroyl moiety because the chemical shifts of C-4″, C-3″,5″ (δ 118.0) changed by +0.2 and-2.6 ppm, respectively. Moreover, the HMBC spectrum provided further confirmation with a significant correlation between H-1‴ (δ 4.93, *d*, $J = 7.6$ Hz) of this glucopyranosyl unit and C-4″ of the coumaroyl moiety. Consequently, the structure of compound **6** was assigned as 6-*O*- (4″-*O*-β-glucopyranosyl)-*trans*-*p*-coumaroyl-8-*O*-acetylshanzhiside methyl ester, named saletpangponoside A.



	R_1	R_2
9	H	H
10	H	Ac
11	OH	Ac



12

The molecular formula of compound **7** had the same elemental composition as **6**, $C_{34}H_{44}O_{19}$, by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectral data were very similar to those of **6**. The only significant difference was the coupling constants of the olefinic protons (δ 5.86 and 6.94, $J = 12.9$ Hz). Therefore, the structure of compound **7** was determined as the *cis* isomer of **6**, named saletpangponoside B.

The molecular formula of compound **8** was determined as $C_{26}H_{34}O_{13}$ by HR–FAB mass spectrometry. The ^{1}H and ^{13}C NMR spectral data indicated that **8** is a derivative of shanzhiside methyl ester (**1**), with an ester moiety on the 8-hydroxy group because of the downfield shift of C-8 (+10.5 ppm). The ester moiety showed the presence of an AA′BB′ system (δ 6.64 and 6.98, $J = 8.6$ Hz from ^{1}H NMR), two methylenes (δ 2.49 and δ 2.76 from ^{1}H NMR, and δ 38.2 and 31.2 from ^{13}C NMR) and a carbonyl carbon (δ 175.0), identified as a *p*-dihydrocoumaroyl moiety. The complete assignments were confirmed by the HMBC spectrum, in which H-α (δ 2.49) showed a three bond correlation to C-1″ (δ 132.6), and H-β (δ 2.76) showed the significant correlation to C-2″,6″ (δ 130.3). Thus, the structure of compound **8**

was elucidated as 8-*O*-*p*-dihydrocoumaroylshanzhiside methyl ester, named saletpangponoside C.

The molecular formula of compound **12** was determined as $C_{19}H_{28}O_{11}$ by HR–FAB mass spectrometry. The ^1H and ^{13}C NMR spectra indicated an iridoid structure. The chemical shifts were very similar to those of mussaenoside (Damtoft et al., 1984a) except that the acetyl group was observed in the spectra. The attachment of the acetyl group was assigned to C-8 (δ 91.0), the carbon signal of which was shift to downfield by 10.6 ppm. Therefore, the structure of compound **12** was identified as 8-*O*-acetylmussaenoside.

3. Experimental

3.1. General

NMR spectra were recorded in CD_3OD using a JEOL JNM A-400 spectrometer (400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR) with tetramethylsilane (TMS) as internal standard. MS were recorded on a JEOL JMS-SX 102 spectrometer. Optical rotations were measured with a Union PM-1 digital polarimeter. Preparative HPLC was carried out on columns of ODS (20×150 mm i.d., YMC) with a Tosoh refraction index (RI-8) detector. For CC, silica gel G 60 (Merck), YMC-gel ODS (50 mm, YMC), and highly porous copolymer of styrene and divinylbenzene (Mitsubishi Chem. Ind. Co. Ltd) were used. The solvent systems were: (I) EtOAc–MeOH (9:1), (II) EtOAc–MeOH–H_2O (4:1:0.1), (III) EtOAc–MeOH–H_2O (7:3:0.3), (IV) 20–35% MeCN, (V) 15% MeCN, (VI) 25% MeCN, (VII) 10–35% MeCN, (VIII) 10% MeCN and (IX) 40% MeOH. The spray reagent used for TLC was 10% H_2SO_4 in 50% EtOH.

3.2. Plant material

Barleria lupulina Lindl. was collected in September 1999 from the Botanical gardens, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand. The identification of the plant was confirmed by Prof. Vichiara Jirawongse, Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University. A voucher sample (KKU-0018) is kept in the Herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand.

Table 1
^1H NMR spectral data of compounds **5**–**8** and **12** (400 MHz, CD_3OD)

H	5	6	7	8	12
1	5.89, *d* (2.7)[b]	5.85, *d* (3.2)	5.85, *d* (3.2)	5.82, *d* (2.4)	5.71, *d* (3.4)
3	7.49, *d* (1.5)	7.48, *d* (1.5)	7.50, *d* (1.5)	7.38, *d* (1.5)	7.43, *d* (1.0)
5	3.33*[a]	3.30*	3.25*	2.94*	3.12, *m*
6	5.40, *m*	5.34, *m*	5.34, *m*	4.25, *m*	1.75, *m*
7	2.37, *bd* (15.4)	2.38, *bd* (15.4)	2.38, *bd* (15.6)	2.11, *bd* (14.9)	2.05, *m*
	2.05, *dd* (15.4, 5.3)	2.09, *dd* (15.4, 5.3)	2.09, *dd* (15.6, 5.4)	1.97, *dd* (14.9, 5.4)	
9	3.01, *dd* (8.5, 2.7)	3.00, *dd* (8.6, 3.2)	2.90, *dd* (8.6, 3.2)	2.94*	2.68, *dd* (8.5, 3.4)
10	1.51, *s*	1.53, *s*	1.53, *s*	1.38, *s*	1.54, *s*
COOMe		3.65, *s*	3.68, *s*	3.66, *s*	3.69, *s*
1′	4.65, *d* (7.8)	4.63, *d* (8.1)	4.66, *d* (7.8)	4.59, *d* (7.8)	4.59, *d* (7.8)
2′	3.18, *dd* (9.0, 7.8)	3.16, *dd* (9.0, 8.1)	3.22, *dd* (9.0, 7.8)	3.14, *dd* (9.0, 7.8)	3.14, *dd* (9.0, 7.8)
3′	3.37, *dd* (9.0, 9.0)	3.34*	3.34*	3.32, *dd* (9.0, 8.8)	3.32, *dd* (9.0, 8.8)
4′	3.26, *dd* (9.0, 9.0)	3.23, *dd* (9.5, 9.5)	3.26*	3.22, *dd* (9.5, 8.8)	3.22, *dd* (9.5, 8.8)
5′	3.31, *m*	3.31, *m*	3.31, *m*	3.26, *m*	3.26, *m*
6′	3.88, *dd* (12.2, 2.2)	3.88, *dd* (11.9, 2.2)	3.90, *dd* (12.0, 2.0)	3.85, *dd* (12.2, 2.2)	3.85, *dd* (12.2, 2.2)
	3.66, *dd* (12.2, 6.1)	3.66, *dd* (11.9, 6.1)	3.71, *dd* (12.0, 5.6)	3.61, *dd* (12.2, 6.4)	3.61, *dd* (12.2, 6.4)
2″,6″	7.40, *d* (8.6)	7.53, *d* (8.8)	7.65, *d* (8.8)	6.98, *d* (8.5)	
3″,5″	6.77, *d* (8.6)	7.08, *d* (8.8)	7.07, *d* (8.8)	6.64, *d* (8.5)	
α	6.28, *d* (15.9)	6.37, *d* (15.9)	5.86, *d* (12.9)	2.49, *m*	
β	7.56, *d* (15.9)	7.61, *d* (15.9)	6.94, *d* (12.9)	2.76, *m*	
1‴		4.93, *d* (7.6)	4.96, *d* (7.6)		
2‴		3.42*	3.48*		
3‴		3.34*	3.34*		
4‴		3.40*	3.45*		
5‴		3.26*	3.28*		
6‴		3.81, *dd* (12.0, 2.0)	3.87, *dd* (12.0, 1.7)		
		3.64, *dd* (12.0, 6.4)	3.71, *dd* (12.0, 5.6)		
OAc		1.92, *s*	1.89, *s*		1.99, *s*

[a] *Chemical shift obtained approximately from HSQC.
[b] *J* (Hz) in parentheses.

3.3. Extraction and isolation

The dried aerial part (900 g) of *B. lupulina* was extracted with hot MeOH. After removal of the solvent by evaporation, the residue (100 g) was defatted with Et_2O. The aqueous layer was subjected to a column of highly porous copolymer of styrene and divinylbenzene and eluted with H_2O, MeOH and Me_2CO, successively. The fraction eluted with MeOH (29.0 g) was subjected to a column of silica gel (systems I, II and III, respectively) affording five fractions. Fraction 2 (7.4 g) was applied to a RP-18 column using system IV to provide six fractions, together with compound **4** (5.1 g). Fractions 2–5 were purified by prep. HPLC–ODS (system VI) to afford compounds **8** (43 mg) and **12** (27 mg). Fraction 3 (8.4 g) was subjected to a column of RP-18 (system IV) to give eight fractions, along with compounds **2** (2.6 g) and **10** (596 mg). Fractions 3-1 and 3-2 were further purified by prep. HPLC-ODS (system V) to provide compound **3** (398 mg). Fraction 3-8 was purified by prep. HPLC-ODS (system VI) to afford compound **16** (92 mg). Fraction 4 (8.2 g) was applied to a RP-18 column (system VII) to afford fifteen fractions, and compound **1** (1.9 g). Fraction 4-5 was purified by prep. HPLC–ODS (system VIII) to give compounds **9** (50 mg) and **18** (59 mg). Fraction 4-8 was purified by prep. HPLC–ODS (system V) to provide compounds **11** (32 mg) and **17** (27 mg). Fraction 4-9 was further purified by prep. HPLC-ODS (system IX) to give compounds **13** (162 mg), **14** (73 mg) and **15** (138 mg). Finally, fractions 4-13 and 4-14 were purified by prep. HPLC-ODS (system VI) to provide compound **5** (23 mg), **6** (90 mg) and **7** (73 mg).

Table 2
^{13}C NMR spectral data of compounds **5–8** and **12** (100 MHz, CD_3OD)

C	5	6	7	8	12
1	95.3	95.4	95.4	95.7	95.5
3	154.6	154.5	154.5	153.6	153.0
4	108.4	108.5	108.4	109.8	112.2
5	39.8	39.9	39.8	42.2	32.9
6	78.8	78.9	78.8	76.0	29.7
7	45.2	45.1	45.0	47.7	39.6
8	89.6	89.6	89.6	89.7	91.0
9	50.3	50.3	50.3	49.9	51.0
10	21.9	21.8	21.8	22.2	21.2
11	169.7	168.4	168.4	169.0	169.0
OMe		51.9	51.9	51.8	51.7
1′	100.3	100.3	100.3	100.4	100.2
2′	74.6	74.6	74.5	74.7	74.7
3′	77.8	77.9	77.8	78.0	78.0
4′	71.5	71.6	71.6	71.4	71.6
5′	78.2	78.3	78.3	78.2	78.3
6′	62.9	62.9	62.9	63.0	62.9
1″	127.0	129.8	130.3	132.6	
2″,6″	131.1	130.8	132.9	130.3	
3″,5″	115.4	118.0	117.1	116.2	
4″	161.1	160.9	159.7	156.7	
α	116.8	117.2	118.8	38.2	
β	146.5	145.9	144.0	31.2	
CO	168.5	168.1	167.4	175.0	
1‴		101.8	101.9		
2‴		74.8	74.8		
3‴		77.9	77.8		
4‴		71.2	71.2		
5‴		78.1	78.1		
6‴		62.4	62.4		
Oac	173.0	172.9	172.9		173.0
	22.2	22.3	22.2		22.2

3.4. 8-O-Acetyl-6-O-trans-p-coumaroylshanzhiside (*5*)

Amorphous powder, $[\alpha]_D^{21}$ −75.6° (MeOH, *c* 1.58); ^1H NMR (CD_3OD): Table 1 and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m/z*: 579.1710 $[M–H]^-$ ($C_{27}H_{31}O_{14}$ requires 579.1713).

3.5. Saletpangponoside A (*6*)

Amorphous powder, $[\alpha]_D^{21}$ −90.3° (MeOH, *c* 1.79); ^1H NMR (CD_3OD): Table 1 and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m/z*: 755.2413 $[M–H]^-$ ($C_{34}H_{43}O_{19}$ requires 755.2398).

3.6. Saletpangponoside B (*7*)

Amorphous powder, $[\alpha]_D^{21}$ −104.8° (MeOH, *c* 2.43); ^1H NMR (CD_3OD): Table 1 and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m/z*: 755.2413 $[M–H]^-$ ($C_{34}H_{43}O_{19}$ requires 755.2398).

3.7. Saletpangponoside C (*8*)

Amorphous powder, $[\alpha]_D^{21}$ −58.9° (MeOH, *c* 2.86); ^1H NMR (CD_3OD): Table 1 and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m/z*: 553.1979 $[M–H]^-$ ($C_{26}H_{33}O_{13}$ requires 553.1921).

3.8. 8-O-Acetylmussaenoside (*12*)

Amorphous powder, $[\alpha]_D^{21}$ −53.2° (MeOH, *c* 1.84); ^1H NMR (CD_3OD) Table 1 and ^{13}C NMR (CD_3OD): Table 2; negative HR–FAB–MS, *m/z*: 431.1576 $[M–H]^-$ ($C_{19}H_{27}O_{11}$ requires 431.1553).

Acknowledgements

We would like to thank the Research Center for Molecular Medicine, Hiroshima University for the use of its NMR facilities. Also, we are grateful to Mr. Bamrung Thavinchiua of the Department of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, for help in obtaining the plant material.

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