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LI Jin[a]; HAI-SHENG Chen[a]; YONG-SHENG Jin[a]; SHUANG Liang[a]; ZHAO-BAO Xiang[a]; JIA Lu[a]
[a] College of Pharmacy, Second Military Medical University, Shanghai, China

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Chemical constituents from *Belamcanda chinensis*

LI JIN, HAI-SHENG CHEN*, YONG-SHENG JIN, SHUANG LIANG, ZHAO-BAO XIANG and JIA LU

College of Pharmacy, Second Military Medical University, Shanghai 200433, China

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One new flavone and one new isoflavone glycoside were isolated along with 15 known compounds from the rhizome of *Belamcanda chinensis* (Iridaceae), and their structures were characterised as 5,4′-dihydroxy-6,7-methylenedioxy-3′-methoxyflavone (**1**) and 3′,5′-dimethoxy irisolone-4′-*O*-β-D-glucoside (**2**) on the basis of spectroscopic methods.

Keywords: *Belamcanda chinensis*; Iridaceae; Flavone; Isoflavone glycoside

1. Introduction

The dried rhizomes of *Belamcanda chinensis* (L.) DC (Iridaceae) have been used in China as folk medicine for the treatment of coughing and pharyngitis [1]. As for the chemical constituents of the plant, the occurrence of iridal-type triterpenoids [2–4] and isoflavonoids [5–12] in the rhizomes, and phenols, benzoquinones and benzofurans [3,13] in the seeds was reported. Further investigation of the rhizomes resulted in the isolation of a new flavone, 5,4′-dihydroxy-6,7-methylenedioxy-3′-methoxyflavone (**1**) and a new isoflavone glycoside, 3′,5′-dimethoxy irisolone-4′-*O*-β-D-glucoside (**2**), together with 15 known compounds (figure 1). We report herein the structure elucidation of the two new compounds.

2. Results and discussion

The dried rhizomes were chopped and extracted with 80% EtOH three times under reflux. The EtOH extract was partitioned with chloroform and ethyl acetate. Column chromatography of the chloroform and ethyl acetate soluble portion over silica gel, Sephadex LH-20, and octadecylsilyl (ODS) gel gave 17 compounds including **1** and **2**. Among them 15 were identified as irigenin (**3**) [5], iridin (**4**), tectorigenin (**5**), tectoridin (**6**) [14], irisflorentin (**7**) [6], nonirisflorentin (**8**) [7], iristectogenin A (**9**) [14], dichotomitin (**10**)

*Corresponding author. Email: haishengc@hotmail.com

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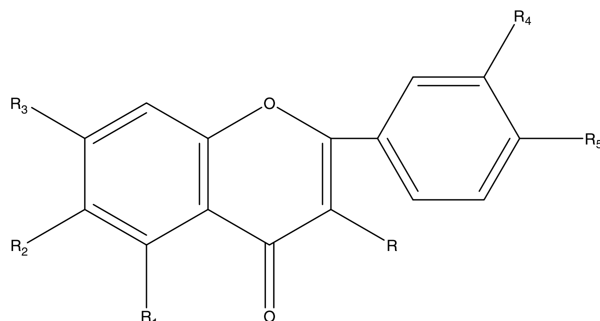
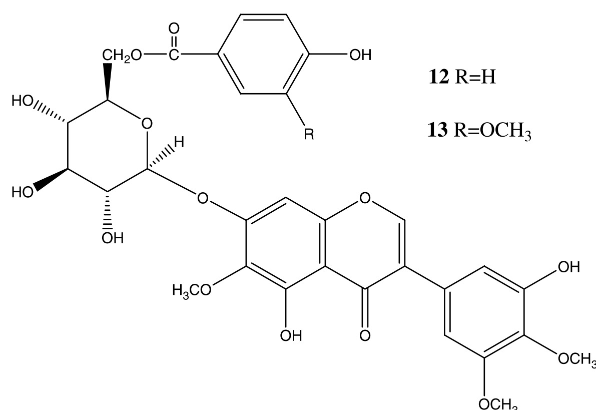


1 R=H, R1, R_5=OH, R_2, R_3=OCH_2O, R_4=OCH_3 **15** R=OH, R_1, R_3, R_5=OH, R_4=OCH_3

14 R=H, R_1, R_3, R_5=OH, R_2=OCH_3, R_4=H **16** R=OH, R_1, R_5=OH, R_3, R_4=OCH_3

	R_1	R_2	R_3	R_4	R_5	R_6
2	OCH_3	O-CH_2-O		OCH_3	Oglc	OCH_3
3	OH	OCH_3	OH	OH	OCH_3	OCH_3
4	OH	OCH_3	O-glc	OH	OCH_3	OCH_3
5	OH	OCH_3	OH	H	OH	H
6	OH	OCH_3	O-glc	H	OH	H
7	OCH_3	O-CH_2-O		OCH_3	OCH_3	OCH_3
8	OH	O-CH_2-O		OCH_3	OCH_3	OCH_3
9	OH	OCH_3	OH	OH	OCH_3	H
10	OH	O-CH_2-O		OH	OCH_3	OCH_3
11	OH	OH	OH	OH	OCH_3	H



12 R=H

13 R=OCH_3

Figure 1. Structures of compounds **1**–**16**.

[15], 5,6,7,3′-tetrahydroxy-4′-methoxyisoflavone (**11**), 6″-*O*-vanilloyiridin (**12**), 6″-*O*-*p*-hydroxybenzoyliridin (**13**) [8], hispidulin (**14**), isorhamnetin (**15**) [9], rhamnazin (**16**) [16], and acetovaninone (**17**) [10] on the basis of their NMR spectral data and by comparison of their physical properties with those reported in the literature.

Compound **1**, a yellow powder, exhibited a $[M-H]^-$ ion peak at *m/z* 327 in EI-MS and the molecular formula $C_{17}H_{12}O_7$ was determined by HRESI-MS at *m/z* 327.0502 $[M-H]^-$.

Its IR spectrum showed the presence of hydroxyl (3460 cm^{-1}), conjugated carbonyl (1685 cm^{-1}), methylenedioxy (928 cm^{-1}) and an aromatic ring system (1623 cm^{-1}). The UV spectrum had maxima at 343 and 286 nm, respectively, indicating it to be a flavone. The ^{13}C NMR spectrum also revealed carbons signals (C-2: δ 164.0, C-3: δ 103.0, C-4: δ 182.5) of a skeleton of flavone, one methoxyl (δ 56.0) and one methylenedioxy (δ 102.7) groups. The ^{1}H NMR spectrum showed a typical three substituents in ring B at δ 7.56 (d, $J = 2.0$ Hz), δ 6.93 (d, $J = 9.0$ Hz), δ 7.57 (dd, $J = 2.0$, 9.0 Hz), which were assigned to H-2′, H-5′, and H-6′, respectively. One proton singlet at δ 6.96 was ascribable to H-3. The extreme downfield characteristic signal at δ 13.03 could be assigned to 5-OH due to the intramolecular hydrogen bond. This was further supported by strong and broad IR absorption bands for hydroxyl groups (3460 cm^{-1}). The appearance of RDA fragment ions at *m/z* 180 and 148 in the mass spectrum established the presence of one methylenedioxy in ring A and one methoxy and one hydroxy in ring B. The positions of the methoxyl group at C-3′ and the hydroxyl group at C-4′ in the ring B were ascertained by the NOESY spectrum, which showed a clear correlation between the methoxyl proton signal (δ 3.90) and H-2′ (δ 7.56, d, $J = 2.0$ Hz). The methylenedioxy group positioned at C-6, C-7 on account of the downfield proton signal at δ 6.96 (H-8) and the upfield carbon signal at δ 89.76 (C-8), which was very similar to those reported for kanzakiflavone-2 [17]. In the HMBC spectrum, the methylenedioxy protons (δ 6.17) were also found to be coupled with C-6 (δ 129.5) and C-7 (δ 152.6) and the methoxyl protons were (δ 3.90) coupled with C-3′ (δ 148.1) (Table 1). Consequently, compound **1** was deduced to be 5,4′-dihydroxy-6,7-methylenedioxy-3′-methoxyflavone.

Compound **2** was obtained as a yellow amorphous powder. Its molecular formula $C_{25}H_{26}O_{13}$ was determined by HRESI-MS at *m/z* 557.1264 $[M + Na]^{+}$. The presence

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Table 1. ^{13}C NMR spectral data (125 MHz, DMSO-d_6, δ ppm) and HMBC correlations for compounds **1** and **2**.

	1		*2*	
Position	δ_C	*HMBC*	δ_C	*HMBC*
2	164.0		152.1	H-2/C-3, 4, 9, 1′
3	103.0	H-3/C-2, 4, 10, 1′	127.3	
4	182.5		173.7	
5	153.7		140.5	
6	129.5		135.9	
7	152.6		152.6	
8	89.8	H-8/C-6, 7, 9, 10	93.5	H-8/C-6, 7, 9, 10
9	141.1		153.8	
10	106.7		113.2	
1′	121.3		123.9	
2′	110.3	H-2′/C-2, 1′, 3′, 4′, 6′	107.9	H-2′/C-3, 1′, 3′, 4′, 6′
3′	148.1		152.2	
4′	150.9		134.4	
5′	115.8	H-5′/C-1′, 3′, 4′, 6′	152.2	
6′	120.4	H-6′/C-2, 1′, 3′, 4′, 6′	107.9	H-6′/C-3, 1′, 2′, 3′, 4′
glc-1″			102.6	H-1″/C-4′
2″			74.1	
3″			77.2	
4″			69.8	
5″			76.5	
6″			60.7	
6,7-(OCH_2O)	102.7	6,7-(OCH_2O)/C-6, 7	102.6	6,7-(OCH_2O)/C-6, 7
5-OMe			60.7	5-OMe/C-5
3′-OMe	56.0	3′-OMe/C-3′	56.5	3′-OMe/C-3′
5′-OMe			56.5	5′-OMe/C-5′

of an isoflavone skeleton was suggested from the UV spectrum (λ_{max} 293, 322 nm). The ^{1}H NMR and ^{13}C NMR spectra of 2 also showed a number of signals characteristic of sugar and isoflavone moieties. Strong IR absorptions at 1656 cm^{-1} (C=O), 1581 cm^{-1} (C=C), 3490 cm^{-1} (OH) and 937 cm^{-1} (OCH_2O), along with a broad C—O stretching band in the region 1012–1280 cm^{-1} further confirmed the presence of an isoflavone moiety. In the ^{1}H NMR spectrum the characteristic isoflavone signal for H-2 was observed at δ 8.34 and one aromatic proton signal at δ 7.02 for H-8, indicating three substituents of the A-ring. A represented two-proton singlet at δ 6.86 (H-2′, 6′) indicated a symmetric trisubstituted B-ring. The spectrum also revealed the presence of three methoxyl groups (δ 3.91, δ 3.80, δ 3.80) and a methylenedioxy (δ 6.19) in the structure. Represented six-proton singlet at δ 3.80 for two methoxyl groups could be attached to C-3′, 5′. Thus, the methylenedioxy group must be located on ring A. The anomeric proton of the β-glucose moiety appeared as a doublet at δ 4.97 ($J = 5.9$ Hz) and other characteristic carbons signals were at δ 74.1 (C-2″), δ 77.2 (C-3″), δ 69.8 (C-4″), δ 76.5 (C-5″), δ 60.7 (C-6″). By the HMBC spectrum, the correlation of anomeric proton (δ 4.97) with C-4′ (δ 134.4) indicated that the sugar unit was connected to C-4′. So the third methoxyl group should be attached to C-5, and this was further confirmed by the interaction of the methoxyl protons (δ 3.91) with C-5 (δ 140.5). The other important correlations in the HMBC spectrum were the methylenedioxy protons (δ 6.19) with C-6 (δ 135.9) and C-7 (δ 152.6) (Table 1). On the basis of the above evidence, compound **2** was elucidated as 3,5-dimethoxy irisolone-4-*O*-β-D-glucoside.

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3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a WZZ-2S/2SS digital automatic polarimeter. IR were recorded on KBr discs with a Bruker Vector 22 spectrometer. UV were obtained in MeOH on a UV-210A spectrometer. EI-MS and HRESI-MS were performed with a Mat-212 and a Micromass Auto Spec Q-TOF spectrometer, respectively. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer with tetramethylsilane (TMS) as an internal standard and DMSO-d_6, CD_3OD as solvents. Chemical shifts were given in δ (ppm) values.

3.2 Plant material

The plant material was purchased in October 2000 from Bozhou, Anhui province and identified as the rhizomes of *Belamcanda chinensis* (L.) DC by Professor Zhen Hanchen, College of Pharmacy, Second Military Medical University. A voucher specimen has been deposited in the herbarium of School of Pharmacy, Second Military Medical University, Shanghai (No. 20001022.).

3.3 Extraction and isolation

The dried rhizomes of *B. chinensis* (4 kg) were chopped, extracted with 80% EtOH three times under reflux, and concentrated under vacuum to yield an EtOH extract (200 g). The extract was diluted in H_2O and extracted successively with petroleum ether, $CHCl_3$ and EtOAc to give petroleum ether (22 g), $CHCl_3$ (65 g), and EtOAc (28 g) extract. The $CHCl_3$

extract (65 g) was subjected to column chromatography over silica gel using a gradient solvent system $CHCl_3 \rightarrow CHCl_3$/MeOH (20:1) to afford three fractions (c1–c3). The fraction c1, eluted by $CHCl_3$, was chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) to afford five subfractions (c1-1–c1-5). The subfraction c1-1 was further chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) to yield compounds **7** (980 mg), **17** (133 mg) and **11** (15 mg). The subfraction c1-2 was recrystallised in MeOH to yield compounds **1** (12 mg), **8** (51 mg), and **16** (39 mg). The subfraction c1-4 was also chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and then recrystallised in MeOH to yield compounds **3** (1.1 g) and **9** (318 mg). The fraction c2, eluted by $CHCl_3$/MeOH (80:1), was chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) to afford three subfractions (c2-1–c2-3). The subfraction c2-1 was purified by recrystallisation from MeOH to obtain compound **10** (95 mg). The subfraction c2-2 was further chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) to obtain **5** (920 mg) and **15** (8 mg). The fraction c3, eluted by $CHCl_3$/MeOH (40:1), was separated by preparative TLC, using $CHCl_3$/MeOH (20:1) as mobile phrase, to give compound **14** (21 mg).

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The EtOAc extract (28 g) was subjected to column chromatography over silica gel using a solvent system $CHCl_3$/MeOH (20:1 → 1:1) in stepwise gradient mode to afford three fractions (e1–e3). The fraction e1 was chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and then purified by preparative TLC ($CHCl_3$/MeOH, 5:1) to yield compound **2** (30 mg). The e2 fraction was chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and then separated by C_{18} column chromatography (MeOH/H_2O, 50:50) to yield compounds **12** (10 mg) and **13** (18 mg). The fraction e3 was also chromatographed over Sephadex LH-20 using $CHCl_3$/MeOH (1:1) and then separated by C_{18} column chromatography (MeOH/H_2O, 50:50) to yield compounds **4** (660 mg) and **6** (500 mg).

3.3.1 5,4′-Dihydroxy-6,7-methylenedioxy-3′-methoxyflavone (1). Yellow powder, $C_{17}H_{12}O_7$. IR (KBr) ν_{max} cm^{-1}: 3490 (OH), 1656 (C=O), 1581 (C=C), and 937 (OCH_2O). UV λ_{max} (MeOH) nm: 343, 286. ^{1}H NMR (DMSO-d_6) δ: 13.03 (1H, s, 5-OH), 9.99 (1H, s, 4′-OH), 7.57 (1H, dd, $J = 2.0$, 9.0 Hz, H-6′), 7.56 (1H, d, $J = 2.0$ Hz, H-2′), 6.96 (1H, s, H-3), 6.96 (1H, s, H-8), 6.93 (1H, d, $J = 9.0$ Hz, H-5′), 6.17 (2H, s, —OCH_2O—), 3.90 (3H, s, OCH_3). ^{13}C NMR: see table 1. EI-MS *m/z* (%): 327 $[M-H]^-$ (100). HRESI-MS *m/z*: 327.0502 $[M - H]^-$ (calcd for $C_{17}H_{11}O_7$, 327.0505).

3.3.2 3′,5′-Dimethoxy irisolone-4′-*O*-β-D-glucoside (2). Yellow amorphous powder, $C_{25}H_{26}O_{13}$. ($[\alpha]_D$ + 46.7 (*c* 0.73, MeOH). IR (KBr) ν_{max} cm^{-1}: 3460 (OH), 1685 (C=O), 1582 (C=C), and 928 (OCH_2O). UV λ_{max} (MeOH) nm: 322, 293. ^{1}H NMR (DMSO-d_6) δ: 8.34 (1H, s, H-2), 7.02 (1H, s, H-8), 6.86 (2H, s, H-2′, 6′), 6.19 (2H, s, —OCH_2O—), 4.97 (1H, d, $J = 5.9$ Hz, glc H-1″), 3.91, 3.80, 3.80 (each 3H, s, OCH_3), ^{13}C NMR: see table 1. ESI-MS *m/z*: 557.08 $[M + Na]^+$, 569.07 $[M + Cl]^-$. HRESI-MS *m/z* 557.1264 $[M + Na]^+$ (calcd for $C_{25}H_{26}O_{13}$ Na, 557.1271).

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