Isoflavonoids from *Belamcanda chinensis*

Hideyuki Ito, Satomi Onoue, and Takashi Yoshida*

Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700—8530, Japan.
Received May 9, 2001; accepted June 19, 2001

Four new isoflavonoids were isolated along with six known related compounds from a rhizome of *Belamcanda chinensis* (Iridaceae), and their structures were characterized as 6″-*O*-*p*-hydroxybenzoyliridin, 6″-*O*-vanilloyliridin, 5, 6, 7, 3′-tetrahydroxy-4′-methoxyisoflavone and 2, 3-dihydroirigenin, respectively, on the basis of spectroscopic methods and chemical evidence.

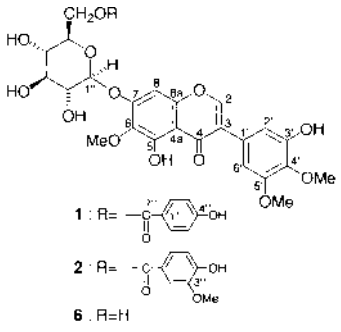
Key words *Belamcanda chinensis*; Iridaceae; isoflavonoid glucoside; isoflavone; isoflavanone

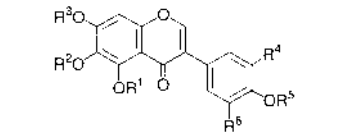
The dried rhizomes of *Belamcanda chinensis* (L.) DC have been used as folk medicine for the treatment of coughing and pharyngitis in China.[1] As for the chemical constituents of the plant, the occurrence of iridal-type triterpenoids[2—4] and isoflavonoids[5,6] in the rhizomes, and phenols, benzoquinones and benzofurans[3,7] in the seeds was reported. We also reported the isolation and characterization of several iridals as ichthyotoxic components from the rhizomes of *B. chinensis*.[8] Further investigation of the rhizomes has resulted in the isolation of four new isoflavonoids, 6″-*O*-*p*-hydroxybenzoyliridin (**1**), 6″-*O*-vanilloyliridin (**2**), 5,6,7,3′-tetrahydroxy-4′-methoxyisoflavone (**3**) and 2,3-dihydroirigenin (**4**), together with six known compounds. We report herein the structure elucidation of these new compounds.

Fresh rhizomes of *B. chinensis* were soaked successively in *n*-hexane and MeOH. The MeOH extract was partitioned with ether and water. Column chromatography of the ether soluble portion over silica and octadecylsilyl (ODS) gels gave ten compounds including **1**—**4**. Six among them were identified as irigenin (**5**),[9] iridin (**6**),[9] tectorigenin (**7**),[10] tectorigin (**8**),[11] irisflorentin (**9**)[12] and rhamnocitrin (**10**),[13] by comparison of their physicochemical data with those reported in the literature.

Compound **1** was obtained as a pale yellow amorphous powder. The presence of an isoflavone skeleton was suggested from the UV spectrum (λ_{max} 259, 320 nm). Its molecular formula $C_{31}H_{30}O_{15}$ was determined by high-resolution electrospray ionization (HR-ESI) MS (*m*/*z* 643.1650 $[M+H]^+$). The ^{1}H-NMR spectrum of **1** showed two singlets (δ 8.16, 6.85), two *meta*-coupled doublets (J=2 Hz) (δ 6.80, 6.79) and a chelated hydroxyl proton signal (δ 12.96) in the aromatic region. The spectrum also indicated the presence of three methoxyl groups (δ 3.87, 3.81, 3.80) and an anomeric proton of sugar [δ 5.27 (d, J=6.5 Hz)]. These signals were very similar to those of iridin (**6**), except for extra AA′BB′-type signals at δ 7.93 and 6.93 (each 2H, d, J=8.5 Hz) in **1**. The presence of a *para*-substituted benzoyl unit in **1** was revealed by the ^{13}C-NMR spectrum (Table 1), which showed the resonances of an ester carbonyl and six sp^2 carbons besides those due to the iridin moiety. Based on these spectral data taking a $[M+H]^+$ ion peak in the ESI-MS, which is 104 mass units ($C_7H_4O_2$) larger than that of **6** into consideration, compound **1** was assumed to be *p*-hydroxybenzoyliridin. The location of the acyl group in **1** was evidenced by downfield shifts of H-6″ signals of the glucose unit relative to those of **6**. Acid hydrolysis of **1** afforded *p*-hydroxybenzoic acid along with irigenin (**5**) and iridin (**6**). Based on these findings, compound **1** was characterized as 6″-*O*-*p*-hydroxybenzoyliridin.

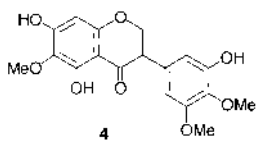
Compound **2**, a pale yellow amorphous powder, exhibited a $[M+H]^+$ ion peak at *m*/*z* 673 in ESI-MS, and the molecular formula $C_{32}H_{32}O_{16}$ was established by HR ESI-MS. The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **2** were almost superimposable on those of compound **1**. The distinguishable feature of **2** from **1** in the ^{1}H-NMR spectrum was the presence of an



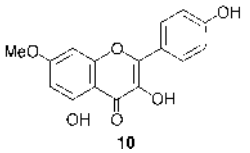


	R^1	R^2	R^3	R^4	R^5	R^6
3	H	H	H	OH	Me	H
5	H	Me	H	OH	Me	OMe
7	H	Me	H	H	H	H
8	H	Me	Glc	H	H	H
9	Me	$-CH_2-$		OMe	Me	OMe
11	H	Me	H	OH	Me	H

Glc: glucopyranosyl



4



10

Chart 1

* To whom correspondence should be addressed. e-mail: yoshida@pheasant.pharm.okayama-u.ac.jp

Table 1. ^{13}C-NMR Spectral Data of **1**—**4** and **6** (126 MHz, Acetone-d_6)

Carbon	**1**	**2**	**6**	**3**	**4**
Aglycone					
C-2	155.2	155.2	155.5	154.5	72.0
C-3	122.3	122.3	123.4	123.5	51.6
C-4	181.8	181.7	181.6	182.0	198.1
C-4a	108.0	107.8	107.7	106.5	103.3
C-5	154.8[a)]	154.8[b)]	153.9[c)]	146.3	160.0[d)]
C-6	134.1	133.7	133.4	123.6	130.0
C-7	157.7	157.7	157.3	157.9	156.7[d)]
C-8	94.9	94.8	95.0	94.4	95.3
C-8a	153.9[a)]	153.9[b)]	153.8[c)]	154.2	159.5[d)]
C-1′	127.4	127.2	127.1	121.5	132.3
C-2′	105.9	105.8	105.6	116.0	110.0
C-3′	151.1	151.0	150.9	145.6	151.3
C-4′	137.4	137.4	137.3	146.3	136.8
C-5′	153.6	153.6	153.6	111.5	154.2
C-6′	110.7	110.7	110.6	117.3	105.5
Glucose					
C-1″	101.4	101.3	101.2		
C-2″	74.4	74.1	73.8		
C-3″	77.9	77.5	77.6		
C-4″	71.5	71.3	70.4		
C-5″	75.3	75.2	76.9		
C-6″	64.6	64.7	61.8		
Acyl group					
C-1‴	123.7	123.7			
C-2‴	132.6	113.6			
C-3‴	116.0	154.3			
C-4‴	162.7	152.2			
C-5‴	116.0	115.6			
C-6‴	132.6	124.7			
C-7‴	166.3	166.5			
OMe	56.3	56.2	56.2	60.6	56.2
	60.7	56.3	60.6		60.6
	60.8	60.6	61.1		60.7
		60.9			

a—*d*) Assignments interchangeable.

extra 3H singlet due to a methoxyl group, and the ABX-type protons instead of the AA′BB′-type protons of **1**. Compound **2** was thus suggested to be a derivative of **1** possessing a 4-hydroxy-3-methoxy or 3-hydroxy-4-methoxybenzoyl group at position 6″ of the glucose moiety in the molecule. The nuclear Overhauser effect spectroscopy (NOESY) spectrum of **2** showed a correlation between a signal at δ 7.55 (d, J=2 Hz, H-2‴) and a methoxyl signal at δ 3.84, establishing the location of the methoxyl group at the C-3‴ position. Consequently, compound **2** was deduced to be 6″-*O*-vanilloyliridin, which was confirmed by acid hydrolysis yielding vanillic acid besides **5** and **6**.

Compound **3** showed a pseudo-molecular ion $[M+H]^+$ peak at *m/z* 317 in the ESI-MS and its molecular formula was determined to be $C_{16}H_{12}O_7$ by HR-ESI-MS. The UV, ^{1}H-, and ^{13}C-NMR spectra of **3** were similar to those of iristectorigenin A (**11**), which was isolated from *Iris spuria*.[14] The spectral comparison of **3** and **11** revealed the lack of a methoxyl group in the former. The methoxyl group in **3** was allocated to the C-4′ position based on the NOESY spectrum which showed a clear correlation between the methoxyl proton signal (δ 3.87) and H-5′ signal (δ 6.87, d, J=8 Hz). On the basis of these data, compound **3** was characterized as 5,6,7,3′-tetrahydroxy-4′-methoxyisoflavone.[15]

Compound **4** was obtained as a pale yellow amorphous powder and shown to have the molecular formula $C_{18}H_{18}O_8$ by HR-ESI-MS (*m/z* 363.1113 $[M+H]^+$). The UV spectrum of **4** displayed maxima at 253 and 288 nm indicative of an isoflavanone skeleton. The ^{1}H-NMR spectrum of **4** showed mutually coupled methine [δ 3.92 (dd, J=5.5, 7 Hz)] and methylene proton signals [δ 4.61 (dd, J=7, 11.5 Hz), δ 4.59 (dd, J=5.5, 11.5 Hz)] as well as aromatic proton signals due to H-8 (δ 6.00), H-2′ (δ 6.51) and H-6′ (δ 6.56). The presence of three methoxyl signals (δ 3.80, 3.76, 3.74) was also indicated. Compound **4** was thus assumed to be 2, 3-dihydroirigenin and this assumption was substantiated by catalytic hydrogenation of irigenin (**5**) with Pd/C to yield a dihydro derivative, which was shown to be identical with **4** by HPLC and NMR. Based on these data, compound **4** was characterized as racemic 2,3-dihydroirigenin (5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavanone).[16]

Experimental

Optical rotations were measured with a JASCO DIP-1000 polarimeter. UV spectra were measured on a HITACHI U-2001 spectrophotometer. ESI-MS was performed with a Micromass Auto Spec OA-TOF spectrometer using 50% MeOH containing 0.1% NH_4OAc as a solvent. ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian VXR-500 instrument (500 MHz for ^{1}H and 126 MHz for ^{13}C) and chemical shifts are given in δ (ppm) values relative to that of the solvent [acetone-d_6 (δ_H 2.04; δ_C 29.8)] on a tetramethylsilane scale. The circular dichroism (CD) spectrum was recorded on a JASCO J-720 W spectrometer. Normal-phase HPLC was conducted on a YMC-Pack SIL A-003 (YMC Co., Ltd.) column (4.6 i.d.×250 mm) developed with *n*-hexane–MeOH–tetrahydrofuran–formic acid (55 : 33 : 11 : 1) containing oxalic acid (450 mg/l) (flow rate, 1.5 ml/min; detection 280 nm) at room temperature. Reversed-phase HPLC was performed on a YMC-Pack ODS-A A-302 (YMC Co., Ltd.) column (4.6 i.d.×150 mm) developed with 0.01 M H_3PO_4–0.01 M KH_2PO_4–CH_3CN (42.5 : 42.5 : 15) (flow rate, 1.0 ml/min; detection 280 nm) at 40 °C.

Plant Material Rhizomes of *B. chinensis* cultivated at the herbarium of the Faculty of Pharmaceutical Sciences, Okayama University, were collected in January, 1995. A voucher specimen (OPH-I03) is kept at the same herbarium.

Extraction and Isolation The fresh rhizomes (1 kg) of *B. chinensis* were chopped and soaked in hexane (2 l) three times (for each 24 h) at room temperature to yield a hexane extract (2.5 g). The residue was further extracted with MeOH (2 l×3). The concentrated solution was diluted with H_2O and extracted successively with ether and *n*-BuOH to give Et_2O (9.5 g), *n*-BuOH (13 g), and H_2O (36.8 g) extract. The Et_2O extract was subjected to column chromatography over silica gel using a solvent system $CHCl_3$→$CHCl_3$–acetone→acetone–MeOH in stepwise gradient mode. The eluate with $CHCl_3$–acetone (4 : 1) was further purified by repeated column chromatography over silica gel using $CHCl_3$–acetone to afford irigenin (**5**) (22.9 mg), tectorigenin (**7**) (42.5 mg), and irisflorentin (**9**) (22.6 mg). The acetone–MeOH (9 : 1) eluate was rechromatographed over silica gel with $CHCl_3$–MeOH to give 6″-*O*-*p*-hydroxyiridin (**1**) (7.6 mg), 5,6,7,3′-tetrahydroxy-4′-methoxyisoflavone (**3**) (2.6 mg), **5** (63 mg), **7** (13 mg), and **9** (12 mg). The eluate with acetone–MeOH (1 : 1) was purified by repeated column chromatography over YMC-gel ODS AQ 120 S50 (solvent: aqueous MeOH) to furnish **1** (7.6 mg), 6″-vanilloyliridin (**2**) (3.3 mg), **3** (2.6 mg), and iridin (**6**) (1.8 mg).

The residue after extraction with MeOH was further extracted with hot MeOH. The extract was diluted with H_2O and extracted with ether to give ether and H_2O extracts. The ether extract was purified in a way similar to that described above to afford **1** (24.1 mg), **2** (1.4 mg), **3** (3.5 mg), 2,3-dihydroirigenin (**4**) (3.5 mg), tectoridin (**8**) (4 mg), **9** (17 mg), and rhamnocitrin (**10**) (5.3 mg).

6″-Hydroxybenzoyliridin (1) A pale yellow amorphous powder, $[\alpha]_D$ +3.4° (c=0.5, MeOH), ESI-MS *m/z*: 665 $(M+Na)^+$, 643 $(M+H)^+$. HR-ESI-MS *m/z*: 643.1650 $(M+H)^+$, Calcd for $C_{31}H_{30}O_{15}$+H, 643.1663. UV λ_{max} (MeOH) nm (log ε): 259 (4.22), 320 (3.96). ^{1}H-NMR (acetone-d_6) δ: 12.96 (1H, s, 5-OH), 8.16 (1H, s, H-2), 7.93 (2H, d, J=8.5 Hz, H-2‴, 6‴), 6.93 (2H, d, J=8.5 Hz, H-3‴, 5‴), 6.85 (1H, s, H-8), 6.80 (1H, d, J=2 Hz, H-2′ or H-6′), 6.79 (1H, d, J=2 Hz, H-2′ or H-6′), 5.27 [1H, d, J=6.5 Hz, glucose (glc) H-1″], 4.74 (1H, dd, J=2, 11.5 Hz, glc H-6″), 4.36 (1H, dd, J=8, 11.5 Hz, glc H-6″), 4.04 (1H, d*t*, J=2, 8 Hz, glc H-5″), 3.87, 3.81, 3.80 (each

3H, s, OCH_3), 3.65 (2H, m, glc H-2″, 3″), 3.53 (1H, t, J=9 Hz, glc H-4″). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 1 A solution of **1** (0.7 mg) in 2.5% H_2SO_4 (1 ml) was heated at 80 °C for 8 h. The reaction mixture was analyzed by normal- and reversed-phase HPLC to detect peaks identical with those of irigenin (**5**), iridin (**6**), and *p*-hydroxybenzoic acid.

6″-Vanilloyliridin (2) A pale yellow amorphous powder, $[\alpha]_D$ +5.7° (c=1.0, MeOH), ESI-MS m/z: 673 $(M+H)^+$. HR-ESI-MS m/z: 673.1794 $(M+H)^+$, Calcd for $C_{32}H_{32}O_{16}$+H, 673.1769. UV ν_{max} (MeOH) nm (log ε): 263 (4.03), 320 (3.40). ^{1}H-NMR (acetone-d_6) δ: 12.96 (1H, s, 5-OH), 8.12 (1H, s, H-2), 7.61 (1H, dd, J=1.5, 8.5 Hz, H-6‴), 7.55 (1H, d, J=2 Hz. H-2‴), 6.95 (1H, d, J=8.5 Hz, H-5‴), 6.81 (2H, br s, H-8, 2′), 6.79 (1H, d, J=2 Hz, H-6′), 5.25 (1H, d, J=6.5 Hz, glc H-1″), 4.74 (1H, dd, J=1.5, 12 Hz, glc H-6″), 4.39 (1H, dd, J=8, 12.5 Hz, glc H-6″), 4.04 (1H, d*t*, J=2, 8 Hz, glc H-5″), 3.88, 3.84, 3.81, 3.80 (each 3H, s, OCH_3), 3.64 (2H, m, glc H-2″, 3″), 3.52 (1H, m, glc H-4″). ^{13}C-NMR: see Table 1.

Acid Hydrolysis of 2 A solution of **2** (1.0 mg) in 2.5% H_2SO_4 (1 ml) was heated at 80 °C for 4 h . Normal- and reversed-phase HPLC of the reaction mixture showed peaks identical with those of irigenin (**5**), iridin (**6**), and vanillic acid.

5,6,7,3′-Tetrahydroxy-4′-methoxyisoflavone (3) A pale yellow amorphous powder, ESI-MS m/z: 317 $(M+H)^+$. HR-ESI-MS m/z: 317.0683 $(M+H)^+$, Calcd for $C_{16}H_{12}O_7$+H, 317.0661. UV ν_{max} (MeOH) nm (log ε): 267 (4.29), 294 (4.02). ^{1}H-NMR (acetone-d_6) δ: 13.25 (1H, s, 5-OH), 8.14 (1H, s, H-2), 7.14 (1H, d, J=2 Hz, H-2′), 6.94 (1H, dd, J=2, 8 Hz, H-6′), 6.87 (1H, d, J=8 Hz, H-5′), 6.48 (1H, s, H-8), 3.87 (3H, s, OCH_3). ^{13}C-NMR: see Table 1.

2,3-Dihydroirigenin (4) A pale yellow amorphous powder, $[\alpha]_D$ ±0° (c=0.5, MeOH), ESI-MS m/z: 363 $(M+H)^+$. HR-ESI-MS m/z: 363.1113 $(M+H)^+$, Calcd for $C_{18}H_{18}O_8$+H, 363.1080. UV λ_{max} (MeOH) nm (log ε): 253 (3.77), 288 (4.09). ^{1}H-NMR (acetone-d_6) δ: 12.35 (1H, s, 5-OH), 6.56 (1H, d, J=2 Hz, H-6′), 6.51 (1H, d, J=2 Hz, H-2′), 6.00 (1H, s, H-8), 4.61 (1H, dd, J=7, 11.5 Hz, H-2a), 4.59 (1H, dd, J=5.5, 11.5 Hz. H-2b), 3.92 (1H, dd, J=5.5, 7 Hz, H-3), 3.80, 3.76, 3.74 (each 3H, s, OCH_3). ^{13}C-NMR: see Table 1.

Preparation of 4 from Irigenin (5) A solution of **5** (15 mg) in MeOH (2 ml) containing 10% Pd/C was stirred overnight at room temperature under hydrogen atmosphere. The catalyst was filtered off, and the reaction mixture was evaporated *in vacuo*. The product was purified by preparative TLC ($CHCl_3$–MeOH, 5 : 1) to yield the hydrogenated derivative (0.7 mg), which was identified with **4** by co-chromatography (HPLC) and direct comparison of the ^{1}H-NMR spectra.

Acknowledgements The authors are grateful to the SC-NMR Laboratory of Okayama University for the NMR experiments. This work was supported in part by a Grant-in-Aid for Scientific Research (No. 10557207) from the Ministry of Education, Science, Sports and Culture of Japan.

References and Notes

1) Chang S., "Dictionary of Chinese Crude Drugs," ed. by Shanghai Scientific Technologic Publisher, Shanghai, 1977, p. 1883.
2) Takahashi K., Hoshino Y., Suzuki S., Hano Y., Nomura T., *Phytochemistry*, **53**, 925—929 (2000).
3) Seki K., Haga K., Kaneko R., *Phytochemistry*, **38**, 703—709 (1995).
4) Abe F., Chen R.-F., Yamauchi T., *Phytochemistry*, **30**, 3379—3382 (1991).
5) Woo W. S., Woo E. H., *Phytochemistry*, **33**, 939—940 (1993).
6) Shirane S., Ohta S., Matsuo T., Hirose R., Koga D., Ide A., Yagishita K., *Agric. Biol. Chem*., **46**, 2595—2597 (1982).
7) Fukuyama Y., Okino J., Kodama M., *Chem. Pharm. Bull*., **39**, 1877—1879 (1991).
8) Ito H., Onoue S., Miyake Y., Yoshida T., *J. Nat. Prod*., **62**, 89—93 (1999).
9) Ali A. A., El-Emary N. A., El-Moghazi M. A., Darwish F. M., Frahm A. W., *Phytochemistry*, **22**, 2061—2063 (1983).
10) Mabry T. J., Kagan J., Resler H., *Phytochemistry*, **4**, 177—183 (1965).
11) Shawl A., Kumar T., *Phytochemistry*, **31**, 1399—1401 (1992).
12) Morita N., Arisawa M., Kondo Y., Takemoto T., *Chem. Pharm. Bull*., **21**, 600—603 (1973).
13) Barbera O., Sanz J. F., Sanchez-Parareda J., Alberto Marco J., *Phytochemistry*, **25**, 2361—2365 (1986).
14) Shawl A. S., Vishwapaul, Zaman A., Kalla A. K., *Phytochemistry*, **23**, 2405—2406 (1984).
15) This compound was reported as a synthetic product. Horie T., Sasagawa M., Torii F., Kawamura Y., Yamashita K., *Chem. Pharm. Bull*., **44**, 486—491 (1996).
16) Although this compound was registered in Chemical Abstracts (121928—03—8) as noted by reviewer of the manuscript, it is based on an erroneous structural drawing as isoflavanone for isoflavone, irigenin, in the literature cited in the Chemical Abtracts. Therefore, this is regarded as a new natural product.