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CHEMICAL CONSTITUENTS OF *BELAMCANDA CHINENSIS* (L.) DC

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ABSTRACT

*From the ethyl acetate extract of the rhizomes from Belamcanda chinensis (Iridaceae) five compounds irisflorentin (**1**), tectorigenin (**2**), iristectorigenin A (**3**), irigenin (**4**), and acetovanillone(**5**) were isolated. Their structures were elucidated by spectroscopic methods. This is first report of **5** from B. chinensis.*

Keywords: *Belamcanda chinensis, Iridaceae, acetovanillone.*

I - INTRODUCTION

Belamcanda chinensis (L.) DC., is a perennial shrub belonging to Iridaceae family. It is widely distributed in the cold and wet hillsides in Vietnam and in most parts of China, Korea, Japan, India, and eastern of Russia. The dried rhizomes have been used in Vietnamese traditional and folk medicine as an anti-inflammatory, antitussive, and expectorant agent as well as against throat trouble [1]. In China, it is an important traditional medicine used to treat swelling and pain in the throat, cough and so on. Previous phytochemical study on this plant led to the iridal-type triterpenoids and isoflavonoids in the rhizomes, and phenol, benzoquinones and benzofurans in the seed [2, 3].

In our study, the ethyl acetate extract of the rhizomes of *B. chinensis* exhibited the cytotoxic activity against the hepatonema carcinoma cell line (Hep-G2) with the IC_{50} value of 16.18μg/ml and antimicrobial activity against *Staphylococus aureus* with the MIC value of 100 μg/ml. Further study on chemical constituents of this extract led to the isolation of irisflorentin (**1**), tectorigenin (**2**), iristectorigenin A (**3**), irigenin (**4**), and acetovanillone (**5**).

II - MATERIALS AND METHODS

1. Plant material

The rhizomes of *B. chinensis* was collected in Tamdao National Botanical Garden, Vietnam, in February 2007 and identified by biologist Ngo Van Trai, National Institute of Medicinal Materials. A voucher specimen is deposited in the Herbarium of Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

2. General experimental procedures

The NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500MHz for ^{1}H and 125MHz for ^{13}C) using TMS as an internal standard. The ESI mass spectra were obtained using an AGILENT spectrometer. The following adsorbents were used for purification: TLC normal phase Kieselgel 60 F_{254} (Merck 5554, 0.2 mm), CC: normal phase Si gel (Merck, 0.063 - 0.200 mm). The TLC chromatograms were visualized under UV at 254 and 368 nm and sprayed with

solution of $Ce(SO_4)_2$ in H_2SO_4 65%.

3. Extraction and isolation

Air-dried powdered rhizomes of *B. chinensis* (2 kg) were soaked successively in *n*-hexane three times at room temperature to yield a *n*-hexane extract (4.5 g). The residue was further extracted with MeOH to give MeOH residue (65 g). The MeOH residue was diluted with H_2O and then extracted successively with EtOAc and *n*-BuOH to give EtOAc (23 g) and *n*-BuOH (4.7 g) residue after removal of the solvents *in vacuo*. The EtOAc (10 g) was subjected to a column chromatography over silica gel using a solvent system $CHCl_3$—MeOH (99:1 to 90:10, v/v) in stepwise gradient mode to afford ten sub-fractions (B1- B10). The sub-fraction B2 (2.5g) was further separated on a normal-phase silica gel column eluting with $CHCl_3$—MeOH (30:1, v/v) to give compounds **1** (20 mg) as pale yellow powder and **5** (200 mg) as white powder. The sub-fraction B6 (3.5 g) was chromatographied on a normal-phase silica gel column eluting with $CHCl_3$-MeOH (40:1, v/v) to yield 8 smaller fractions (B6A to B6H). Compounds **2** (12 mg) and **3** (7 mg) were obtained as pale yellow plates from fraction B6C (260 mg) by using a normal-phase silica gel column eluting with $CHCl_3$-MeOH (25:1, v/v). Finally, compound **4** (20 mg) was obtained as yellow powder from the fraction B6E (350 mg) by a normal-phase silica gel column eluting with $CHCl_3$-MeOH (20:1, v/v).

Irisflorentin (1): Pale yellow powder, mp. 168-169ºC, $C_{20}H_{18}O_8$, ESI-MS *m/z* 385 $[M-H]^-$.

^{1}H-NMR (CD_3OD and $CDCl_3$): δ (ppm) 7.76 (1H, s, H-2), 6.56 (1H, s, H-8), 6.67 (2H, s, H-2′, H-6′), 5.99 (2H, s, -O-CH_2-O-), 4.00 (3H, s, OCH_3-5), 3.81 (6H, s, OCH_3-3′, 5′), and 3.77 (3H, s, OCH_3-4′).

^{13}C-NMR (CD_3OD and $CDCl_3$): δ (ppm) 56.0 (OCH_3-3′, 5′), 60.6 (OCH_3-4′), 60.9 (OCH_3-5), 92.9 (C-8), 102.1 (-O-CH_2-O-), 106.6 (C-2′, 6′), 113.4 (C-10), 125.4 (C-3), 127.3 (C-1′), 135.2 (C-6), 137.9 (C-4′), 141.5 (C-5), 150.8 (C-2), 152.8 (C-3′, 5′), 152.9 (C-7), 154.5 (C-9), and 175.3 (C-4).

Tectorigenin (2): Pale yellow plates (1,2 g), mp. 235 - 236ºC, $C_{16}H_{12}O_6$, ESI-MS *m/z* 299 $[M-H]^-$.

^{1}H-NMR (CD_3OD and $CDCl_3$): δ (ppm) 3.87 (3H, s, OCH_3-6), 6.41 (1H, s, H-8), 6.84 (2H, dd, *J* = 8.5, 1.5Hz, H-3′, 5′), 7.35 (2H, dd, *J* = 8.5, 1.5Hz, H-2′, 6′), and 7.99 (1H, s, H-2). ^{13}C-NMR (CD_3OD and $CDCl_3$): δ (ppm) 60.9 (OCH_3-6), 94.9 (C-8), 106.6 (C-10), 116.2 (C-2′,6′), 123.1 (C-3), 124.1 (C-1′), 131.3 (C-3′, 5′), 132.7 (C-6), 154.4 (C-5), 154.7 (C-7), 154.8 (C-2), 158.5 (C-9), 158.6 (C-4′), and 182.4 (C-4).

Iristectorigenin A (3): Pale yellow plates (120 mg), mp. 237-238□C, $C_{17}H_{14}O_7$, ESI-MS *m/z* 329 $[M-H]^-$.

^{1}H-NMR (CD_3OD and $CDCl_3$): δ (ppm) 3.89 (3H, s, OCH_3-6), 3.90 (3H, s, OCH_3-3′), 6.46 (1H, s, H-8), 6.88 (1H, d, *J* = 8.0 Hz, H-2′), 6.96 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 7.14 (1H, *J* = 2.0Hz, H-5′), and 8.06 (1H, s, H-2). ^{13}C-NMR (CD_3OD and $CDCl_3$): δ (ppm) 56.4 (OCH_3-3′), 60.9 (OCH_3-6), 94.9 (C-8), 106.6 (C-10), 113.8 (C-5′), 116.1 (C-2′), 122.7 (C-6′), 123.5 (C-3), 124.2 (C-1′), 132.7 (C-6), 147.7 (C-4′), 148.6 (C-3′), 154.4 (C-5), 154.7 (C-7), 154.8 (C-2), 158.6 (C-9), and 182.4 (C-4).

Irigenin (4): Yellow powder, mp. 184-185□C, $C_{18}H_{16}O_8$, ESI-MS *m/z* 359 $[M-H]^-$.

^{1}H-NMR (CD_3OD and $CDCl_3$): δ (ppm) 3,88 (3H, s, OCH_3-4′), 3,89 (3H, s, OCH_3-5′), 3,94 (3H, s, OCH_3-6), 6,48 (1H, s, H-8), 6,68 (2H, dd, *J* = 6.0 and 2.0 Hz, H-2′, 6′), and 7,90 (1H, s, H-2). ^{13}C-NMR (CD_3OD and $CDCl_3$): δ (ppm) 55.7 (OCH_3-6), 60.3 (OCH_3-4′), 60.4 (OCH_3-5′), 93.9 (C-8), 104.9 (C-10), 105.7 (C-2′), 109.4 (C-6′), 122.9 (C-3), 126.4 (C-1′), 131.2 (C-6), 136.2 (C-4′), 149.8 (C-3′), 152.7 (C-5′), 152.8 (C-5), 153.2 (C-9), 153.3 (C-2), 156.7 (C-7), and 180.7 (C-4).

Acetovanillone(5): White powder, $C_9H_{10}O_3$, ESI-MS *m/z* 167 $[M+H]^+$.

^{1}H-NMR ($CDCl_3$): δ (ppm) 2.56 (3H, s, CH_3-8), 3.96 (3H, d, *J* = 7.5Hz, OCH_3-9), 6.06 (1H, s, OH), 6.94 (1H, d, *J* = 8.0Hz, H-6), 7.54 (1H, dd, *J* = 1.8 and 8.0 Hz, H-7), and 7.53 (1H, d, *J*

= 1.8 Hz, H-3). ^{13}C-NMR ($CDCl_3$): δ (ppm) 196.7 (C=O), 130.3 (C-2), 124.1 (C-3), 146.6 (C-4), 150.4 (C-5), 113.8 (C-6), 109.8 (C-7), 26.17 (CH_3-8), and 56.12 (CH_3-9).

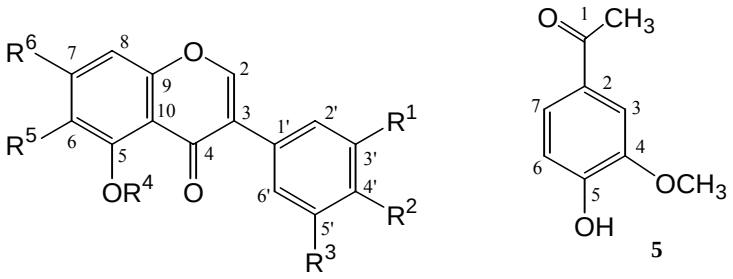
III - RESULTS AND DISCUSSION

Compound **1** was obtained as pale yellow powder from the EtOAc extract of the rhizomes. In the ^{1}H-NMR spectrum, three aromatic protons were assigned at δ 6.56, 7.76 (each 1H, singlet), and at δ 6.67 (2H, singlet), a dioxymethylene group was confirmed by the appearance of a singlet at δ 5.99 (2H), and four methoxyl groups attaching aromatic rings at δ 4.00 (3H), 3.81 (6H), and 3.77 (3H) as singlets. The connecting observation of the proton signal at δ 7.76 with carbon signal at δ 150.8 in the HSQC spectrum was assigned to H-2/C-2 of an isoflavone [4]. The proton signal at δ 6.67 (2H) had HSQC cross peak with only carbon signal at δ 106.6, and two methoxyl groups at δ 3.81 (6H)/δ 56.0 suggesting that the B ring was a tetra-substituted ring with two methoxyl groups at C-3′ and C-5′ [4, 5]. The ^{13}C-NMR spectrum of **1** showed signals of 20 carbon atoms including 4 methoxyl (δ 56.0 x 2, 60.9, and 60.6), 1 dioxymethylen (δ 102.1) and 15 carbon atoms belonging to an isoflavone. In the HMBC spectrum, the H-C long-range correlations between H-2 (δ 7.76) to C-4 (δ 175.3), C-9 (δ 154.5) and C-1′ (δ 127.3), between H-8 (δ 6.56) and C-9 (δ 154.5)/C-7 (δ 152.9)/C-6 (δ 135.2)/C-10 (δ 113.4), and between dioxymethylene proton at δ 5.99 and C-6 (δ 135.2)/C-7 (δ 152.9) were observed confirming the A and C ring assignments of a isoflavone with a dioxymethylene group attached to C-6 and C-7. Furthermore, proton H-2′ at δ_H 6.67 had HMBC correlation with C-3 (δ 125.4)/C-3′ (δ 152.8)/C-4′ (δ 137.9), methoxyl protons at δ 3.81 and 3.77 had HMBC correlations with C-3′ (δ 152.8) and C-4′ (δ 137.9), respectively, confirming that three methoxyl groups attached to C-3′, C-4′ and C-5′, and two left protons were H-2′ and H-6′. From the above data, the suggesting structure of **1** was showed in Fig. 1 with its molecular formula as $C_{20}H_{18}O_8$, which was further confirmed by the exhibition of a quasi molecular ion peak at *m/z* 385 $[M-H]^-$ in the ESI mass spectrum. All the NMR data of **1** were in good agreement with those of 5,3′,4′,5′-tetramethoxy-6,7-methylenedioxyisoflavone or irisflorentin, which was isolated from *B. chinensis* [4, 5].

Compound **2** was obtained as pale yellow plates, mp.235-236□C. The presence of an isoflavone skeleton was suggested from the UV spectrum (λ_{max} 259, 320nm). The ^{1}H-NMR spectrum of **2** showed signals at δ 6.84 (2H, dd, *J* = 8.5, 1.5 Hz, H-3′, 5′) and 7.35 (2H, dd, *J* = 8.5, 1.5 Hz, H-2′, 6′) suggesting a *para*-substitued ring, a singlet at δ 7.99 (1H, s) assigned for H-2, the other singlet at δ 6.41 (1H, s) suggested the A ring was penta-substituted, and one methoxyl group at δ 3.87 (3H, s, OCH_3-6). The ^{13}C-NMR spectrum showed signals of 16 carbon atoms, including one methoxyl and 15 carbons of the isoflavone, which were further confirmed by DEPT 90, DEPT 135 and HSQC spectra. In addition, the ESI mass spectrum exhibited an ion peak at *m/z* 299 $[M-H]^+$ corresponding to the molecular formula of $C_{16}H_{12}O_6$. All the NMR data of **2** were in good agreement with those of 4′,5,7-trihydroxy-6-methoxyisoflavone or tectorigenin [6], whose molecular formula is $C_{16}H_{12}O_6$.

Compound **3** was obtained as a pale yellow plates. The UV, ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2** suggesting that **3** was an isoflavonoid. The proton signals at δ 6.88 (1H, d, *J* = 8.0 Hz, H-2′), 6.96 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), and 7.14 (1H, *J* = 2.0 Hz, H-5′) confirmed that the B ring was 1,3,4-tetra-subtitued, two singlets at δ 6.46 and 8.06 were assigned for H-8 and H-2, respectively, and two methoxyl groups were at δ 3.89 (3H, s, OCH_3-6) and 3.90 (3H, s, OCH_3-4′). The ^{13}C-NMR spectrum of **3** exhibited signals of 17 carbon atoms including two methoxyl groups at δ 56.4 (OCH_3-4′) and 60.9 (OCH_3-6), and the others belonging to a isoflavone skeleton, confirming by DEPT 90, DEPT 135, HSQC, and HMBC spectra. Moreover, the ESI mass spectrum of **3**

showed a quasi molecular ion peak at *m/z* 329 $[M-H]^+$ corresponding to the molecular formula of $C_{17}H_{14}O_7$. On the basis of these data, compound **3** was characterized as iristectorigenin A or (4′,5,7-trihydroxy-3′,6 dimethoxyisoflavone) [6].



1 $R^1 = R^2 = R^3 = OCH_3$, $R^4 = CH_3$, $R^5R^6 = O\text{-}CH_2\text{-}O$
2 $R^1 = R^3 = R^4 = H$, $R^2 = R^6 = OH$, $R^5 = OCH_3$
3 $R^2 = R^6 = OH$, $R^1 = R^5 = OCH_3$, $R^3 = R^4 = H$
4 $R^1 = R^6 = OH$, $R^2 = R^3 = R^5 = OCH_3$, $R^4 = H$

Figure 1: The structures of isolated compounds from *Belamcanda chinensis*

The NMR spectra of **4** were very similar to those of **3** except for the additional signals of the methoxyl group suggesting that **4** was a derivative of **3**. Three methoxyl groups at δ 3.88/136.2, 3.89/152,7, and 3.94/131,2, four methine carbons at δ 7,90 /153,3, 6.48/93.9, 6.68/105,7, and δ 6.68/109,4 were identified from ^{13}C-NMR, DEPT 90, DEPT 135, and HSQC spectra. The suggesting structure of **4** was shown in Fig. 1, and its NMR assignments were assigned by comparing with the corresponding data of 3′,5,7-trihydroxy-4′,5′,6 trimethoxyisoflavone (or irigenin) [6] and found to match. Furthermore, the ESI mass spectrum of **4** showed a quasi molecular ion peak at *m/z* 359 $[M-H]^-$, corresponding to the molecular formula of $C_{18}H_{16}O_8$.

The ^{13}C-NMR spectrum of **5** exhibited signals of 9 carbon atoms. Of which, 6 signals at δ 130.3 (C-2), 124.1 (C-3), 146.6 (C-4), 150.4 (C-5), 113.8 (C-6), and 109.8 (C-7) were assigned for one aromatic ring, and three others at δ 196.7, 26.17, and 56.12 were assigned for the carbonyl, methyl, and methoxyl carbons, respectively. The signals at δ 6.94 (d, J = 8.0 Hz), 7.54 (dd, J = 8.0, 1.8 Hz), and 7.53 (d, J = 1.8 Hz) in the ^{1}H-NMR spectrum confirmed that the aromatic ring was 1,3,4-tri-substituted. The HMBC correlation between H-8 (δ 2.56) and C-2 (δ 130.3), between H-6 (δ 6.94) and C-4 (δ 146.6), between H-7 (δ 7.54) and C-5 (δ 150.4), and between methoxyl proton at δ 3.96 and C-4 were observed. In addition, the HMBC correlation between H-7 and C-4 was not observed. This evidence confirmed that the hydroxyl, methoxyl, and carbonyl groups were attached to C-5, C-4, and C-2 of the aromatic ring, respectively. Furthermore, the ESI mass spectrum of **5** showed a quasi molecular ion peak at *m/z* 167 $[M+H]^+$, corresponding to the molecular formula of $C_9H_{10}O_3$. Thus, compound **5** was identified as acetovanillone.

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