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IRIDOID GLUCOSIDES OF *BARLERIA LUPULINA*

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Key Word Index—*Barleria lupulina*; Acanthacea; leaves; iridoid glucosides.

Abstract—In addition ot the known compounds, barlerin, acetylbarlerin, shanzhiside methyl ester, acetylshanzhiside methyl ester and ipolamiidoside, four iridoid glucosides isolated from the leaves of *Barleria lupulina* have been identified as 6-*O*-*p*-methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester, 6-*O*-*p*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester and 6-*O*-*p*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In earlier work on the constituents of *Barleria lupulina* Lindl., five iridoid glucosids were isolated, i.e. shanzhiside methyl ester (**1**), 8-*O*-acetylshanzhiside methyl ester (barlerin) (**2**), 6,8-*O*,*O*-diacetylshanzhiside methyl ester (acetylberlerin) (**3**) [1], 6-*O*-acetylshanzhiside methyl ester (**4**) and ipolamiidoside (**5**) [2]. This paper describes the isolation and structure elucidation of four new iridoid glucosides, 6-*O*-*p*-methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**6**), 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**7**), 6-*O*-*p*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**8**) and 6-*O*-*p*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester (**9**).

RESULTS AND DISCUSSION

Compound **6** analyzed for $C_{29}H_{36}H_{14}\cdot 2H_2O$ but gave $[M+H]^+$ at *m*/*z* 609 (CIMS). The UV spectrum showed bands at λ_{max} 227 (iridoid enol-ether system with a methoxycarbonyl group), 297 (sh) and 307 nm (cinnamoyl unit) and there were characteristic IR bands at 3400, 1700 and 1638 cm^{-1} indicative of a hydroxyl group, an ester carbonyl and enol-ether system, respectively. A carboxylated iridoid was also supported by the presence of two singlets at δ 7.48 (H-3) and 3.71 (COOMe) in the ^1H NMR spectrum. Two acetalic protons resonating at δ 4.66 (*d*, $J = 7.5$ Hz) and at δ 5.95 (*d*, $J = 1.5$ Hz) were assigned to the anomeric proton of the β-D-glucopyranosyl moiety and to H-1, respectively. Two singlets at δ 1.52 and 1.90 were the protons of a tertiary methyl group (C-10) and of an acetyl group at C-8, respectively. Four aromatic signals appeared as two doublets at δ 6.88 and 7.73 ($J = 9.0$ Hz) (AA′BB′ pattern), two olefinic signals at δ 5.79 ($J = 12.0$ Hz) and 6.86 ($J = 12.0$ Hz) arose from the *cis*-cinnamoyl moiety, and a singlet at δ 3.84 was assignable to a methoxy group on the aromatic ring. These data led to the conclusion that compound **6** had a *p*-methoxy-*cis*-cinnamoyl unit as the acyl function.

The downfield shift of 0.14 ppm in the signal of H-6 (δ 5.41) in the ^1H NMR spectrum of **6** compared the corresponding signal (δ 5.27) in **3** [1] suggested that the acyl unit, *p*-methoxy-*cis*-cinnamoyl, was located at C-6 and not at C-8 in **6**. The structure of **6** was then established to be 6-*O*-*p*-methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester.

The spectral data (UV, IR, MS) of compound **7** were quite similar to those of **6**. The ^1H NMR of **7** differed from that of **6** only by the signals of the two olefinic protons in the cinnamoyl unit. Two doublets of olefinic protons of **7** (δ 6.29 and 7.61, $J = 15.0$ Hz) appeared at lower fields with higher coupling constants values than those of the corresponding values of compound **6** (Table 1). These data led to the conclusion that compound **7** had a *p*-methoxy-*trans*-cinnamoyl moiety as the acyl function attached to C-6. 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester was assigned to compound **7**.

The chemical analysis data ($C_{28}H_{34}O_{14}\cdot 11/2H_2O$) and $[M+H]^+$ (*m*/*z* 595) of compounds **8** and **9** suggested that both compounds had one methylene group (CH_2) less than compounds **6** and **7**. This was con-

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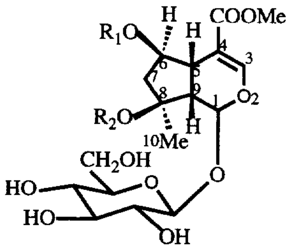
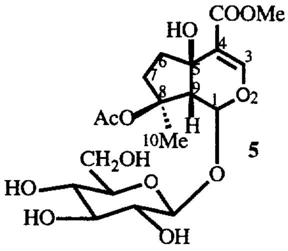


	R_1	R_2
1	H	H
2	H	Ac
3	Ac	H
4	Ac	Ac
6	*p*-OMe-*cis*-cinnamoyl	Ac
7	*p*-OMe-*trans*-cinnamoyl	Ac
8	*p*-*cis*-coumaroyl	Ac
9	*p*-*trans*-coumaroyl	Ac



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firmed by the absence of aromatic methoxyl signals in the ^1H NMR spectra of **8** and **9**. Other peaks in the ^1H NMR spectra of **8** and **9** were almost identical to those of **6** and **7**, respectively (see Table 1). The UV, IR and MS spectra of **8** and **9** were quite similar to those of **6** and **7**. These data led to the conclusion that compound **8** was 6-*O*-*p*-*cis*-coumaroyl-8-*O*-acetylshanzhiside methyl ester and **9** was 6-*O*-*p*-*trans*-coumaroyl-8-*O*-acetylshanzhiside methyl ester.

The ^{13}C NMR data of iridoids **6**–**9** were in agreement with the structures (Table 2).

EXPERIMENTAL

Unless otherwise stated, microanalyses were carried out by the Scientific and Technological Research Equipment Center, Chulalongkorn University, Bangkok, Thailand. Mps: uncorr; MeOH; ^1H NMR: $CDCl_3$ and DMSO-d_6, Bruker AMX 400 (400 MHz), TMS as int. standard; ^{13}C NMR: $CDCl_3$ and DMSO-d_6, 100.62 MHz, TMS as int. standard; CI MS: Finnigan MAT TSQ46 (desorption probe), CH_4 being used as reagent gas; Optical rotation: MeOH; TLC: precoated PF_{254} plates (Merck); spots were detected by spraying with 1% $CeSO_4$ in 10% aq. H_2SO_4 followed by heating; CC: silica gel 70–230 mesh (Merck); Semi-prep. HPLC: Waters LC consisting of 6000A pump, U6K injector, 450 nm variable wavelength UV detector and R401 RI detector, using a Partisil M9 10/50 ODC-2 (Whatman) column, MeOH–H_2O (58:42) and (52:48) at a flow rate of 4 ml min^{-1}. A voucher specimen (Bansiddhi 9763) of

Table 1. ^1H NMR spectral data of iridoids **6**–**9**

H	**6**	**7**	**8**	**9**
1	5.95, *d* (1.5)	6.03, *d* (1.5)	6.05, *d* (1.5)	6.02, *d* (1.5)
3	7.48, *s*	7.50, *s*	7.49, *s*	7.50, *s*
5	3.25, *d* (9.0)	3.31, *d* (9.0)	3.23, *dd* (1.5, 9.0)	3.31, *dd* (1.5, 9.0)
6	5.41, *d* (4.5)	5.46, *d* (4.5)	5.46, *d* (4.5)	5.46, *d* (4.5)
7a	2.01, *dd* (4.5, 15.0)	2.04, *dd* (4.5, 15.0)	1.94, *dd* (4.5, 15.0)	2.01, *dd* (4.5, 15.0)
7b	2.38, *d* (15.0)	2.41, *d* (15.0)	2.36, *d* (15.0)	2.41, *d* (15.0)
9	3.00, *dd* (1.5, 9.0)	3.09, *dd* (1.5, 9.0)	3.01, *dd* (1.5, 9.0)	3.10, *dd* (1.5, 9.0)
10	1.57, *s*	1.53, *s*	1.47, *s*	1.53, *s*
COOMe	3.70, *s*	3.71, *s*	3.72, *s*	3.70, *s*
OAc	1.90, *s*	1.96, *s*	1.88, *s*	1.96, *s*
α	5.79, *d* (12.0)	6.29, *d* (15.0)	5.77, *d* (12.0)	6.22, *d* (15.0)
β	6.86, *d* (12.0)	7.61, *d* (15.0)	6.82, *d* (12.0)	7.58, *d* (15.0)
2 × ArH	6.88, *d* (9.0)	6.97, *d* (9.0)	6.83, *d* (9.0)	6.86, *d* (9.0)
2 × ArH	7.73, *d* (9.0)	7.49, *d* (9.0)	7.61, *d* (9.0)	7.39, *d* (9.0)
OMe	3.84, *s*	3.86, *s*		
1′	4.66, *d* (7.5)	4.69, *d* (7.5)	4.69, *d* (7.5)	4.70, *d* (7.5)

Table 2. ^{13}C NMR chemical shifts of iridoids **6** **9** in $CDCl_3/DMSO$-d_6

C	6	7	8	9
1	92.4	93.4	93.8	93.4
3	151.5	152.5	152.3	152.4
4	105.3	106.1	105.8	106.0
5	37.1	37.8	37.6	37.6
6	75.2	76.3	76.0	77.4
7	42.5	43.5	43.8	43.5
8	86.4	87.2	87.0	87.0
9	47.2	48.1	48.0	48.0
10	20.0	21.4	21.2	21.2
1′	97.4	98.5	98.9	98.6
2′	71.6	72.5	72.2	72.3
3′	75.6	76.1	76.0	76.1
4′	68.7	69.8	69.9	69.6
5′	77.2	76.3	76.1	76.1
6′	60.2	61.3	61.7	61.2
1″	125.2	126.5	124.6	125.0
2″,6″	128.4	131.6	128.9	131.7
3″,5″	113.0	113.8	115.2	114.3
4″	159.8	159.7	158.9	158.1
α	114.0	116.3	113.5	115.0
β	142.0	142.6	144.0	142.9
C=O	164.6	164.8	165.6	164.8
	165.0	165.9	165.7	165.7
	169.2	170.2	170.3	170.1
OMe	49.8	50.6	50.4	50.5
	53.9	54.7	—	—
OAc	19.8	20.8	20.8	20.8

the plant material has been deposited at the Herbarium, the Division of Medicinal Plant Research and Development, Department of Medical Science, Nonthaburi 11000, Thailand.

Extraction and isolation

Fresh leaves of *B. lupulina* (1.62 kg) were blended with 95% EtOH at room temp. and filtered. Removal of EtOH *in vacuo* gave a dark green solid (209.3 g). The solid was dissolved in H_2O (800 ml) and extracted with Et_2O (4 × 600 ml). The H_2O layer was extracted with *n*-BuOH (4 × 600 ml). Removal of *n*-BuOH *in vacuo* gave a dark green solid (85.6 g). A portion of the *n*-BuOH extract (59.1 g) was subjected to CC on silica gel (700 g) using CH_2Cl_2–MeOH (20 : 1 and 20 : 2) and CH_2Cl_2–MeOH–H_2O (20 : 3 : 1, 15 : 3 : 1, 10 : 3 : 1, 7 : 3 : 1 and 6 : 4 : 1) as the eluent to give 11 frs as dark solids (12.3, 5.6, 2.6, 3.7, 3.4, 1.5, 1.7, 1.3, 3.5, 4.6 and 11.3 g respectively).

6-*O*-*p*-Methoxy-*cis*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**6**) *and* 6-*O*-*p*-methoxy-*trans*-cinnamoyl-8-*O*-acetylshanzhiside methyl ester (**7**). Fr. 1 (12.3 g) was chromatographed on silica gel (600 g) using CH_2Cl_2–MeOH–H_2O (30 : 3 : 1) as the eluent to give a mixture of glucosides **6** and **7** as a dark green solid (413 mg), a mixture of **6**, **7** and acetylbarlerin (**3**) as a dark green solid (754 mg) and compound **3** as a light green solid (7.5 g). The mixture of **6** and **7** (413 mg) was rechromatographed on silica gel using CH_2Cl_2, CH_2Cl_2–MeOH (50 : 1, 40 : 1, 30 : 1 and 25 : 1) as the eluent to give as a colourless solid (317 mg) a mixture of **6** and **7**. Purification of this mixture (200 mg) by RP HPLC gave **6** (57 mg) and **7** (99 mg) as colourless solids. Compound **6**, colourless powder: (found: C, 53.9; H, 6.2. $C_{29}H_{36}O_{14}\cdot 2H_2O$ requires C, 54.0; H, 6.2%), $[\alpha]_D^{24} = -129.7°$ (c, 0.35). UV λ_{max} nm (log ε): 227 (4.36), 297 (sh) (4.25), 307 (4.27); IR ν_{max} cm^{-1}: 3400, 1700, 1640, 1600, 1270, 1180, 1080, 1020; CI-MS m/z (rel. int.): 609 $[M+H]^+$ (12), 549 (12), 447 (12), 387 (22), 371 (70), 227 (40), 209 (100), 191 (92), 185 (40), 179 (11), 161 (40); ^1H NMR: Table 1; ^{13}C NMR: Table 2. Compound **7** colourless powder: (found: C, 54.0, H, 5.9. $C_{29}H_{36}O_{14}\cdot 2H_2O$ requires C, 54.0; H, 6.2%), $[\alpha]_D^{25} = -103.0°$ (c, 0.26). UV λ_{max} nm (log ε): 227 (4.36), 297 (sh) (4.35), 310 (4.38); IR ν_{max} cm^{-1}: 3400 (broad), 1715, 1645, 1602, 1260, 1190, 1030; CI-MS m/z (rel. int.): 609 $[M+H]^+$ (12), 549 (22), 447 (25), 387 (22), 371 (47), 227 (55), 209 (100), 191 (85), 179 (11), 161 (25); ^1H NMR: Table 1; ^{13}C NMR: Table 2.

6-*O*-*p*-*cis*-Coumaroyl-8-*O*-acetylshanzhizide methyl ester (**8**) *and* 6-*O*-*p*-trans-coumaroyl-8-*O*-acetylshanzhside methyl ester (**9**). Fr. 3 (2.6 g) was subjected to CC on silica gel (150 g) using CH_2Cl_2–MeOH (40 : 1, 30 : 1, 20 : 1, 15 : 1, 10 : 1 and 10 : 2, respectively) as the eluent to give acetylbarlerin (**3**) as a colourless solid (372 mg) and a mixture of compounds **8**, **9** and barlerin (**2**) as a green solid (1.7 g). The mixture was further purified on a column of silica gel (100 g) using CH_2Cl_2–MeOH (50 : 1, 45 : 1, 40 : 1, 30 : 1, 20 : 1, 10 : 1 and 5 : 1 respectively) as the eluent to give a mixture of **8** and **9** as a colourless solid (274 mg), and a mixture of **8**, **9** and barlerin (**2**) as a colourless solid (460 mg) and compound **2** as a colourless solid (164 mg). The mixture of **8** and **9** (153 mg) was repurified by RP HPLC to give **8** and **9** as colourless solids (29 and 117 mg, respectively). Compound **8**, colourless solid, mp. 133–135° (found: C, 54.2; H, 5.6. $C_{28}H_{34}O_{14}$ 1 1/2 H_2O requires C, 54.1; H, 6.0 %), $[\alpha]_D^{23} = -143.6°$ (c, 0.10). UV λ_{max} nm (log ε): 228 (4.33), 299 (sh) (4.24), 312 (4.29); IR ν_{max} cm^{-1}: 3400, 1700, 1640, 1605, 1290, 1180, 1090, 1030; CI-MS m/z (rel. int.): 595 $[M+H]^+$ (30), 535 (20), 433 (17), 371 (20), 269 (10), 209 (70), 191 (100), 165 (10), 147 (10); ^1H NMR: Table 1; ^{13}C NMR: Table 2. Compound **9** colourless solid, mp 151–153°C (found: C, 54.0; H, 5.9. $C_{28}H_{34}O_{14}$. 1 1/2 H_2O requires C, 54.1; H, 6.0%). $[\alpha]_D^{24} = -110.9°$ (c, 0.26). UV λ_{max} nm (log ε): 220 (4.33), 298 (sh) (4.30), 312 (4.33); IR ν_{max} cm^{-1}: 3400, 1700, 1638, 1602, 1290, 1170, 1080, 1040; CIMS m/z (rel. int.): 595 $[M+H]^+$ (30), 535 (25), 433 (20), 371 (20), 269 (10), 209 (60), 191 (100), 165 (12), 147 (12); ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

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