

Isoflavonoids of *Belamcanda chinensis*

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Abstract—Tectorigenin, irigenin, their glucosides and 5, 3'-dihydroxy-4', 5'-dimethoxy-6, 7-methylenedioxyisoflavone were isolated from the rhizomes of *Belamcanda chinensis*.

Keywords—*Belamcanda chinensis*, • Iridaceae • isoflavonoids • tectoridin • tectorigenin • iridin • irigenin • 5, 3'-dihydroxy-4', 5'-dimethoxy-6, 7-methylenedioxyisoflavone.

Belamcanda chinensis (Iridaceae) is found in the hills in Korea and its rhizomes have been used as a Chinese drug to treat throat troubles such as tonsillitis.¹⁾ However, a great portion of the drug is imported from China, because of shortage of domestic drug supply.

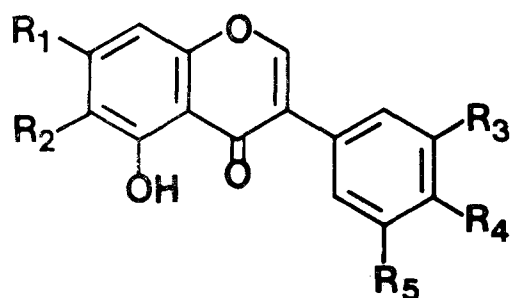
Mannich, *et al.*²⁾ reported isolation of tectoridin from the plant of Chinese origin. However, Kutani and Hayashi³⁾ isolated iridin from the drug collected in Korea and postulated that the discrepancy between two results was attributed to the difference of plant materials.

In order to clarify this matter, chemical study on the flavonoid constituents was undertaken, and led us to a conclusion that isoflavone constituents in both plant materials were identical.

Column chromatography of the chloroform soluble fraction gave three isoflavonoids (compound 1~3).

Compound 1, mp 230°, compound 2, mp 185° and compound 3, mp 241~242°, all showed FeCl₃ positive but negative in Mg+HCl test.

The UV of each compound, exhibiting band II peak at 267~285 nm (Table I), was very similar to those reported for a number of isoflavonoids⁴⁾ and the IR was very similar to each other, showing the presence of OH, con-



	R ₁	R ₂	R ₃	R ₄	R ₅
1	OH	OCH ₃	H	OH	H
2	OH	OCH ₃	OH	OCH ₃	OCH ₃
3		O—CH ₂ —O	OH	OCH ₃	OCH ₃
4	OG	OCH ₃	H	OH	H
5	OG	OCH ₃	OH	OCH ₃	OCH ₃


jugated carbonyl and aromatic ring system, except for the absorption band for methylenedioxy group at 920 cm⁻¹ in IR of compound 3.

Two singlet signals for H-2 and H-8 protons of each compound fell in the normal shift region for the isoflavonoid nucleus, oxygenated at 5, 6 and 7 positions of ring A (Table II). Compound 1 showed two *ortho*-coupled doublets of two protons, indicating a 4'-substituted B ring, whereas compound 2 and 3, two *meta*-coupled doublets of one proton in almost identical region, indicating an asymmetric 3', 4', 5'-tri-

Table I. UV spectral data of compounds

Solvent	1	2	3	4	5
MeOH	268.5(4.57) 323.0 sh(3.98)	269.0(4.14) 330.0 sh(3.34)	284.5(4.45) 330.0 sh(4.05)	267.5(4.58) 333.5 sh(3.62)	268.5(4.45) 331.5 sh(3.47)
+NaOH	276.5(4.54) 328.0(4.23)	274.0(4.10) 338.5(3.76)	279.5(4.47) 364.0(3.65)	273.5(4.67) 360.0(4.15)	268.5(4.46) 361.5(3.51)
+NaOAc	271.5(4.56) 330.0(4.15)	272.5(4.15) 336.0(3.63)	285.0(4.46) 315.5(4.06)	267.0(4.60) 334.0 sh(3.63)	268.5(4.46) 330.0(3.48)
+NaOAc/H ₃ BO ₃	267.0(4.57) 324.5 sh(3.97)	270.0(4.14) 332.5(3.49)	285.0(4.46) 315.5(4.06)	267.0(4.60) 334.0 sh(3.63)	268.5(4.46) 330.0(3.48)
+AlCl ₃	275.5(4.55) 320.5(4.10) 374.0(3.57)	277.0(4.10) 313.5(3.57) 381.0(3.46)	284.0(4.43) 335.0(4.03)	278.0(4.56) 315.5(3.90) 381.0(3.62)	273.0(4.43) 310.0 sh(3.89) 385.0(3.46)
+AlCl ₃ /HCl	277.0(4.53) 317.5 sh(4.10) 374.0(3.57)	278.5(4.11) 321.0 sh(3.50) 379.0(3.12)	285.0(4.45) 335.0(4.00)	279.5(4.57) 317.5 sh(4.10) 385.0(3.60)	280.0(4.44) 321.5(3.49) 386.5(3.44)

Table I. ¹H-NMR data of compounds (80 MHz, DMSO-d₆)

Proton No.	1	2	3	4	5
H-2	8.32	8.38	8.46	8.43	8.50
H-8	6.50	6.52	6.87	6.83	6.92
H-2'	7.40	6.71	6.72	7.40	6.75
(and 6')	d(9)	d(2)	d(1.2)	d(9)	d(2)
H-3' and 5'	6.82 d(9)			6.83 d(9)	
H-6'		6.68 d(2)	6.67 d(1.2)		6.74 d(2)
H-5-OH	13.05	12.90	12.33	12.91	12.80
H-3'-OH(or 4'-OH)	9.50	9.17	9.22	9.57	9.24
OCH ₃	3.76	3.71 3.77 3.80	3.69 3.72	3.77	3.71 3.77 3.80
			6.17		
H-1''				5.10 d(5)	5.09 d(5.5)
H-2''~6''				4.3~5.0	4.3~5.0

All signals are singlet except for protons of B ring and sugar. (d=doublet, figures in parentheses are coupling constants in Hz).

oxygenated B ring. Each NMR revealed the presence of one methoxy group in compound 1, three methoxy groups in compound 2 and one methoxy and one methylenedioxy groups in compound 3. Appearance of retro-Diels-Alder

fragment ions at *m/z* 182, 182 and 180 in MS spectra of the compounds, respectively, indicated that one methyl group was located on ring A in compound 1 and 2, and one methylenedioxy group on ring A in compound 3.

Table III. ^{13}C -NMR chemical shifts of compounds (20 MHz, DMSO- d_6)

Carbon No.	1	2	3	4	5
2	154.0	154.3	154.8	154.7	155.0
3	122.1	121.9	122.2	122.3	122.0
4	180.7	180.8	180.6	180.8	180.8
5	152.9	152.8	141.4	152.7	152.9
6	131.7	131.4	129.7	132.8	132.5
7	153.4	153.1	152.9	153.1	152.9
8	94.1	93.8	89.4	94.3	94.0
9	157.6	157.7	154.0	156.8	156.6
10	105.1	105.8	107.5	106.8	106.9
1'	121.5	126.1	125.8	121.3	126.0
2'	130.3	110.7	110.5	130.3	110.7
3'	115.3	150.5	150.2	115.3	150.4
4'	157.6	136.0	136.7	157.7	136.0
5'	115.3	152.5	152.9	115.3	152.5
6'	130.3	104.8	105.0	130.3	104.8
6-OCH ₃	60.1	60.4	—	60.5	60.3
4'-OCH ₃	—	60.1	60.0	—	60.1
5'-OCH ₃	—	55.8	56.0	—	55.6
O-CH-O	—	—	102.9	—	—
1''	—	—	—	100.6	100.3
2''	—	—	—	73.4	73.0
3''	—	—	—	76.9	76.9
4''	—	—	—	70.0	70.1
5''	—	—	—	77.5	77.9
6''	—	—	—	61.0	61.0

A bathochromic shift of band II in the presence of AlCl_3 and appearance of an exchangeable proton signal around δ 13 in each compound indicated that 5-hydroxy group was not substituted.

In compound 1, a bathochromic shift of band II in the presence of NaOAc and appearance of the methyl carbon signal at δ 60.1 (Table III) indicated 6-hydroxy group was methylated, therefore compound 1 was identified as tectorigenin.

In compound 2, a bathochromic shift of band II in the presence of NaOAc and appearance of two methyl carbon signals around δ 60 indi-

cated that 6, 4' and 5' hydroxy groups were methylated, therefore compound 2 was identified as irigenin. In compound 3, one methyl carbon signal appeared at δ 60, indicating that 4' and 5' hydroxy groups were methylated, therefore compound 3 was identified as 5, 3'-dihydroxy-4', 5'-dimethoxy-6, 7-methylenedioxy isoflavone, which was first isolated from *Iris germanica*.⁵⁾

Column chromatography of the butanol soluble fraction afforded two isoflavone glycosides (compound 4, mp 257~258° and 5, mp 208°).

Acid hydrolysis of each compound gave glucose as the sugar and tectorigenin from compound 4 and irigenin from compound 5 as the aglycone. The lack of shift in UV spectrum of each compound on the addition of NaOAc indicated that glucose was attached to 7-hydroxy group, therefore compound 4 and 5 were identified as tectoridin and iridin, respectively. ^{13}C -chemical shifts are in agreement with the values for the structures (Table III).

Experimental

Mps were determined on a Mitamura-Ricken apparatus and are uncorrected. IR absorption spectra were obtained in KBr pellets on a Perkin-Elmer Model 283 B spectrophotometer. UV spectra were run with Gilford System 2600 spectrophotometer. NMR spectra were recorded with a Varian FT-80A spectrometer. MS spectra were obtained on a Hewlett Packard GC/MS spectrometer Type 5985 B.

Plant materials

The rhizomes of *B. chinensis* were collected in the vicinity of Seoul. The Chinese origin was purchased from a drug store in Seoul.

Extraction and isolation

The air-dried each plant sample was extracted with MeOH and evaporated. The residue was partitioned between equal volumes of hexane and 10% aqueous methanol. The aqueous me-

thanol layer was partitioned with chloroform, then with butanol. Chloroform and butanol layers were separately dried with dry Na_2SO_4 and concentrated under reduced pressure before chromatography over SiO_2 column. The columns were eluted with CHCl_3 and subsequently with a gradient of CHCl_3 -MeOH to afford compound 1 to 3 from the chloroform fraction and compound 4 and 5 from the butanol fraction.

Characterization of compounds

Tectorigenin (compound 1), crystallized from methanol as pale yellow needles, mp. 230° , Rf, 0.22 (CHCl_3 -MeOH=97:3, Si gel); IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3415(OH), 1665(conjugated C=O), 1600, 1520, 1475(aromatic); MS m/z (rel. int.): 300 (M^+ , 57.3), 182(RDA with ring A, trace), 118 (RDA with ring B, 26.4); UV, NMR: see Tables.

Irigenin(compound 2) crystallized from MeOH as pale yellow needles, mp 185° , Rf, 0.32 (CHCl_3 -MeOH=97:3, Si gel); IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3415 (OH), 1665 (conjugated C=O), 1600, 1455, 1425(aromatic); MS m/z (rel. int.): 360(M^+ , 39.6), 183 (RDA+H, 7.7); UV, NMR: see Tables.

5, 3'-Dihydroxy-4', 5-dimethoxy-6, 7-methylenedioxy isoflavone(compound 3), crystallized from MeOH as yellow needles, mp $241\sim 242^\circ$: Rf, 0.58 (CHCl_3 -MeOH=97:3, Si gel); IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3420(OH), 1680 (conjugated C=O), 1620, 1460(aromatic), 920(methylenedioxy); MS m/z (rel. int.): 358 (M^+ , 100), 180 (RDA with ring A, 10.7), 178 (RDA with ring B, 1.6); UV, NMR: see Tables.

Tectoridin (compound 4), crystallized from MeOH as pale yellow needles, mp $257\sim 258^\circ$; IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3375(OH), 1665(conjugated C=O), 1620, 1585 (aromatic) 1000~1100 (glyco-

side); UV NMR: see Tables.

Iridin(compound 5), crystallized from MeOH as pale yellow needles, mp 208° ; IR $\nu_{\text{max}}^{\text{KBr}}$ (cm^{-1}): 3400 (OH), 1665 (conjugated C=O), 1520, 1465, 1430 (aromatic), 1000~1100 (glycoside); UV, NMR: see Tables.

Acid hydrolysis of compound 4 and 5

Twenty mg of each compound was refluxed with 5% H_2SO_4 (50ml) for 3hr. After cooling the reaction mixture was filtered. The aglycone was crystallized from MeOH to give tectorigenin from compound 4 and irigenin from compound 5, each aglycone was confirmed by direct comparison of an authentic sample (TLC, mmp, MS). Each filtrate was neutralized with BaCO_3 , filtered and concentrated *in vacuo*. D-Glucose was identified by TLC (precoated cellulose, pyridine-EtOAc-HOAc- H_2O =36:36:7:21) by comparison with an authentic sugar.

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