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Isoflavonoids of *Belamcanda chinensis* (II)*

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Abstract—Dimethyltectorigenin, irisflorentin, muningin and iristectorigenins A and B were isolated from the rhizomes of *Belamcanda chinensis.*

Keywords—*Belamcanda chinensis* • Iridaceae • isoflavonoids • dimethyltectorigenin • irisflorentin • muningin • iristectorigenins A and B

In the previous paper, isolation of tectorigenin, irigenin and their glucosides together with 5,3′-dihydroxy-4′,5′-dimethoxy-6,7-methylenedioxyisoflavone was reported.[1] A further study on the chloroform soluble fraction has now led to the isolation of four additional isoflavones (Compounds **6**-**9**). Compound **6**, mp 180~182°, Compound **8**, mp 284~286° and Compound **9**, mp 186~188° showed $FeCl_3$ positive but Compound **7**, mp 166~167° negative.

The UV of each compound, exhibiting band II peak at 264~269nm (Table I) was very similar to those reported for a number of isoflavonoids[2] and the IR showed the presence of conjugated carbonyl and aromatic ring system in all.

All compounds showed the hydroxyl group absorption except Compound **7**, which showed, however, the methylenedioxyl group absorption.

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). Compounds **6** and **8** showed two two proton *ortho*-coupled doublets, indicating the presence of a 4′-substituted B ring, Compound **7** showed a two proton singlet, indicating a symmetric trisubstituted B ring, Compound **9** showed an one proton *ortho*-coupled doublet, one proton *meta*-coupled doublet and one proton double doublet, indicating a trisubstituted B ring. Each NMR revealed the presence of three methoxyl group in Compound **6**, four methoxyl group and a methylenedioxyl group in Compound **7**, two methoxyl group in both Compound **8** and Compound **9**, respectively. The appearance of a pair of retro-Diels-Alder fragment ions at

Table I. UV spectral data of compounds [λ_{max}^{MeOH} nm (log ε)]

Solvent	6	8	9
MeOH	266.5(4.21)	263.5(4.39)	269.5(4.73)
	330.0(3.24)	325.0(3.75)	331.0(4.66)
+NaOH	272.0(4.08)	283.5(4.38)	280.5(4.63)
	358.5(3.28)	372.5(3.69)	333.0(4.53)
+NaOAc	266.5(4.21)	263.5(4.37)	275.5(4.73)
	330.0(3.24)	323.5(3.68)	342.0(4.36)
+NaOAc/ H_3BO_3	267.0(4.57)	264.0(4.39)	272.0(4.71)
	330.0(3.24)	325.0(3.76)	342.0(3.95)
+$AlCl_3$	276.5(4.13)	264.0(4.36)	278.5(4.72)
	312.0(3.63)	324.5(3.78)	318.2(4.26)
	374.0(3.27)		366.4(3.85)
+$AlCl_3$/HCl	278.5(4.16)	264.0(4.32)	279.5(4.74)
	316.5(3.52)	333.0(3.76)	318.0(4.27)
	374.0(3.27)		374.4(3.88)

* For previous report see ref. 1.

Table II. ^{1}H-NMR data of compounds (80 MHz)

Proton No.	6 ($CDCl_3$)	PM 6 ($CDCl_3$)	7 ($CDCl_3$)	8 (DMSO-d_6)	9 (DMSO-d_6)
H-2	7.87(1H, s)	7.78(1H, s)	7.78(1H, s)	8.16(1H, s)	8.34(1H, s)
H-8	6.45(1H, s)	6.67(1H, s)	6.63(1H, s)	6.73(1H, s)	6.49(1H, s)
H-2′(H-6′)	6.97(2H, d) J=8.8	6.94(2H, d) J=8.8	6.75(2H, s)	6.88(2H, d) J=8.8	7.14(1H, d) J=1.8
H-3′(H-5′)	7.46(2H, d) J=8.8	7.46(2H, d) J=8.8		7.33(2H, d) J=8.8	
H-5′					6.81(1H, d) J=8.1
H-6′					7.01(1H, dd) J=1.8, 8.1
H-5-OH	12.78(1H, s)				13.02(1H, s)
Other OH				9.40(bs)	9.02(bs)
—OCH_3	3.83(3H, s)	3.81(3H, s)	3.87(9H, s)	3.73(3H, s)	3.75(3H, s)
	3.91(3H, s)	3.89(3H, s)	4.08(3H, s)	3.90(3H, s)	3.79(3H, s)
	3.94(3H, s)	3.94(6H, s)			
—O\ /—CH_2 —O/			6.06(2H, s)		

m/z 196 and 132, 194 and 195, 196 and 118 and 182 and 148 in each MS spectrum, respectively, indicated that two methoxyls and one hydroxyl were located on ring A and one methoxyl on ring B in Compound **6**, one methylenedioxyl and one methoxyl on ring A and three methoxyls on ring B in Compound **7**, two methoxyls and one hydroxyl on ring A and one hydroxyl on ring B in Compound **8** and one methoxyl and two hydroxyl on ring A and one methoxyl and one hydroxyl on ring B in Compound **9**.

In Compound **6**, a bathochromic shift of band II in UV with $AlCl_3$ and appearance of an exchangeable proton signal around δ13 ppm indicated that 5-hydroxy group was not substituted, therefore it was identified as 5-hydroxy-6, 7, 4′-trimethoxyisoflavone. Methylation of Compound **6** with dimethylsulfate gave the same product as 5, 6, 7, 4′-tetramethoxyisoflavone (PM **6**) prepared from tectorigenin. Compound **6** was isolated first from *Dalbergia sissoo*.[3]

In Compound **7**, its UV spectrum was not changed in the presence of shift reagents. Two methoxyl carbon signals appeared around δ60 ppm, indicating the presence of two *ortho*-disubstituted methoxyl groups, which were allocated to C-5 and C-4′ positions, respectively. Therefore it was characterized as 6, 7-methylenedioxy-5, 3′, 4′, 5′-tetramethoxyisoflavone. As expected, methylation of 5, 3′-dihydroxy-4′, 5′-dimethoxy-6, 7-methylenedioxyisoflavone gave Compound **7**. Compound **7** (irisflorentin) was first isolated *Iris florentina*.[4]

In Compound **8**, a bathochromic shift of band I on addition of NaOH was observed but no band shift in the presence of $AlCl_3$ and NaOAc, indicating that hydroxyl groups at C-5 and C-7 were methylated, therefore it was characterized as 6, 4′-dihydroxy-5, 7-dimethoxyisoflavone, and confirmed by derivatization into permethylated tectorigenin (PM **6**). Compound **8** (muningin) was isolated previously from *Pterocarpus angolensis*.[5]

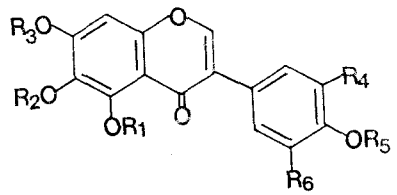
In Compound **9**, a bathochromic shift of band II in the presence of $AlCl_3$ and NaOAc and

increase of intensity of band I in the presence of NaOMe, indicating the presence of 5-hydroxyl, 7-hydroxyl and 4′-hydroxyl groups. Therefore, Compound **9** was assumed to be 5, 7, 4′-trihydroxy-6, 3′-dimethoxyisoflavone (iristectorigenin B, **9b**). However, the melting point of Compound **9** compared badly with that reported the authors.[6] In order to explain this dissimilarity, Compound **9** was subjected to degradation. Oxidation with H_2O_2 resulted in formation of both vanillic acid and isovanillic acid. Thus Compound **9** was concluded to be a mixture of iristectorigenin A(**9a**) and B(**9b**), which were first isolated from *Iris tectorum*.[6,7]

^{13}C-chemical shifts (Table III) of each compound are in agreement with the values for each structure. The assignments were based on shift comparison with literature data.[8,9] The early assignments[1] for C-7 and C-9 in all the compounds previously reported have to be reversed. Original incorrect assignments were purely based on those of the reference 10.

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

Carbon No.	**6** ($CDCl_3$)	PM **6** ($CDCl_3$)	**7** ($CDCl_3$)	**9** (DMSO-d_6)
2	152.55	150.34	152.85	154.3
3	123.50	125.41	125.57	122.0
4	181.06	174.94	174.94	180.7
5	153.54	153.09	141.81	152.9
6	131.90	140.70	135.57	131.8
7	158.99	157.74	154.82	157.7
8	90.47	96.14	93.10	94.1
9	153.70	154.56	150.65	153.4
10	106.77	113.14	113.79	105.1
1′	123.04	124.41	127.40	122.1
2′	130.09	130.20	107.20	113.9
3′	114.21	113.92	153.16	147.6
4′	159.86	159.62	138.62	147.1
5′	114.21	113.92	153.16	115.6
6′	130.09	130.20	107.20	122.0
OCH_3	60.69	61.97	61.05	60.1
		61.30	60.69	
	56.25	56.17	56.35	56.2
	55.32	55.26	56.35	
-O\ /CH$_2$ /O-			102.19	



	R_1	R_2	R_3	R_4	R_5	R_6
6	H	CH_3	CH_3	H	CH_3	H
PM **6**	CH_3	CH_3	CH_3	H	CH_3	H
7	CH_3	—CH_2—		OCH_3	CH_3	OCH_3
8	CH_3	H	CH_3	H	H	H
9a	H	CH_3	H	OH	CH_3	H
9b	H	CH_3	H	OCH_3	H	H

Experimental

Separation of compounds

The $CHCl_3$ soluble fraction of the MeOH extract was chromatographed over SiO_2 column and eluted with a gradient of $CHCl_3$-MeOH to afford fractions, but most of the fractions consisted of mixtures. Among them the fraction containing Compounds **6**-**9** was subjected to SiO_2 column chromatography using hexane-EtOAc (gradient) to give each compound.

Characterization of compounds

5-Hydroxy-6, 7, 4′-trimethoxyisoflavone (Compound **6**)—crystallized from MeOH as pale yellow needles, mp. 180~182°; Rf, 0.18 (hexane-EtOAc=7:3, si gel); IR $\nu_{max}^{KBr}(cm^{-1})$: 3430 (OH), 1665(conjugated C=O), 258, 1460, 1520(aromatic); MS *m/z* (rel. int.): 328(M^+, 100), 196 (RDA with A ring, 2.0), 132 (RDA with B ring); UV, NMR: see Tables I, II and III.

5, 3′, 4′, 5′-Tetramethoxy-6, 7-methylenedioxyisoflavone (Compound **7**)—crystallized from MeOH as needles, mp. 166~167°; Rf, 0.1 (hexane-EtOAc=6:4 si gel); UV λ_{max}^{MeOH} nm (logε): 266.5(4.44), 323.5(3.80), unchanged by shift reagents; IR ν_{max}^{KBr} (cm^{-1}): 1668(conjugated C=O), 1585(conjugated C=C), 929 (methylenedioxy); MS *m/z* (rel. int.):386 (M^+, 100), 194 (RDA with A ring, 3.0), 195 (RDA with B ring, 2.6); NMR: see Tables II and III.

6, 4′-Dihydroxy-5, 7-dimethoxyisoflavone (Compound **8**)—crystallized from MeOH as white needles, mp. 284~286°; Rf, 0.23($CHCl_3$-MeOH=94 : 6, si gel); IR ν_{max}^{KBr} (cm^{-1}) : 3400 (OH), 1630 (conjugated C=O), 1450, 1525 (aromatic); MS *m/z* (rel. int.) : 314 (M^+, 56.2), 196 (RDA with A ring, 5.3), 118 (RDA with B ring, 70.0); UV, NMR : see Tables I and II.

5, 7, 4′-Trihydroxy-6, 3′-dimethoxyisoflavone (Compound **9**)—crystallized from MeOH as yellow needles, mp. 186~188°; Rf, 0.33 ($CHCl_3$-MeOH=94 : 6, si gel); IR ν_{max}^{KBr}(cm^{-1}) : 3430(OH), 1630 (conjugated C=O), 1590, 1530, 1470 (aromatic); MS *m/z* (rel. int.) : 330 (M^+, 100), 182(RDA with A ring, 0.9), 148 (RDA with B ring, 9.0); UV, NMR : see Tables I, II and III.

Methylation of Compound 6 and Compound 8

To a solution of each compound (30 mg) in acetone (10 ml) was added $(CH_3)_2SO_4$ (1.0 ml) and K_2CO_3 (2.4 g), and stirred for 5 hr at 50°. After diluted with water the reaction mixture was extracted with ether. The ether layer was washed with water, dried with Na_2SO_4, evaporated and applied to column chromatography using hexane-EtOAc (7 : 3) to obtain pure permethylated tectorigenin (PM **6**); mp. 176~178°; UV λ_{max}^{MeOH} nm (log ε) : 261.0(4.54), 304.5 (3.84), unchanged by shift reagents; MS *m/z* (rel. int.) : 342 (M^+, 406), 327 ($M-CH_3$, 100), 210 (RDA with A ring, 4.9), 132 (RDA with B ring, 59.5); NMR : see Tables II and III.

Preparation of Compound 7

Methylation of the authentic sample of 5, 3′-dihydroxy-4′, 5′-dimethoxy-6, 7-methylenedioxyisoflavone as described above gave a single product, which was in every respect (mp, MS, TLC and ^{1}HNMR) the same as Compound **7**.

Oxidation of Compound 9

To a solution of Compound **9** (2 mg) in 5% KOH (3 ml) was added 3% H_2O_2 (1 ml) and the mixture was allowed to stand over night.

After decomposition of excess H_2O_2 with MnO_2, the reaction mixture was acidified with dilute HCl, extracted with ether and submitted to TLC to detect vanillic acid and isovanillic acid. Rf, 0.25 and 0.18 (C_6H_6-EtOAc-HOAc= 18 : 1 : 1); 0.42 and 0.32 (C_6H_6-HOAc-H_2O= 6 : 7 : 3); 0.27 and 0.20 (toluene-formic acid-ethylformate=5 : 4 : 1); Color reaction with Gibbs reagent followed by NH_3 fuming : pink and blue, respectively.

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