CHAPTER IV

RESULTS AND DISCUSSION

The methanol extract from the rhizomes of *Belamcanda chinensis* (L.) DC. was isolated using several chromatographic techniques to give sixteen compounds classified as four isoflavones (BC1-BC4), four isoflavone glycosides (BC5-BC8), two flavone glycosides (BC9 and BC10), one stilbene (BC16) and other phenolic compounds (BC11-BC15).

A part of the methanolic extract of the heartwood of *Dalbergia parviflora* Roxb. was purified using several chromatographic techniques to yield forty-one compounds as six isoflavans (DP1, DP2, DP13, DP18, DP25 and DP30), seventeen isoflavones (DP3, DP7, DP9, DP11, DP19, DP20, DP22, DP23, DP24, DP28, DP31, DP33, DP34, DP35, DP36, DP38 and DP41), eight isoflavanones (DP4, DP5, DP6, DP15, DP16, DP17, DP21 and DP29), three flavanones (DP8, DP37 and DP39), three 2,3-dihydroflavonols (DP14, DP27 and DP32), one pterocarpan (DP12), one chalcone (DP40) and two cinnamylphenol (DP10 and DP26).

The structures of all isolates were determined from their UV, IR, NMR and MS data and further confirmed by comparison with literature values. The estrogenic activities of these compounds were evaluated.

1. Structure Determination of Isolated Compounds

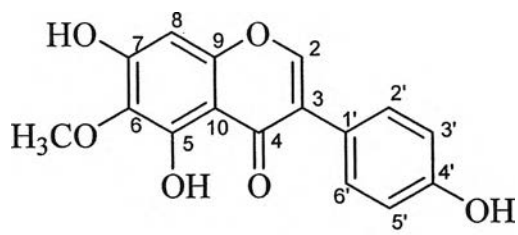
1.1. Structure Determination of Compound BC1

Compound BC1 was obtained as an amorphous powder. A molecular formula of $C_{16}H_{13}O_6$ was deduced from its $[M+H]^+$ ion at *m/z* 301 in the FABMS.

The signals at δ_H 8.29 assignable to H-2 and δ_C 155.0 (C-2) in the ^1H and ^{13}C-NMR spectra, respectively, were suggestive of an isoflavone type skeleton. The ^1H-NMR further revealed the presence of a methoxyl group at δ 3.74, an isolated proton at δ 6.47 (s, H-8), *ortho* coupled AA′BB′-type protons at δ 7.36 and 6.80 (each 2H, d, J = 7 Hz) assignable to H-2′,6′ and H-3′,5′ respectively, in addition to a chelated hydroxyl group proton at δ 13.02.

The ^{13}C NMR spectrum in DMSO-d_6 showed 14 signals for 16 carbon atoms, corresponding to one methoxyl carbon at δ 60.9 (6-O<u>C</u>H$_3$), six hydrogen-bonded aromatic carbons at δ 155.0 (C-2), 95.0 (C-8), 131.4 (C-2′,6′), 116.3 (C-3′,5′), eight aromatic carbons at δ 124.3 (C-3), 154.6 (C-5), 132.8 (C-6), 158.7 (C-7), 154.9 (C-9), 106.8 (C-10), 123.3 (C-1′), 158.8 (C-4′) and a carbonyl carbon at δ 182.6 (C-4).

By comparing the above spectral data with previously reported values (Park *et al.*, 1999), BC 1 was identified as 5,7,4′-trihydroxy-6-methoxyisoflavone (tectorigenin) **[14]**. This compound has been isolated previously from several *Iris* plants such as *Iris pseudacorus* (Hanawa *et al.*, 1991), *I. crocea* (Shawl and Kumar, 1992), *I. tectorum* (Wu and Xu, 1992), *I. germanica* (Pailer *et al.*, 1973) and from some *Pueraria* plants such as *Pueraria thunbergiana* (Park *et al.*, 1999), *P. thomsonii* and *P. lobata* (Morito *et al.*, 2002).



[14]

Table 6 NMR Spectral data of compound BC1 (in DMSO-d_6) and tectorigenin (in DMSO-d_6)

Position	Compound BC1		Tectorigenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.29 (s)	155.0	8.29 (s)	153.9
3	-	124.3	-	123.8
4	-	182.6	-	180.5
5	-	154.6	-	152.7
6	-	132.8	-	131.4
7	-	158.7	-	157.5
8	6.47 (s)	95.0	6.49 (s)	93.8
9	-	154.9	-	153.2
10	-	106.8	-	104.8
1'	-	123.3	-	121.0
2'	7.36 (d,7)	131.4	7.38 (d,8.3)	130.1
3'	6.80 (d,7)	116.3	6.82 (d,8.3)	115.0
4'	-	158.8	-	157.4
5'	6.80 (d,7)	116.3	6.82 (d,8.3)	115.0
6'	7.36 (d,7)	131.4	7.38 (d,8.3)	130.1
6-OMe	3.74 (s)	60.9	3.88 (s)	59.9
5-OH	13.02 (s)	-	-	-

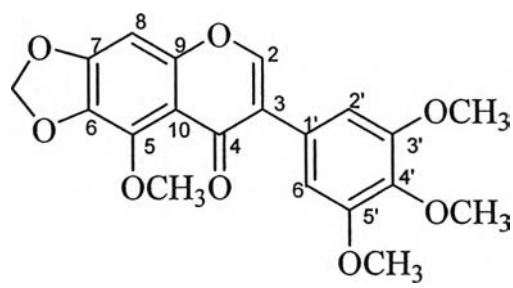
1.2. Structure Determination of Compound BC2

Compound BC2 was obtained as an amorphous powder. Its molecular formula $C_{20}H_{19}O_8$ was established by FABMS with the molecular ion $[M+H]^+$ peak at *m/z* 387.

The ^1H NMR spectrum of BC2 in DMSO-d_6 (Figure 21 and Table 7) exhibited the characteristic signal due to H-2 of an isoflavone structure at δ 8.29 (1H, s) with a singlet (H-8) signal at δ 7.00. The ^1H NMR spectral data, furthermore, revealed the presence of four methoxyl groups at δ 3.68 (3H, s), 3.79 (6H, s) and 3.91 (3H, s) and a methylenedioxy unit at δ 6.17 (2H, s). The appearance of singlet signal at 6.83 (2H) indicated the 3′,4′,5′-oxygenated B ring (Morita, *et al.*, 1973).

The ^{13}C NMR spectral data of BC2 in DMSO-d_6 (Figure 22 and Table 7) exhibited fifteen signals, corresponding to four methoxyl carbons at δ 55.9, 60.0 and 60.7, four hydrogen-bonded *sp2* carbons at δ 93.5 (C-8), 106.8 (C-2′ and C-6′), 153.8 (C-2), ten *sp2* carbons at δ 106.8 (C-10), 124.1 (C-1), 127.4 (C-3 and C-6),135.9 (C-4′), 137.4 (C-5), 152.0 (C-3′ and C-5′), 152.4 (C-9), 152.6 (C-7) and one carbonyl carbon at δ 173.6 (C-4).

By analysis of the above spectroscopic data with reported data (Morota *et al.*, 1973), BC2 was identified as 5,3′,4′,5′-tetramethoxy-6,7-methylene dioxyisoflavone, trivially know as irisflorentin **[7]**. This isoflavone has been isolated previously from *Iris florentina* (Morita *et al.*, 1973), *I. germanica* (Pailer *et al.*, 1973), and *I. dichotomy* (Dewick, 1993).



[7]

Table 7 NMR Spectral data of compound BC2 (in DMSO-d_6) and irisflorentin (in $CDCl_3$)

Position	Compound BC2		Irisflorentin
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
2	8.29(*s*)	153.8	7.81(s)
3	-	127.4	-
4	-	173.6	-
5	-	137.4	-
6	-	127.4	-
7	-	152.6	-
8	7.00(s)	93.5	6.63(s)
9	-	152.4	-
10	-	106.8	-
1'	-	124.1	-
2'	6.83(s)	106.8	6.77(s)
3'	-	152.0	-
4'	-	135.9	-
5'	-	152.0	-
6'	6.83(s)	106.8	6.77(s)
O-CH_2-O	6.17(s)	102.6	6.08(s)
5-OMe	3.91(s)	60.7	4.09(s)
3'-OMe	3.79(s)	55.9	3.89(s)
4'-OMe	3.68(s)	60.0	3.87(s)
5'-OMe	3.79(s)	55.9	3.89(s)

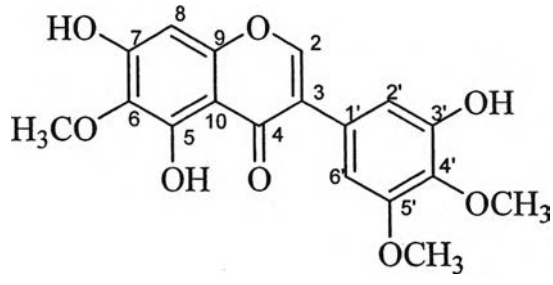
1.3 Structure Determination of Compound BC3

Compound BC3 was obtained as an amorphous powder. A molecular formula of $C_{18}H_{17}O_8$ was deduced from its $[M+H]^+$ ion at *m/z* 361 in the FABMS.

The ^1H NMR spectrum (Table 8 and Figure 23) showed a characteristic singlet proton signal at δ 8.35 (1H, s) for the isoflavone skeleton with a chelated hydroxyl group appeared at δ 13.00. The ^1H NMR spectrum also exhibited a sharp singlet proton signal at δ 6.48, assignable to H-8, three methoxyl groups (δ 3.69, 3.75, 3.78) and aromatic protons on B-ring as *meta*-coupled at δ 6.65 (1H, d, $J_{2',6'}$ = 2 Hz, H-2′) and 6.71 (1H, d, $J_{6',2'}$ = 2 Hz, H-6′).

The ^{13}C NMR spectrum (Table 8 and Figure 24) showed the three methoxyl carbons at δ 55.6 (5′-O<u>C</u>H$_3$), 59.8 (4′ and 6-O<u>C</u>H$_3$), four hydrogen-bonded *sp2* carbons at δ 154.6 (C-2), 93.9 (C-8), 104.6 (C-2′), 110.3 (C-6′), ten *sp2* carbons at δ 121.7 (C-3), 152.8 (C-5), 131.5 (C-6), 157.8 (C-7), 153.2 (C-9), 104.6 (C-10), 126.0 (C-1′), 150.2 (C-3′), 136.4 (C-4′), 152.6 (C-5′), and a carbonyl carbon at δ 180.1 (C-4).

From the above spectral evidence by comparing with reported data (Wollenweber *et al.*, 2003 and Ali *et al.*, 1983), BC3 was identified as 5,7,3′-trihydroxy-6,4′,5′-trimethoxyisoflavone (irigenin) **[5]**. This compound has been isolated previously from several plants in genus *Iris* such as *Iris germanica* (wollenweber *et al.*, 2003; Ali *et al.*, 1983), *I. kumaonensis* (Agarwal *et al.*, 1984), *I. florentina* (Arisawa *et al.*, 1973), and *I. unguiculans* (Arisawa and Morita, 1976)



[5]

Table 8 NMR Spectral data of compound BC3 (in DMSO-d_6) and irigenin (in $CDCl_3$)

Position	Compound BC3		Irigenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.35 (s)	154.6	8.38 (s)	154.9
3	-	121.7	-	122.0
4	-	180.1	-	180.4
5	-	152.8	-	153.1
6	-	131.5	-	131.6
7	-	157.8	-	157.5
8	6.48 (s)	93.9	6.51 (*s*)	94.1
9	-	153.2	-	153.1
10	-	104.6	-	104.9
1'	-	126.0	-	126.3
2'	6.65 (d,2)	104.6	6.66 (d,1.9)	104.9
3'	-	150.2	-	150.3
4'	-	136.4	-	136.6
5'	-	152.6	-	152.8
6'	6.71 (d,2)	110.3	6.71 (d,1.9)	110.5
6-OMe	3.75 (s)[a]	59.8	3.75 (s)	60.1
4'-OMe	3.69 (s)[b]	59.8	3.69 (s)	60.1
5'-OMe	3.78 (s)[c]	55.6	3.79 (s)	56.0
5-OH	13.00(br.s)	-	13.03(br.s)	-
3'-OH	9.20(br.s)	-	9.27(br.s)	-
7-OH	-	-	10.8(br.s)	-

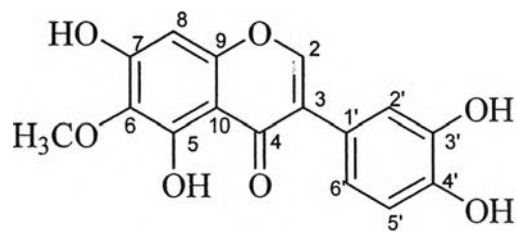
[a-c] assignments may be interchanged.

1.4 Structure Determination of Compound BC4

Compound BC4 was obtained as an amorphous powder. The molecular formula was determined as $C_{16}H_{13}O_7$ from its $[M+H]^+$ ion at *m/z* 317 in the FABMS.

The ^1H NMR spectrum of BC4 in DMSO- d_6 (Table 9 and Figure 25) showed singlet signals at δ 8.27 (1H, s) and 13.07 (1H, s) suggesting the compound as 5-hydroxy isoflavone. Besides of a singlet signal-at δ 6.48 (1H, s, H-8), three ABC-type protons at δ 6.99 (1H, d, $J_{2',6'}$ = 2 Hz, H-2′) 6.76 (1H, d, $J_{5',6'}$ = 8 Hz, H-5′) and 6.78 (1H, dd, $J_{6',5'}$ = 8 Hz and $J_{6',2'}$ = 2 Hz, H-6′) in addition to a methoxyl proton signal at δ 3.75 (3H, s) were observed. The ^{13}C NMR in DMSO-d_6 (Table 9 and Figure 26) displayed signals for 16 carbons, which consisted of one methoxyl carbon at δ 59.8 (6-O<u>C</u>H$_3$), five hydrogen-bonded *sp2* carbons at δ 153.9 (C-2), 93.7 (C-8), 115.3 (C-2′), 116.5 (C-5′), 121.9 (C-6′), nine *sp2* carbons at δ 121.6 (C-3), 152.6 (C-5), 131.4 (C-6), 157 (C-7), 153.2 (C-9), 104.8 (C-10), 119.9 (C-1′), 144.8 (C-3′), 145.4 (C-4′) and one carbonyl carbon at δ 180.5 (C-4).

The above results were in good agreement with published data in good agreement (Choudhary *et al.*, 2001) BC4 was identified as 5,7,3′,4′-tetrahydroxy-6-methoxyisoflavone (irilin D) **[412]**. It was first isolated from *Iris bungei* (Choudhary *et al.*, 2001).



[412]

Table 9 NMR Spectral data of compound BC4 (in DMSO-d_6) and irilin D (in DMSO-d_6)

Position	Compound BC4		Iridin D	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.27 (s)	153.9	8.27 (s)	155.3
3	-	121.6	-	120.8
4	-	180.5	-	180.1
5	-	152.6	-	152.8
6	-	131.4	-	131.5
7	-	157.4	-	155.4
8	6.48 (s)	93.7	6.30 (s)	93.9
9	-	153.2	-	153.1
10	-	104.8	-	104.2
1'	-	119.9	-	120.5
2'	6.99 (d,2)	115.3	6.62 (d,2.2)	115.4
3'	-	144.8	-	145.4
4'	-	145.4	-	143.8
5'	6.76 (d,8)	116.5	6.64 (d,8.3)	118.6
6'	6.78 (dd,8,2)	121.9	7.80 (d,8.3)	121.8
6-OMe	3.75 (s)	59.8	3.76 (s)	60.2
5-OH	13.07 (s)	-	12.59 (s)	-

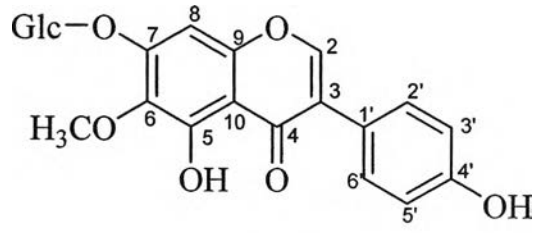
1.5 Structure Determination of Compound BC5

Compound BC5 was obtained as an amorphous powder and showed the molecular ion $[M+H]^+$ in the FABMS spectrum at *m/z* 463 corresponding to the molecular formula of $C_{22}H_{23}O_{11}$.

The ^1H NMR spectrum of BC5 in DMSO-d_6 (Table 10 and Figure 27) exhibited *ortho* coupled AA'BB'-type proton signals at δ 7.39 (d, $J_{2',3'} = J_{5',6'} = 9$ Hz, H-2',6') and 6.82 (d, $J_{3',2'} = J_{6',5'} = 9$ Hz, H-3',5') indicating that the B-ring was substituted at C-4', as in BC1. Besides of the characteristic H-2 signal observed at δ 8.42 (1H, s), a doublet signal assignable to anomeric proton was observed at δ 5.08 (1H, d, $J = 7$ Hz). As shown in the case of BC-1, The H-8 proton signal of 5,7-dihydroxy isoflavones, which is a common substitution pattern of this plant metabolites and usually appeared around δ 6.3- 6.5, while the signal is shifted downfield to the range of δ 6.5- 6.9 when the compound was *O*-glycosylated at C-7. According to the downfield shifted H-8 signal at δ 6.87 (δ 6.47 in BC1), the compound was concluded to be *O*-glycosylated at C-7. And from the coupling constant of the anomeric proton ($J = 7$ Hz) the glycosidic linkage was indicated as β (α-linkage exhibits couplings in the range 2-3 Hz) (Markham, 1982). In addition, the two hydroxyl signals were shown at δ 12.90 (s, 5-OH) and 9.54 (s, 4'-OH).

The ^{13}C NMR spectrum of BC5 in DMSO-d_6 (Table 10) showed twenty-two carbon atoms. A comparative elucidation with BC1 indicated BC5 as BC1 (tectorigenin) glycoside **[14]**. The presence of β-D-glucose was evident from the anomeric carbon signal at δ 100.2 along with signals for oxymethine carbons at δ 73.1 (C-1''), 76.7 (C-3''), 69.6 (C-4'') and 77.2 (C-5'') and 60.6 (C-6'').

Based on the above spectral data by and comparing with reported data (Park *et al.*, 1999), BC5 was identified as 5,7,4'-trihydroxy-6-methoxyisoflavone-7-*O*-β-D-glucopyranoside (tectoridin) **[13]**. It has been isolated from several plants such as *Pueraria thumbergiana* (Park *et al.*, 1999), *Iris germanica*, *I. tectorum*, *Baptisia nuttalliana* and *Dalbergia riparia* (Farnsworth *et al.*, 1975).



[13]

Table 10 NMR Spectral data of compound BC5 (in DMSO-d_6) and tectoridin (in DMSO-d_6)

Position	Compound BC5		Tectoridin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
Aglycone moiety				
2	8.42 (s)	154.6	8.43 (s)	154.6
3	-	122.0	-	122.1
4	-	180.7	-	180.8
5	-	152.8	-	152.9
6	-	132.5	-	132.5
7	-	156.6	-	156.6
8	6.87 (s)	94.0	6.88 (s)	94.0
9	-	152.4	-	152.4
10	-	106.4	-	106.5
1'	-	121.0	-	121.0
2'	7.39 (d,9)	130.1	7.40 (d,8.4)	130.1
3'	6.82 (d,9)	115.0	6.83 (d,8.4)	115.1
4'	-	157.4	-	157.5
5'	6.82 (d,9)	115.0	6.83 (d,8.4)	115.1
6'	7.39 (d,9)	130.1	7.40 (d,8.4)	130.1
Sugar moiety				
1"	5.08 (d,7)	100.2	5.09 (d,7.5)	100.2
2"	3.33 (m)	73.1	3.34 (m)	73.1
3"	3.33 (m)	76.7	3.34 (m)	76.7
4"	3.17 (m)	69.6	3.20 (m)	69.7
5"	3.44 (m)	77.2	3.45 (m)	77.3
6"a	3.46 (m)	60.6	3.49 (m)	60.7
6"b	3.71 (m)	-	3.69 (m)	-
6-OMe	3.77 (s)	60.2	3.78 (s)	-
5-OH	12.90 (s)	-	-	-
4'-OH	9.54(s)	-	-	-

1.6 Structure Determination of Compound BC6

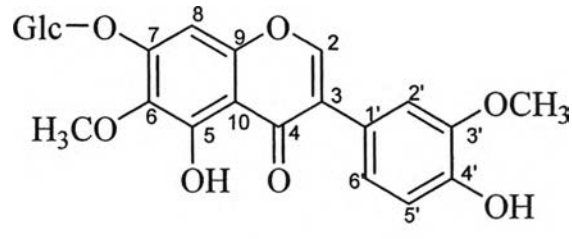
Compound BC6, an amorphous powder, was deduced its molecular formula as for $C_{23}H_{25}O_{12}$ from its $[M+H]^+$ ion at *m/z* 493 in FABMS.

The ^1H NMR spectrum of BC6 in DMSO-d_6 (Table 11 and Figure 28) showed characteristic pattern of an 5-hydroxy isoflavone with a singlet proton signals at δ 8.45 (H-2) and 12.91 (5-OH). Besides of H-8 signal at δ 6.88 (1H, s), a characteristic ABX pattern signals at δ 7.15 (1H, d, $J_{2',6'}$ = 2 Hz, H-2′), 6.83 (1H,d, $J_{5',6'}$ = 8 Hz, H-5′) and 7.00 (1H, dd, $J_{6',5'}$ = 8 Hz, $J_{6',2'}$ = 2 Hz, H-6) were observed. Correlations of these protons were confirmed from the ^1H-^1H connectivity (COSY) spectrum (Figure 30). A doublet signal at δ 5.08 (1H, d, J = 7.5 Hz) was referred to anomeric proton of β-linked glucose from its coupling constant value. The ^{13}C NMR of BC6 in DMSO-d_6 (Table 11, Figure 29) showed 23 carbon signals, corresponding to two methoxyl carbons at δ 55.7 (3′-O<u>C</u>H$_3$), 60.1 (6-O<u>C</u>H$_3$), a methylene carbon at δ 60.6 (C-6″), five aromatic carbons with a hydrogen at δ 154.6 (C-2), 93.5 (C-8), 113.4 (C-2′), 115.2 (C-5′), 121.7 (C-6′), oxymethine carbons at δ 73.1 (C-2″), 77.2 (C-3″), 69.6 (C-4″), 76.6 (C-5″), nine aromatic carbons with non hydrogen at δ 122.1 (C-3), 152.4 (C-5), 132.5 (C-6), 156.5 (C-7), 152.4 (C-9), 106.5 (C-10), 121.5 (C-1′), 147.2 (C-3′), 146.7 (C-4′), one carbonyl carbon at δ 180.6 (C-4) and an anomeric carbon at δ 100.1.

The HMQC spectrum (Figure 31) for identifying directly connected ^1H-^{13}C nuclei and the HMBC spectrum (Table 11 and Figure 32) for correlating coupled ^1H-^{13}C over 2- or 3- bonds were employed. The anomeric proton signal at δ 5.08 which was identified direct connectivity with a signal at δ 100.1 (C-1″) from the HMQC spectrum represented the correlation with C-7 (δ 156.5), and the results indicated that the sugar unit was connected to C-7. Across the three-bond correlation between a methoxyl group signals (δ 3.77) and C-6 (δ 132.5) displayed the methoxyl group attached to C-6 in A-ring. The another methoxyl group (δ 3.79) was placed at C-3′, as indicated by correlation with C-3′ in its HMBC spectrum

By comparing the above spectroscopic data with reported values (Morita *et al.*, 1972), BC6 was identified as 5,4′-dihydroxy-6,3′-dimethoxyisoflavone-7-*O*-β -

glucopyranoside (iristectorin B) **[413]**. This compound was first reported from *Iris tectorum* (Morita *et al.*, 1972)



[413]

Table 11 NMR Spectral data of compound BC6 (in DMSO-d_6) and iristectorigenin B (in 10% CCl_4)

Position	Compound BC6		Iristectorigenin B	HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	(correlation with ^{1}H)
Aglycone moiety				
2	8.45 (s)	154.6	7.74(s)	-
3	-	122.1	-	H-6'
4	-	180.6	-	H-2,H-8
5	-	152.4	-	H-8
6	-	132.5	-	H-8, 6-OMe
7	-	156.5	-	H-8*, H-1"
8	6.88 (s)	93.5	6.46(s)	-
9	-	152.4	-	H-8*,H-2
10	-	106.5	-	H-8
1'	-	121.5	-	H-5',H-2
2'	7.15 (d,2)	113.4	7.06(d,2.5)	H-6'
3'	-	147.2	-	3'-OMe , H-5'
4'	-	146.7	-	H-2', H-6'
5'	6.83 (d,8)	115.2	6.82(d,8)	-
6'	7.00 (dd,8,2)	121.7	6.89(dd,2.5,8)	H-2' , H-5'*
Sugar moiety				
1"	5.08 (d,7.5)	100.1	-	-
2"	3.33 (m)	73.1	-	H-3"*
3"	3.33 (m)	77.2	-	-
4"	3.18 (m)	69.6	-	H-2"
5"	3.46 (m)	76.6	-	H-3", H-4"*, H-6"a*
6"a	3.46 (m)	60.6	-	-
6"b	3.70 (m)	-	-	-
6-OMe	3.77 (s)	60.1	3.75(s)	-
3'-OMe	3.79 (s)	55.7	3.83(s)	-
5-OH	12.91 (br.s)	-	-	-
4'-OH	9.10(br.s)	-	-	-

* Across two bonds correlations

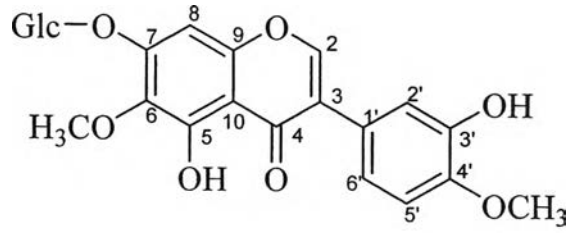
1.7 Structure Determination of Compound BC7

Compound BC7 was obtained as an amorphous powder. It showed molecular ion $[M+H]^+$ peak at *m/z* 493 in the FABMS, suggesting the molecular formula as $C_{23}H_{25}O_{12}$.

The ^1H NMR of compound BC7 in DMSO-d_6 (Table 12 and Figure 33) confirmed the 5-hydroxy isoflavones nucleus from the signals for H-2 at δ 8.42 (1H, s) and a chelated hydroxyl group at δ 12.90 (br.s) together with the aromatic proton signal at δ 6.87 (1H, s), assignable to H-8. The aromatic proton signals at δ 7.04 1H, s, H-5′) and 6.96 (2H, s, H-2′, H-6′) were assigned for three protons in B-ring (as no coupling constant was observed, it is hard to explain substitution pattern of B-ring from the PMR. Furthermore, aliphatic proton signals of sugar moiety [δ 5.08 (1H, d, J = 7.5 Hz, H-1″), 3.20 (2H, m, H-2″, H-3″), 3.18 (1H, m, H-4″), 3.43 (2H, m, H-5″, H-6″a), 3.75 (1H, m, H-6″b)] and two methoxyl moiety δ 3.77 (3H, s), 3.79 (3H, s) were observed. The coupling constant of H-1″ suggested it as β-anomeric proton. And the sugar moiety was attached to the nucleus with *O*-linkage as supported by the chemical shift value of C-1″ at δ 100.2, while around δ 130 were reported for *C*-glycoside (Agrawal et al., 1989).

The connectivity of seven sugar protons were determined by the ^1H-^1H COSY experiment (Figure 31), and their directly bonded carbons were assigned from the HMQC experiment (Figure 32). The HMBC spectrum (Table 12, Figure 33) showed correlation across three-bonds between hydroxylated *sp2* carbon (δ 156.5, C-7) and H-1″, and the results indicated the compound as isoflavone 7-*O*-glycoside. The two methoxyl groups (δ 3.77 and 3.79) were deduced to be attached at C-6 (δ 132.5) and C-4′ (δ 147.7) from ^1H-^{13}C correlations observed in the HMBC spectrum, respectively. The hydroxyl at δ 9.01 (1H, br.s) was placed at C-3′ as suggested by its two-bonds correlation signal from H-2′.

By comparison of these facts with reported data (Morita *et al.*, 1972; Shawl *et al.*, 1984), BC7 was identified as 5,3′-dihydroxy-6,4′-methoxyisoflavone-7-*O*-β-D-glucopyranoside (iristectorin A) **[414]**. This compound has been reported from *Iris tectorum* (Morita *et al.*, 1972) and *I. spuria* (Shawl *et al.*, 1984).



[414]

Table 12 NMR Spectral data of compound BC7 (in DMSO-d_6) and iristectorin A (in CCl_4 and DMSO-d_6)

Position	Compound BC7		Iristectorin A		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	
Aglycone moiety					
2	8.42 (s)	154.8	7.72 (s)	154.2	-
3	-	123.1	-	121.8	H-6', H-2*, H-2'
4	-	180.6	-	180.5	H-2
5	-	152.8	-	152.7	6-OMe
6	-	132.5	-	131.5	H-8, 6-OMe
7	-	156.5	-	153.3	H-8*, H-1"
8	6.87 (s)	94.0	6.62 (s)	93.9	-
9	-	152.3	-	157.5	H-2, H-8*
10	-	106.4	-	104.9	H-8
1'	-	121.9	-	121.9	H-5'
2'	6.96 (s)	116.3	6.81 (s)	115.3	-
3'	-	146.1	-	146.7	H-2'*
4'	-	147.7	-	147.3	H-2, H-5', 4'-OMe
5'	7.04 (s)	112.0	7.14 (s)	113.3	-
6'	6.96 (s)	119.8	6.81 (s)	121.8	H-5'*
Sugar moiety					
1"	5.08 (d,7.5)	100.2	5.0 (m)	-	-
2"	3.32 (m)	73.1	-	-	H-3"*
3"	3.32 (m)	77.2	-	-	-
4"	3.18 (m)	69.6	-	-	-
5"	3.46 (m)	76.6	-	-	-
6"a	3.46 (m)	60.6	-	-	-
6"b	3.75 (m)	-	-	-	-
6-OMe	3.77 (s)	60.2	3.75 (s)	60.6	-
4'-OMe	3.79 (s)	55.6	3.85 (s)	55.8	-
5-OH	12.90(br.s)	-	-	-	-
3'-OH	9.01(br.s)	-	-	-	-

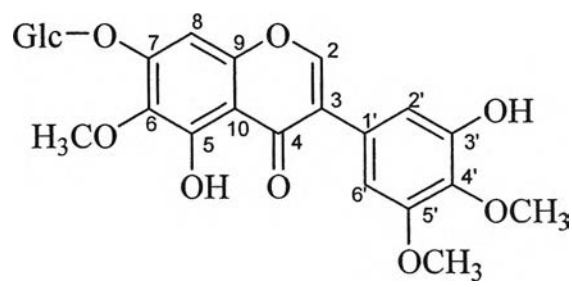
* Across two bonds correlations

1.8 Structure Determination of Compound BC8

Compound BC8, an amorphous powder, showed a molecular ion $[M+H]^+$ in the FABMS spectrum at *m/z* 523, corresponding to the molecular formula $C_{24}H_{27}O_{13}$.

The ^1H NMR spectrum in DMSO-d_6 (Table 13 and Figure 38) revealed the signals for three methoxyl groups at δ 3.77, 3.70, 3.79 (3H, s, each), H-2 of an isoflavone nucleus at δ 8.46 (1H, s) and a singlet at δ 6.88. And *meta*-coupled two aromatic proton signals at δ 6.69 (1H, d, J = 2 Hz, H-2′) and 6.73 (1H, d, J = 2 Hz, H-6′) indicated the presence of 3,4,5-trioxygenated B-ring. A doublet signal at δ 5.09 (1H, d, J = 7 Hz) was assigned to the H-1″ of β-linked glucose with the signal at δ 100.2 in the ^{13}C NMR in DMSO-d_6 (Table 13 and Figure 39). The ^{13}C NMR spectrum of the aglycone signals were in good accordance with BC3 (irigenin). And the attached position of glucose was assigned to C-7 because the H-8 was shifted into downfield compared with its aglycone irigenin **[5]**.

Compound BC8 was irigenin-7-*O*-β-D-glucopyranoside (iridin) **[4]** based on the above spectral data. The ^1H and ^{13}C NMR spectra were in good agreement with published values (Atta-Ur-Rahman *et al.*, 2002; Ali *et al.*, 1983). This compound has been isolated commonly from *Iris* genus plants *e.g. I. florentiana*, *I. germanica*, *I. kumaonin*, *I. nepalensis*, *I. pallida* and *I. tectorum* (Farnsworth *et al.*, 1975; Agarwal *et al.*, 1984).



[4]

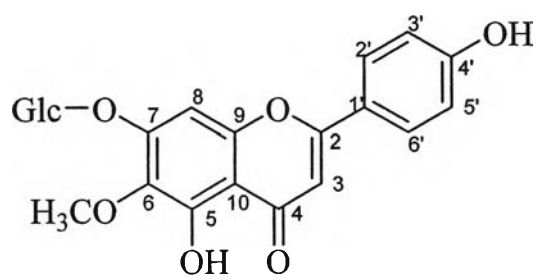
Table 13 NMR Spectral data of compound BC8 (in DMSO-d_6) and iridin (in CD_3OD and DMSO-d_6)

Position	Compound BC8		Iridin	
	^1H(*mult.*, *J* in Hz)	^{13}C	^1H(*mult.*, *J* in Hz)	^{13}C
Aglycone moiety				
2	8.46 (s)	155.1	8.21 (s)	155.4
3	-	122.1	-	122.1
4	-	180.2	-	178.2
5	-	152.8	-	153.1
6	-	132.5	-	130.9
7	-	156.4	-	156.8
8	6.88 (s)	93.8	6.98	94.2
9	-	152.8	-	153.1
10	-	106.5	-	104.8
1'	-	125.9	-	125.9
2'	6.69 (d,2)	104.6	6.69 (d,2.8)	104.8
3'	-	150.2	-	150.3
4'	-	136.4	-	136.7
5'	-	152.5	-	152.4
6'	6.73 (d,2)	110.3	6.70 (d,2.8)	110.5
Sugar moiety				
1"	5.09 (d,7)	100.2	5.10 (d,7)	100.3
2"	3.32 (m)	73.1	3.44 (m)	73.2
3"	3.32 (m)	77.2	3.51 (m)	77.4
4"	3.18 (m)	69.6	3.55 (m)	69.8
5"	3.46 (m)	76.6	3.47 (m)	76.8
6"a	3.46 (m)	60.7	3.72 (dd,4,12)	60.8
6"b	3.73 (m)	-	3.93 (dd,6,12)	-
6-OMe	3.77 (s)	60.2	3.80 (s)	60.0
4'-OMe	3.70 (s)	59.8	3.84 (s)	60.0
5'-OMe	3.79 (s)	55.8	3.88 (s)	55.95
5-OH	12.88 (br.s)	-	-	-
3'-OH	9.20(br.s)	-	-	-

1.9 Structure Determination of Compound BC9

Compound BC9 was isolated as an amorphous powder. The molecular formula was determined as $C_{22}H_{23}O_{11}$ by FABMS of its $[M+H]^+$ ion at *m/z* 463.

The ^1H NMR spectrum in DMSO-d_6 of BC9 (Table 14 and Figure 40) revealed a chelated hydroxyl proton signal at δ 12.95 (br.s), indicating a 5-hydroxyflavone structure. The signals of H-8 appeared as singlet at δ 7.00 and an olefinic singlet signal at δ 6.83 was assigned to H-3, while *ortho* coupled AA′BB′-type proton signals at δ 7.93 (2H, $J_{2',3'} = J_{6',5'}$ = 8 Hz, H-2′, H-6′) and 6.93 (2H, $J_{3',2'} = J_{5',6'}$ = 8 Hz, H-3′, H-5′) indicated 4′-oxygenated B-ring. The anomeric proton signal was appeared at δ 5.10 (1H, d, J = 7 Hz), and presence of sugar moiety was recognized from the ^{13}C NMR signals at δ 100.2, 73.1 (C-2″), 77.2 (C-3″), 69.6 (C-4″), 76.7 (C-5″) and 60.6 (C-6″) (Table 14 and Figure 41). The sugar moiety should be attached at C-7 because the glycosylation shift to downfield was observed with H-8 signal. Based on above spectral data, BC9 was identified as 5,4′-dihydroxy-6-methoxyflavone-7-*O*-β-glucopyranoside (hispiduloside, hispidulin) **[415]** by comparison of its spectroscopic data with the published values (Hiermann, 1978). This compound has been isolated from *Digitalis lanata* (Hiermann, 1978).



[415]

Table 14 NMR Spectral data of compound BC9 (in DMSO-d_6) and hispiduloside (in $CDCl_3$)

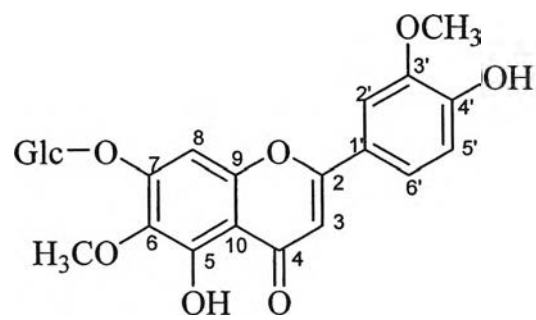
Position	Compound BC9		Hispiduloside
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
Aglycone moiety			
2	-	164.2	-
3	6.83 (s)	102.6	6.20 (s)
4	-	182.2	-
5	-	152.4	-
6	-	132.5	-
7	-	156.4	-
8	7.00 (s)	94.3	6.73 (s)
9	-	152.0	-
10	-	105.6	-
1'	-	121.0	-
2'	7.93 (d,8)	128.4	7.83-7.95 (m)
3'	6.93 (d,8)	115.9	6.85-7.10 (q)
4'	-	161.3	-
5'	6.93 (d,8)	115.9	6.85-7.10 (q)
6'	7.93 (d,8)	128.4	7.83-7.95 (m)
Sugar moiety			
1"	5.10 (d,7)	100.2	5.33 (m)
2"	3.46 (m)	73.1	5.33 (m)
3"	3.46 (m)	77.2	5.33 (m)
4"	3.20 (m)	69.6	5.33 (m)
5"	3.70 (m)	76.7	3.96 (s)
6"a	3.70 (m)	60.6	4.30 (m)
6"b	3.79 (m)	-	-
6-OMe	3.76 (s)	60.2	3.85 (s)
5-OH	12.95 (br.s)	-	-

1.10 Structure Determination of Compound BC10

Compound BC10 was obtained as an amorphous powder. A molecular formula of $C_{23}H_{25}O_{12}$ was deduced from its $[M+H]^+$ ion at *m/z* 493 in the FABMS. Its ^1H NMR spectrum in DMSO-d_6 (Table 15 and Figure 42) showed a chelated phenolic proton at δ 12.95 (br.s), two methoxyl signals at δ 3.77, 3.88 (each 3H, s), two singlet protons at δ 6.95 and 7.03, assignable to H-3 and H-8, respectively. In addition, three ABX-type protons at δ 7.58 (1H, dd, $J_{ortho-\ meta}$ = 8,2 Hz), 7.57 (1H, J_{meta} = 2 Hz) and 6.95 (1H, d, J_{ortho} = 8 Hz) were assigned to H-6′,2′ and 5′, respectively. Furthermore, the presence of β-linked sugar moiety was suggested from the signal at δ 5.09 (1H, d, J = 8).

The ^{13}C NMR spectrum of BC10 in DMSO-d_6 (Table 15) displayed the signals for 23 carbons, composing one carbonyl carbon, six aromatic hydrogen-bonded carbons at eight aromatic carbons, an anomeric carbon, five oxymethine carbons and two methoxyl carbons .

The ^1H-^1H and one bond ^1H-^{13}C correlations of sugar unit were confirmed from the ^1H-^1H COSY and HMQC spectra (Figure 44 and 45). Regarding the attached position of two methoxyl groups, one methoxyl was recognized to be placed at C-6 (δ 133.9) according to its HMBC correlation (Figure 46) from the signal at δ 3.77. The another methoxyl was indicated to be attached at C-3′ (δ 148.0) from the nuclear Overhauser effect (NOE) enhancement (Figure 43) of H-2′ signal (δ 7.57) by irradiating the signal at δ 3.88 (3′-OCH_3), and it was further confirmed from the HMBC. Comparing above spectral data with reported values (Hiermann, 1978), BC10 was identified as 5,4′-dihydroxy-6,3′-dimethoxyflavone-7-*O*-β-D-glucopyranoside (jacecoside) **[416]**.



[416]

Table 15 NMR Spectral data of compound BC10 (in DMSO-d_6) and jaceoside (in $CDCl_3$)

Position	Compound BC10		Jaceoside	HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	(correlation with ^{1}H)
Aglycone moiety				
2	-	164.2	-	H-3*, H-6'
3	6.95 (s)	103.0	6.60 (s)	
4	-	182.2	-	H-3*, 5-OH
5	-	152.4	-	-
6	-	133.9	-	6-OMe, H-8, 5-OH
7	-	156.5	-	H-8*
8	7.03 (s)	94.3	7.18 (s)	-
9	-	152.0	-	H-8*
10	-	105.8	-	H-3, H-8, 5-OH
1'	-	120.5	-	
2'	7.57 (d,2)	121.5	7.4-7.55 (m)	
3'	-	148.0	7.28 (s)	H-5',3'-OMe
4'	-	150.9	-	
5'	6.95 (d,8)	115.9	-	
6'	7.58 (dd,8,2)	110.5	7.4-7.55 (m)	H-2'*
Sugar moiety				
1"	5.09 (d,8)	100.5	5.33 (m)	-
2"	3.33 (m)	73.5	5.33 (m)	H-3"*
3"	3.33 (m)	77.0	5.33 (m)	-
4"	3.27 (m)	69.5	5.33 (m)	-
5"	3.45 (m)	77.5	3.95 (s)	-
6"a	3.46 (m)	60.6	4.30 (m)	-
6"b	3.72 (m)	-	4.30 (m)	-
6-OMe	3.77 (m)	60.2	3.87 (s)	-
5'-OMe	3.88 (m)	56.0	3.87 (s)	-
5-OH	12.95 (br.s)	-	-	-

* Across two bond correlations

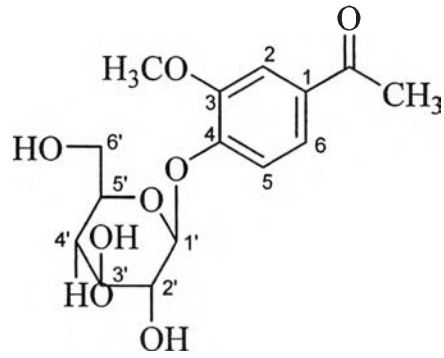
1.11 Structure determination of Compound BC11

Compound BC11 was obtained as an amorphous powder and showed a molecular ion $[M+H]^+$ in the FABMS spectrum at *m/z* 329 corresponding to the molecular formula of $C_{15}H_{21}O_8$.

The ^1H NMR spectrum in DMSO-d_6 (Table 16 and Figure 47) showed a methyl ketone at δ 2.52 (3H, s, 8-CH_3), a methoxyl group at δ 3.82 (3H, s), three aromatic protons at δ 7.46 (1H, d, J = 2Hz, H-2), 7.17 (1H, d, J = 9Hz, H-5) and 7.56 (1H, dd, J = 9,2 Hz, H-6) characteristic for a 1,3,4-trisubstitued aromatic ring protons, a doublet proton at δ 4.96 (J = 8 Hz) and oxymethine proton signals at δ 3.18-3.66 indicating the presence of a sugar moiety. The NOE effect was observed at H-2 (δ 7.46) (Figure 48) by irradiating the methoxyl group (δ 3.82) indicating the methoxyl group position as C-3.

The ^{13}C NMR spectrum of compound BC11 in DMSO-d_6 (Table 16 and Figure 49) exhibited fifteen signals, corresponding to one methyl carbon at δ 26.3, three aromatic carbons with a hydrogen at δ 111.1, 114.2, 122.6, three aromatic carbons at δ 130.8, 148.7, 150.6, one carbonyl carbon at δ 196.3, an anomeric carbon at δ 99.5, sugar methine carbons at δ 60.5, 69.5, 73.0, 76.8, 77.1 and one methoxyl carbon at δ 55.6.

The obtained spectral data and the recorded values (Morita *et al.*, 1972; De Rosa *et al.*, 1996) are shown in Table 16. Compound BC11 was thus identified as 4-hydroxyl-3-methoxyacetophenone-4-*O*-β-D-glucopyranoside (acetovanillone-4-*O*-β-D-glucopyranoside, androsin) **[417]**. This compound has been isolated from several plants such as *Iris tectorum* (Morita *et al.*, 1972), *Penstemon pinifolius*, *Apocynum androsaemifolium* and *Neolloydia texensis* (De Rosa *et al.*, 1996).



[417]

Table 16 NMR Spectral data of compound BC11 (in DMSO-d_6) and androsin (^{1}H NMR in CCl_4 and ^{13}C NMR in CD_3OD+D_2O)

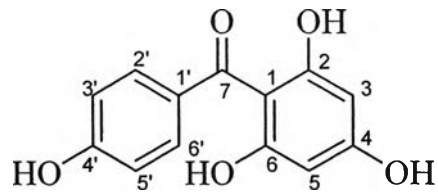
Position	Compound BC11		Androsin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
Aglycone moiety				
1	-	130.8	-	132.6
2	7.46 (d,2)	111.1	7.54 (m)	112.4
3	-	148.7	-	149.6
4	-	150.6	-	151.6
5	7.17 (d,9)	114.2	7.06 (d,9)	115.7
6	7.56 (dd,2,9)	122.6	7.54 (m)	124.9
7	-	196.3	-	202.2
8	2.52 (s)	26.3	2.51 (s)	26.7
Sugar moiety				
1'	4.96 (d,8)	99.5	5.02 (d,7)	101.0
2'	3.27 (m)	73.0	3.45-3.90 (m)	73.9
3'	3.27 (m)	76.8	3.45-3.90 (m)	77.4
4'	3.18 (m)	69.5	3.45-3.90 (m)	70.8
5'	3.35 (m)	77.1	3.45-3.90 (m)	77.2
6'a	3.45 (m)	60.5	3.45-3.90 (m)	61.9
6'b	3.66 (m)	-	3.45-3.90 (m)	-
3-OCH_3	3.82 (s)	55.6	3.94 (s)	56.8

1.12 Structure Determination of Compound BC12

Compound BC12 was obtained as an amorphous. Its molecular formula $C_{13}H_{11}O_5$ was established by FABMS with the molecular ion $[M+H]^+$ at *m/z* 247 (Figure 50).

The ^1H NMR spectrum in DMSO-d_6 (Table 17 and Figure 51) of this compound displayed a singlet proton at δ 5.82 (2H, H-3, 5) and *ortho* coupled AA'BB'-type protons at δ 7.53 and 6.76 (each 2H, d, J = 9 Hz), assignable to H-2',6' and H-3', 5' respectively, indicating the presence of two symmetrical aromatic rings. The ^{13}C NMR spectrum in DMSO-d_6 (Table 17 and Figure 52) supported the symmetrical ring systems from the signals at δ 157.9 (C-2,6), 131.3 (C-2',6'), 114.5 (C-3',5') and 94.2 (C-3,5). The H-C direct connectivity and H-C long range connectivity across two or three bonds were shown from the HMQC and HMBC spectra, respectively (Figure 53-54).

Based on the above spectral data, this compound was identified as 2,4,6,4'-tetrahydroxybenzophenone (iriflophenone) **[418]**. Its ^1H NMR data are in good agreement with published data (Arisawa *et al.*, 1973) which was an isolate from *Iris florentina*.



[418]

Table 17 NMR Spectral data of compound BC12 (in DMSO-d_6) and iriflophenone (in CCl_4)

Position	Compound BC12		Iriflophenone	HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	(correlation with ^{1}H)
1	-	106.5	-	H-3, H-5
2	-	157.9	-	H-3*
3	5.82 (s)	94.2	5.78 (s)	H-5
4	-	160.4	-	H-3*, H-5*
5	5.82 (s)	94.2	5.78 (s)	H-3
6	-	157.9	-	H-5*
7	-	194.3	-	H-2', H-6'
1'	-	130.6	-	H-3', H-5'
2'	7.53 (d,9)	131.3	7.55 (d,9)	-
3'	6.76 (d,9)	114.5	6.65 (d,9)	H-5'
4'	-	161.3	-	H-2',H-6',H-3'*,H-5'*
5'	6.76 (d,9)	114.5	6.65 (d,9)	H-3'
6'	7.53 (d,9)	131.3	7.55 (d,9)	-

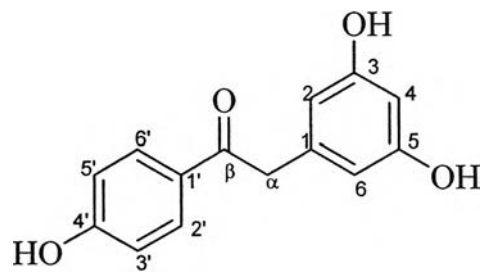
* Across two bonds correlations

1.13 Structure Determination of Compound BC13

Compound BC13, an amorphous powder, showed its $[M+H]^+$ at *m/z* 245.0820 in the HRFABMS (Figure 55), indicating a molecular formula of $C_{14}H_{13}O_4$ (calcd for 245.0814). The UV spectral data exhibited maximum absorption bands at 219 (sh) and 278 nm.

The ^1H NMR spectrum of BC13 in DMSO-d_6 (Table 18 and Figure 56) showed the presence of two symmetrical aromatic rings from 1,4-substituted aromatic proton signals at δ 6.82 (2H, d, J = 9 Hz, H-3′,5′), 7.86 (2H, d, J = 9 Hz, H-2′,6′) and 1,3,5-trisubstituted aromatic proton signals at δ 6.03 (1H, t, J = 2 Hz, H-4), 6.09 (2H, d, J = 2 Hz, H-2,6). And one methylene group at δ 3.98 (2H, s, H-α) bridging benzophenone and aromatic ring was observed. The 2D NMR techniques such as the ^1H-^1H COSY, HMQC and HMBC (Figure 58-60) were performed to assign all protons and carbons, and the results are shown in Table 18. From the HMBC spectrum, H-C long-range couplings were observed between the H-2,6 (δ 6.09) and the methylene carbon (δ 44.5) and between H-2′,6′ (δ 7.86) and the conjugated carbonyl carbon (δ 195.7).

According the results above mentioned, the structure of compound BC13 were defined as 1-(4-hydroxyphenyl)-2-(3,5-dihydroxyphenyl) ethanone (belamphenone) **[419]**. This is the first report on the isolation of this compound as a natural product, but it is known as a synthetic product (Eddarir *et al.*, 2001).



[419]

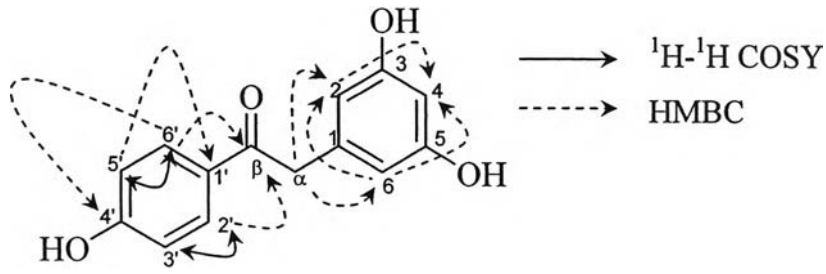


Figure 5 ^{1}H-^{1}H COSY and HMBC correlations of compound BC13

Table 18 NMR Spectral data of compound BC13 (in DMSO-d_6)

Position	Compound BC13		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	
1	-	137.2	H-2,6*
2	6.09 (d,2)	107.3	H-6, H-α, H-4
3	-	158.2	-
4	6.03 (t,2)	100.7	H-2, H-6
5	-	158.2	-
6	6.09 (d,2)	107.3	H-2, H-α, H-4
α	3.98 (s)	44.5	H-2,H-6
β	-	195.7	H-α*, H-2', H-6'
1'	-	127.9	H-3', H-5'
2'	7.86 (d,9)	131.0	-
3'	6.82 (d,9)	115.1	-
4'	-	161.9	H-2, H-6'
5'	6.82 (d,9)	115.1	-
6'	7.86 (d,9)	131.0	-

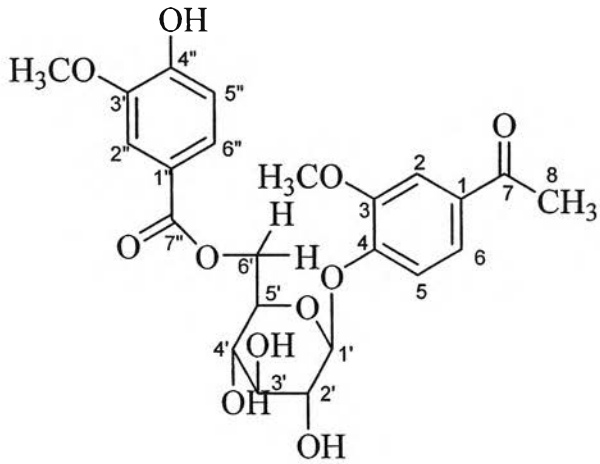
* Across two bonds correlations

1.14 Structure Determination of Compound BC14

Compound BC14 was obtained as an amorphous powder. The HRFABMS of BC14 suggested a molecular formula of $C_{23}H_{26}O_{11}Na$ from its molecular ion $[M+Na]^+$ peak at *m/z* 501.1357 (calcd for 501.1373). The UV spectral data revealed absorption bands at 221 (sh), 264 and 294 nm.

The ^1H and ^{13}C NMR spectra of BC14 in DMSO-d_6 (Figure 61-62 and Table 19) were very similar to those of compound BC11 (androsin) except for one more aromatic ester moiety, which exhibited signals for an ABX pattern at δ 7.40 (1H, d, $J_{2'',6''}$ = 2 Hz, H-2″), 6.87 (1H, d, $J_{5'',6''}$ = 9 Hz, H-5″) and 7.46 (1H, dd, $J_{6'',2''}$ = 2 Hz, $J_{6'',5''}$ = 9 Hz, H-6″) along with correlations in the HMQC spectrum (Figure 60) and the HMBC spectrum (Figure 65). Two methoxyl groups were assigned to be attached at C-3 and C-3″, respectively, as supported by three-bonds coupling of methoxyl protons at δ 3.80 (3H, s) with C-3 (δ 148.6), and of methoxyl protons at δ 3.76 (3H, s) with C-3″(δ 147.3). It was suggested the structure as androsin vanillic acid ester.

Regarding the sugar unit, the connectivities of oxymethine protons were determined by the ^1H-^1H COSY experiment (Figure 63) and their one bond correlations were confirmed from the HMQC experiment. The H-C long-range correlation in the HMBC spectrum between proton signals at δ 4.20 (dd, J = 12,8 Hz) and the carbonyl carbon (δ 165.2) of vanillic acid and between the anomeric proton at δ 5.10 (d, J = 8 Hz) and the oxygenated aromatic carbon (δ 150.2) of aglycone, acetovanillone, indicated that the vanillic acid was attached to the 6′-OH of 4-*O*-glucosylated acetovanillon. The ester and aglycone moieties of BC14 were confirmed by direct comparison with authentic samples after acid hydrolysis. And the sugar component of BC14 was identified as D-glucose by GC analysis after conversion to a thiazolidine derivative. All data are consistent with the structure of BC14, and it was thus assigned as a new compound, acetovanillone-1-*O*-β-D-(6-*O*-vanilloyl) glucopyranoside and has been given the name belalloside A **[420]**.



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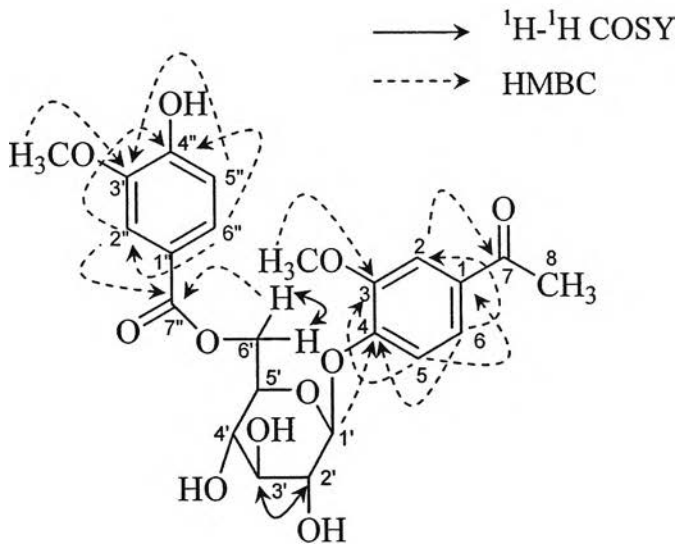


Figure 6 ^{1}H-^{1}H COSY and HMBC correlations of compound BC14

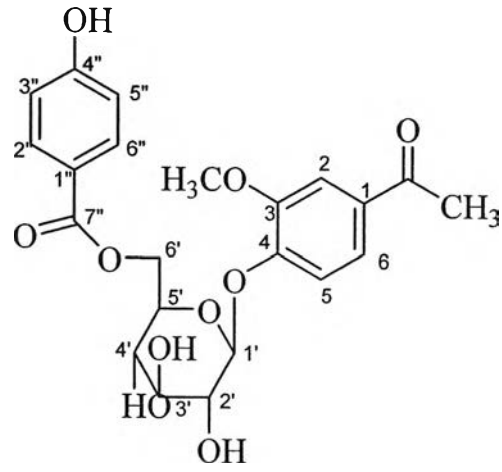
Table 19 NMR Spectral data of compound BC14 (in DMSO-d_6)

Position	Compound BC10		HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	(correlation with ^{1}H)
Aglycone moiety			
1	-	130.8	H-5
2	7.42 (d,2)	110.9	H-6
3	-	148.6	H-5, 3'-OMe
4	-	150.2	H-2, H-6, H-1'
5	7.11(d,9)	114.1	-
6	7.26 (dd,2,9)	122.1	H-2
7	-	196.0	H-2
8	2.48 (s)	26.1	-
Sugar moiety			
1'	5.10 (d,8)	99.1	-
2'	4.20 (dd,8,12)	72.9	-
3'	3.80 (m)	76.6	-
4'	3.34 (m)	70.1	-
5'	4.55 (dd,2,12)	73.9	-
6'a	4.20 (dd,8,12)	63.7	-
6'b	4.55 (dd,2,12)	-	-
Ester moiety			
1"	-	120.4	H-5"
2"	7.40 (d,2)	112.9	H-6"
3"	-	147.3	H-5", 3"-OMe
4"	-	151.6	H-2",H-6"
5"	6.87 (d,9)	115.0	-
6"	7.46 (dd,2,9)	123.5	H-2"
7"	-	165.2	H-2", H-6"
3-OMe	3.80 (s)	55.5	-
3"-OMe	3.76 (s)	55.6	-

1.15 Structure Determination of Compound BC15

Compound BC15, an amorphous powder, was assigned the molecular formula as $C_{22}H_{24}O_{10}Na$ determined from their molecular ion $[M+Na]^+$ peak at *m/z* 471.1247 (calcd for 471.1267) in the HRFABMS. The UV spectral data exhibited absorption bands at 224 (sh) 260 and 299 nm.

The ^1H and ^{13}C NMR spectrum of compound BC15 in DMSO-d_6 (Figures 66-67 and Table 20) were very similar to those of BC14 except for absence of a methoxyl group and suggested its structure as androsin 4-hydroxybenzoic acid ester. The ^1H NMR spectrum showed the 1,4-disubstituted aromatic proton signals at δ 7.78 (2H, d, J = 9 Hz, H-2″,6″) and 6.84 (2H, d, J = 9 Hz, H-3″,5″). The ABX splitting system at δ 7.43 (d, $J_{2,6}$ = 2 Hz, H-2), 7.13 (d, $J_{5,6}$ = 9 Hz, H-5) and 7.32 (dd, $J_{6,5}$ = 9 Hz, $J_{6,2}$ = 2 Hz, H-6) derived from androsin were also observed. The HMBC spectrum showed correlations between proton signal at δ 4.19 (dd, J = 12,8 Hz, H-6′) of glucose and the carbonyl carbon (δ 165.2) of 4-hydroxybenzoic acid and between the anomeric proton at δ 5.10 (d, J = 8 Hz) of glucose and the oxygenated aromatic carbon (δ 150.2) of acetovanillone indicating that the 4-hydroxybenzoic acid was attached to the 6′-OH of 4-*O*-glucosylated acetovanillon. In addition, the ester and aglycone moieties of compound BC15 were confirmed by direct comparison with authentic samples after acid hydrolysis. The sugar moiety of BC15 was identified as D-glucose by GC analysis after conversion to a thiazolidine derivative. From the above spectroscopic data, compound BC15 was confirmed as a new compound, acetovanillone-1-*O*-β-D-(6-*O*-4-hydroxybenzoyl) glucopyranoside and has been named belalloside B **[421]**.



[421]

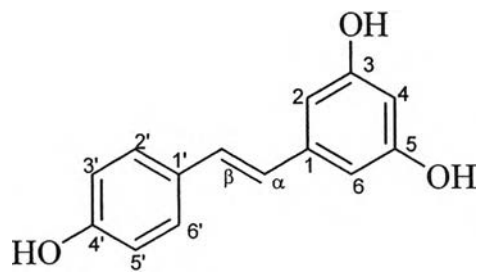
Table 20 NMR Spectral data of compound BC15 (in DMSO-d_6)

Position	Compound BC10	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
Aglycone moiety		
1	-	130.8
2	7.43 (d,2)	110.0
3	-	148.6
4	-	150.2
5	7.13(d,9)	114.2
6	7.32 (dd,2,9)	122.1
7	-	196.1
8	2.50 (s)	26.2
Sugar moiety		
1'	5.10 (d,8)	99.1
2'	4.19 (dd,8,12)	72.9
3'	3.77 (m)	76.6
4'	3.34 (m)	70.1
5'	4.52 (dd,2,12)	73.9
6'a	4.19 (dd,8,12)	63.5
6'b	4.52 (dd,2,12)	
Ester moiety		
1"	-	120.1
2"	7.78 (d,9)	131.4
3"	6.84 (d,9)	115.2
4"	-	162.0
5"	6.84 (d,9)	115.2
6"	7.78 (d,9)	131.4
7"	-	165.2
3-OMe	3.80 (s)	55.5

1.16 Structure Determination of Compound BC16

Compound BC16 was obtained as an amorphous powder. The molecular formula of $C_{14}H_{13}O_3$ was deduced from its $[M+H]^+$ ion at *m/z* 229 in the FABMS.

The ^1H NMR spectrum in DMSO-d_6 (Table 21 and Figure 68) displayed three protons belong to 1,3,5-trisubstituted benzene ring system at δ 6.37 (2H, d, J = 2 Hz, H-2,6) and 6.10 (1H, J = 2 Hz, H-4). A pair of two aromatic proton signals at δ 7.37 (d, $J_{2',3'} = J_{6',5'}$ = 9 Hz, H-2′,6′) and 6.74 (d, $J_{3',2'} = J_{5',6'}$ = 9 Hz, H-3′,5′) could be attributed to a 4-hydroxyphenyl moiety. In addition, two doublets at δ 6.79 (J = 16 Hz) and 6.91 (J = 16 Hz) indicated the presence of a *trans* double bond between C-α and -β. Compound BC16 was identified as 3,5,4′-tridroxystilbene (resveratrol)**[57]**. Its ^1H NMR data are in good agreement with published data (Anjaneyulu *et al.*, 1984).



[57]

Table 21 NMR Spectral data of compound BC16 (in DMSO-d_6) and resveratrol (in DMSO-d_6)

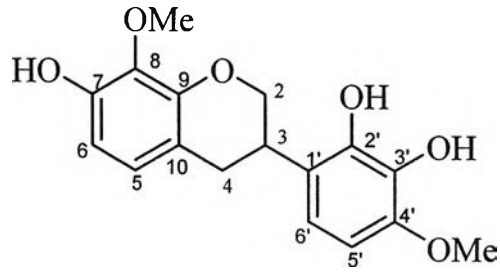
Position	Compound BC16		Resveratrol
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
1	-	139.2	-
2	6.37 (d,2)	104.2	6.42 (d,2)
3	-	158.4	-
4	6.10 (t,2)	101.7	6.15 (t,2)
5	-	158.4	-
6	6.37 (d,2)	104.2	6.42 (d,2)
α	6.79 (d,16)	125.6	6.71 (d,17)
β	6.91 (d,16)	127.8	6.91 (d,17)
1'	-	128.0	-
2'	7.37 (d,9)	127.7	7.43 (d,8)
3'	6.74 (d,9)	115.4	6.79 (d,8)
4'	-	157.1	-
5'	6.74 (d,9)	115.4	6.79 (d,8)
6'	7.37 (d,9)	127.7	7.43 (d,8)
3-OH	9.13 (br.s)		9.34 (br.s)
5-OH	9.13 (br.s)		9.37 (br.s)
4'-OH	9.5 (br.s)		9.34 (br.s)

1.17 Structure Determination of Compound DP1

Compound DP1 was obtained as an amorphous powder. It showed an molecular ion $[M]^+$ peak at *m/z* 318.1115 in the HRFABMS, indicating a molecular formula of $C_{17}H_{18}O_6$ (calcd 318.1103). The UV spectrum showed the absorption bands at 227 and 230 (sh) nm.

In the ^1H NMR spectrum in DMSO-d_6 (Table 22 and Figure 71), characteristic signals assignable to CH_2-CH-CH_2 coupling system of the 3-arylchroman were observed at δ 3.93 (1H, t, J = 10 Hz, H-2a), 4.23 (1H, dd, J = 10,2 Hz, H-2b), 3.76 (1H, m, H-3), 2.72 (1H, dd, J = 16,5 Hz, H-4a) and 2.88 (1H, dd, J = 16,11 Hz, H-4b) indicating DP1 as an isoflavan. The *ortho*-coupled aromatic protons at δ 6.60 (1H, H-5) and 6.33 (1H, H-6) were assigned in ring A, whereas another pair of signals at δ 6.42 (1H, H-5′) and 6.53 (1H, H-6′) were suggested in ring B. The signals at δ 3.66 and 3.74 (3H, s, each) were attributed to the methoxyl groups at C-8 and C-4′, respectively, from the HMQC and HMBC spectra (Figure 74-75). Moreover, NOE was observed at H-5′ (δ 6.42) by irradiating 4′-O$\underline{C}H_3$ (δ 3.74) in the difference NOE spectrum (Figure 72).

The ^{13}C NMR spectrum of compound DP1 in DMSO-d_6 (Figure 73 and Table 22) showed signals for seventeen carbons, corresponding to two methylene carbons at δ 29.9 (C-4), 69.2 (C-2), one methine carbon at δ 31.3 (C-3), four hydrogen-bonded aromatic carbons at δ 123.5 (C-5), 103.1 (C-6), 108.1 (C-5′), 116.4 (C-6′), eight aromatic carbons at δ 148.5 (C-7), 135.6 (C-8), 147.7 (C-9), 114.1 (C-10), 143.8 (C-2′), 133.8 (C-3′), 147.1 (C-4′) and two methoxyl carbons at δ 59.7 (8-O$\underline{C}H_3$) and 55.7 (4′-O$\underline{C}H_3$). Based on the above spectral evidence, compound DP1 was identified as a new compound and has been named (±)-(7,2′,3′-trihydroxy-8,4′-dimethoxyisoflavan (khriol A) **[422]**.



[422]

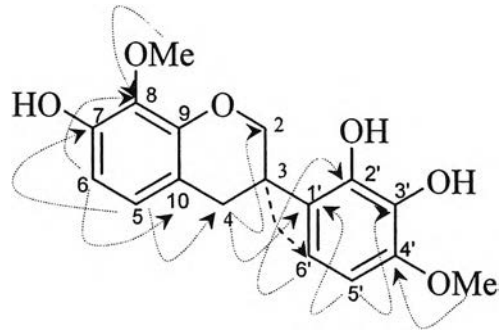


Figure 7 HMBC correlations of compound DP1

Table 22 NMR Spectral data of compound DP1 (in DMSO-d_6)

Position	Compound DP1		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2a	3.93 (t,10)	69.2	H-4
2b	4.23(dd,10,2)		
3	3.76(m)	31.3	H-6', H-4*
4a	2.72 (dd,16,5)	29.9	H-2 , H-5
4b	2.88 (dd,16,11)		
5	6.60 (d,9)	123.5	H-4
6	6.33 (d,9)	103.1	-
7	-	148.5	H-5 , H-6*
8	-	135.6	H-6, 8-OMe
9	-	147.7	H-4 , H-5
10	-	114.1	H-4* , H-6
1'	-	120.9	H-4 , H-5'
2'	-	143.8	H-6'
3'	-	133.8	H-5'
4'	-	147.1	H-6', H-5', 4'-OMe
5'	6.42 (d,9)	108.1	-
6'	6.53 (d,9)	116.4	-
8-OMe	3.66(s)	59.7	
4'-OMe	3.74(s)	55.7	

* Two bond coupling

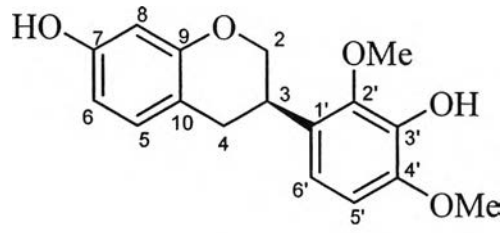
1.18 Structure Determination of Compound DP2

Compound DP2, $[\alpha]_D^{25}$ +19.3°, was obtained as an amorphous powder. Its molecular formula of $C_{17}H_{18}O_5$ was established by FABMS which showed the $[M]^+$ peak at *m/z* 302 (Figure 76).

The ^1H NMR spectrum of compound DP2 in DMSO-d_6 (Table 23 and Figure 77) showed characteristic signals for 3-arylchroman at δ 3.90 (1H, t, J = 10 Hz, H-2a), 4.10 (1H, dd, J = 10,2 Hz, H-2b), 3.28 (1H, m, H-3), 2.72 (1H, dd, J = 16,5 Hz, H-4a), 2.83 (1H, dd, J = 16,11 Hz, H-4b), and it was supported by ^1H-^1H COSY, HMQC and HMBC spectra (Figure 80-82). The aromatic protons coupled with ABX splitting pattern were observed at δ 6.85 (1H, d, J = 9 Hz, H-5), 6.28 (1H, dd, J = 9,2 Hz, H-6), 6.19 (1H, d, J = 2 Hz, H-8) assignable to A-ring and the signals with AB splitting pattern at δ 6.68 (1H, d, J = 9 Hz, H-5′), 6.58 (1H, d, J = 9 Hz, H-6′) were assigned to B-ring. Furthermore, two methoxyl groups at δ 3.73 and 3.75 (3H, each) were placed at C-2′ and C-4′, respectively from the HMBC spectra. In the difference NOE spectrum of DP2, NOE was observed at δ H-5′ [δ 6.68 (1H, d, J = 9 Hz)] by irradiating at δ 3.75 (4′-OC<u>H</u>$_3$).

The ^{13}C NMR spectrum in DMSQ-d_6 (Table 23 and Figure 79) showed seventeen carbons signals corresponding to two methylene carbons, one methine carbon, four aromatic carbons, eight aromatic carbons and two methoxyl carbons. The absolute configuration at C-3 of DP2 was then determined as 3(*R*) by a negative Cotton effect ($[\theta]_{230}$ −14600) and a positive Cotton effect ($[\theta]_{283}$ 3700) in the circular dichroism (CD) (Donnelly *et al.*, 1973).

By comparing the above spectroscopic data with reported values (Hamburger *et al.*, 1987), this compound was identified as (3*R*)-(+)-7,3′-dihydroxy-2′,4′-dimethoxyisoflavan ((3*R*)-(+)-mucronulatol) **[77]**. It has been isolated from several plants in genus *Dalbergia* such as *D. Variabilis*, (Kurosawa *et al.*, 1968) and genus *Machaerium* such as *M. mucromulatum* (Kurosawa *et al.*, 1978).



[77]

Table 23 NMR Spectral data of compound DP2 (in DMSO-d_6) and mucronulatol (in DMSO-d_6)

Position	Compound DP2		Mucronulatol		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	3.90 (t,10) 4.10 (dd,10 2)	69.7	3.90 (dd ,10.3 , 10.3)	69.7	H-4 , H-3*
3	3.28 (m)	31.3	3.30 (ddd,11.2,10.3,3)	31.3	H-4*
4	2.72 (dd,16,5) 2.83(dd,16,11)	30.8	2.71(dd,13.5) 2.84(dd,13,11.3)	30.8	H-2
5	6.85 (d,9)	129.9	6.86(d,8.3)	130.1	H-4
6	6.28(dd,9,2)	107.9	6.29(dd,8.2,2.4)	107.9	H-8
7	-	156.4	-	156.5	H-5 , H-6*, H-8*
8	6.19(d,2)	102.5	6.19(d,2.3)	102.5	H-6
9	-	154.4	-	154.5	H-2, H-4, H-5, H-8*
10	-	112.6	-	112.7	H-4*
1'	-	126.8	-	126.8	H-4 , H-5' , H-3*
2'	-	146.0	-	146.0	H-3, H-6', 2'-OMe
3'	-	139.2	-	139.2	H-5' , H-6'
4'	-	147.7	-	147.7	4'-OMe, H-6', H-5'*
5'	6.68(d,9)	107.6	6.60-6.69	107.5	-
6'	6.58(d,9)	116.1	6.60-6.69	116.2	H-3, H-5'*
2'-OMe	3.73(s)	55.9	3.73(s)	55.9	-
4'-OMe	3.75(s)	60.2	3.75(s)	60.3	-
7'-OH	9.10(s)	-	9.18(s)	-	-
3'-OH	8.55(s)	-	8.66(s)	-	-

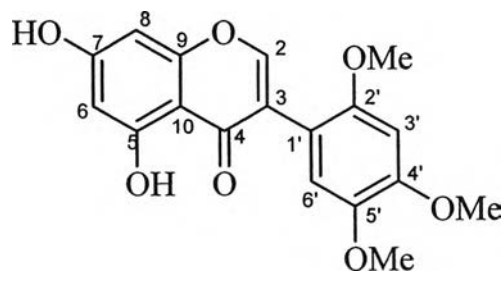
* Across two bonds correlations

1.19 Structure Determination of Compound DP3

Compound DP3 was obtained as an amorphous powder. The FABMS analysis of compound DP3 showed a molecular ion $[M]^+$ peak at *m/z* 344 (Figure 83), corresponding to the formula $C_{18}H_{16}O_7$.

The signals at δ_H 8.04 assignable to H-2 and δ_C 156.0 (C-2) in the ^1H and ^{13}C NMR spectra, respectively, were suggestive of an isoflavone type skeleton (Table 24 and Figure 84 and 86). Appearance of the signal in low field was due to the α - inductive effect of the oxygen and mesomeric electron withdrawing effect of the β carbonyl group. These data were useful to distinguish isoflavones from flavones (Jha *et al.*, 1980). Furthermore a chelated hydroxyl group at δ 13.00 (H-5), two *meta*-coupled aromatic protons at δ 6.27 (1H, d, J = 2 Hz, H-6) and 6.40 (1H, d, J = 2 Hz, H-8) were observed with two singlet aromatic signals at δ 6.79 and 6.97 assignable to H-3′,6′ in B-ring and the fragment ion at *m/z* 152 resulting from *retro*-Diels-Alder cleavage of C-ring. In the difference NOE spectrum of DP3, NOEs were observed at δ H-3′ [δ 6.79 (1H, s)], H-6′ [δ 6.97 (1H, s)] by irradiation at the δ 3.76 (2′,5′-OC<u>H</u>$_3$), and at H-3′ [δ 6.79 (1H, d, J = 9 Hz)] by irradiation at the δ 3.88 (4′-OC<u>H</u>$_3$).

Based on the above spectroscopic data, compound DP3 was identified as 5,7-dihydroxyl-2′,4′,5′-trimethoxyisoflavone (7-demethylrobustigenin) **[423]**. It was first found naturally from *Erythrina sacleuxii* as naturally occurring substance (Yenesew *et al.*, 1998).



[423]

Table 24 NMR Spectral data of compound DP3 (in acetone-d_6) and 7-demethylrobustigenin (in acetone-d_6)

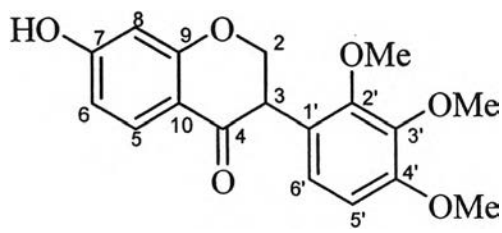
Position	Compound DP3		7-Demethylrobustigenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.04(s)	156.0	8.07(s)	155.7
3	-	121.5	-	122.2
4	-	181.4	-	182.1
5	-	163.8	-	164.5
6	6.27(d,2)	99.9	6.29(d,2.1)	100.5
7	-	165.1	-	165.6
8	6.40(d,2)	94.5	6.42(d,2.1)	95.2
9	-	159.1	-	159.7
10	-	106.2	-	106.8
1'	-	112.2	-	112.7
2'	-	153.5	-	154.0
3'	6.79(s)	99.8	6.81(s)	100.2
4'	-	151.7	-	152.3
5'	-	144.2	-	144.7
6'	6.97(s)	117.8	6.98(s)	118.2
2'-OMe	3.76(s)	56.5	3.89	57.1
4'-OMe	3.88(s)	56.9	3.80	57.6
5'-OMe	3.76(s)	57.2	3.78	57.8
5-OH	13.00(s)	-	13.04(s)	-

1.20 Structure Determination of Compound DP4

Compound DP4 was obtained as an amorphous powder. The FABMS spectrum (Figure 87) showed a molecular $[M+H]^+$ ion peak at *m/z* 331, suggesting a molecular formula of $C_{18}H_{19}O_6$.

The ^1H NMR spectrum in acetone-d_6 exhibited non-equivalent methylene protons at δ 4.13 (1H, dd, J = 11,6 Hz, H-2ax) and 4.45 (1H, dd, J = 11,6 Hz, H-2eq) with a triplet signal at δ 4.57 (1H, t, J = 6 Hz) suggesting the isoflavanone skeleton. The aromatic protons coupled with ABX pattern were observed at δ 7.80 (1H, d, J = 9 Hz, H-5), 6.60 (1H, dd, J = 9,2 Hz, H-6) and 6.40 (1H, d, J = 2 Hz, H-8) indicating the 7-oxygenated structure in A-ring. The presence of *ortho*-coupled protons at δ 6.72 (1H, d, J = 9 Hz) and 6.86 (1H, d, J = 9 Hz) were assigned to H-5′ and H-6′, respectively by irradiating at δ 3.82 (4′-OC<u>H</u>$_3$) in the difference NOE spectrum (Figure 88). Moreover, the mass spectral fragmentation pattern of DP4 with *retro*-Diels-Alder type suggested monohydroxylated ring A (*m/z* 137) and trimethoxylated ring B (*m/z* 193).

By analysis of the above spectroscopic data, this compound was identified as (±)-7-hydroxy-2′-3′,4′-trimethoxyisoflavanone ((±)-3′-methoxyl violanone) **[197]** which was isolated from *Dalbergia cearensis*. Its ^1H NMR data are in good agreement with published data (Guimaraes *et al.*, 1975).



[197]

Table 25 NMR Spectral data of compound DP4 (in acetone-d_6) and 3'-methoxyviolanone (in acetone-d_6)

Position	Compound DP4		3'-Methoxyviolanone	HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	(correlation with ^{1}H)
2a	4.13(dd,11,6)	72.0	4.5(t,12)	-
2b	4.45(dd,11,6)		4.68(dd,12,5)	-
3	4.57(t,6)	48.9	4.19(dd,12,5)	H-2*
4	-	191.2	-	H-2 , H-3*
5	7.80(d,9)	130.0	7.90(d,8.5)	-
6	6.60(dd,9,2)	111.3	6.70(dd,8.5,2.5)	H-8
7	-	165.1	-	H-5, H-2*, H-6*, H-8*
8	6.40(d,2)	103.5	6.53(d,2.5)	H-6
9	-	164.7	-	H-2, H-3, H-8*
10	-	115.7	-	H-6, H-8
1'	-	123.0	-	H-2 , H-5'
2'	-	152.9	-	H-2, H-5', H-6', 2'-OMe
3'	-	143.4	-	H-5', H-6', 3'-OMe
4'	-	154.5	-	4'-OMe
5'	6.72(d,9)	108.6	6.80(d,8.5)	-
6'	6.86(d,9)	125.3	7.00(d,8.5)	H-2
2'-OMe	3.78(s)	61.0	3.85(s)	-
3'-OMe	3.78(s)	60.6	3.85(s)	-
4'-OMe	3.82(s)	56.3	3.85(s)	-

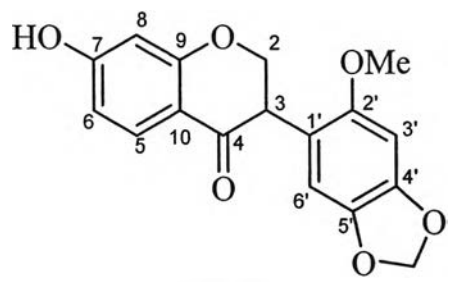
* Across two bonds correlations

1.21 Structure Determination of Compound DP5

Compound DP5, an amorphous powder, exhibited a molecular ion $[M]^+$ peak at *m/z* 314 in the FABMS (Figure 93), corresponding to $C_{17}H_{14}O_6$.

The ^1H NMR spectrum (Table 26 and Figure 94) showed the characteristics of isoflavanone nucleus with signals for H-2 at δ 4.20 (1H, dd, J = 11,6 Hz, H-2a), 4.43 (1H, dd, J = 11,6 Hz, H-2b) and a triplet signal at δ 4.53 (J = 6 Hz, H-3). Besides of three protons coupled with ABX pattern at δ 7.78 (1H, d, J = 9 Hz), 6.56 (1H, dd, J = 9,2 Hz), 6.40 (1H, d, J = 2 Hz), assignable H-5, 6 and 8, respectively, two singlet protons at δ 6.70 and 6.65 were observed with a methoxyl group at δ 3.73 (3H, s) and methylenedioxy unit at δ 5.92 (2H, s). In the difference NOE spectrum of DP5, NOE was observed at δ H-3′ [δ 6.70 (1H, s)] by irradiation at the δ 3.73 (2′-OC<u>H</u>$_3$). From these results, the methylenedioxy was assigned to be attached to C-4′ and C-5′.

Based on the above spectral evidence, compound DP5 was identified as (±)-7-hydroxy-2′-methoxy-4′,5′-methylenedioxyisoflavanone ((±)-onogenin) **[252]**, which was isolated from *Dalbergia stevensonii* (Donnelly *et al.*, 1973).



[252]

Table 26 NMR Spectral data of compound DP5 (in acetone-d_6) and onogenin (in $CDCl_3$)

Position	Compound DP5		Onogenin
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
2a	4.20(dd,11,6)	71.7	-
2b	4.43(dd,11,6)		
3	4.53(t,6)	48.1	-
4	-	191.1	-
5	7.78(d,9)	130.0	8.1(d,9)
6	6.56(dd,9,2)	111.3	6.6-7.0(m)
7	-	165.2	-
8	6.40(d,2)	103.5	6.6-7.0(m)
9	-	164.6	-
10	-	115.7	-
1'	-	120.5	-
2'	-	142.1	-
3'	6.70(s)	102.1	6.6-7.0(m)
4'	-	153.9	-
5'	-	148.4	-
6'	6.65(s)	117.2	6.6-7.0(m)
2'-OMe	3.73(s)	57.1	3.77(s)
OCH_2O	5.92(s)	96.3	5.98(s)

1.22 Structure Determination of Compound DP6

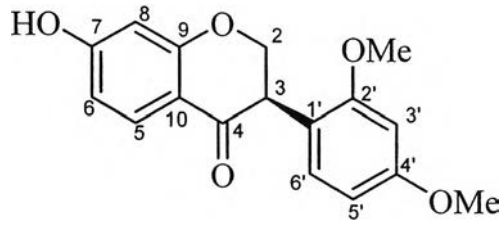
Compound DP6, $[\alpha]_D^{25}$ + 9.7° was obtained as an amorphous powder. The FABMS showed the molecular ion $[M+H]^+$ peak at *m/z* 301, consistent to the molecular formula $C_{17}H_{17}O_5$ (Figure 96).

The ^1H and ^{13}C NMR spectra of DP6 showed a very similar signal pattern to that of DP4 and DP5 and suggested that DP6 was a 7-hydroxyisoflavanone, and it was supported from characteristic proton signals as shown in Table 27. Moreover, the mass spectral fragmentation pattern of DP6 with *retro*-Diels-Alder type cleavage suggested monohydroxylated ring-A (*m/z* 137) and dimethoxylated ring B (*m/z* 165).

In the difference NOE spectrum of DP6, NOEs were observed at δ H-3′ [δ 6.57 (1H, d, J = 2 Hz)] by irradiation at the δ 3.71 (2′-OC<u>H</u>$_3$), and at H-3′ [δ 6.57 (1H, d, J = 2 Hz)], H-5′ [δ 6.47(1H, dd, J = 9,2 Hz)] by irradiation at the δ 3.75 (4′-OC<u>H</u>$_3$). These results were confirmed by the three-bonds correlation from 2′-OC<u>H</u>$_3$ (δ 3.71) to C-2′ (δ 158.1) and from 4′-OC<u>H</u>$_3$ (δ 3.75) to C-4′ (δ 159.9) in the HMBC spectrum (Figure 101).

The absolute configuration at C-3 of DP6 has been proved to be, 3(*R*) by a negative Cotton effect ($[\theta]_{230}$ −12500) and a positive Cotton effect ($[\theta]_{283}$ 5200) in the CD spectrum.

From the above ^1H and ^{13}C NMR data, together with the information from ^1H-^1H COSY, HMQC and HMBC (Figure 99-101) experiments, compound DP6 was identified as (3*R*)-(+)-7-hydroxy-2′,4′-dimethoxyisoflavanone ((3*R*)-(+)-sativanone) **[175]**. They were in close agreement with previously published values (Jain and Nayyar, 1987). It was a synthetic compound and was isolated first from *Dalbergia stevensonii* as natural product (Donnelly *et al.*, 1973).



[175]

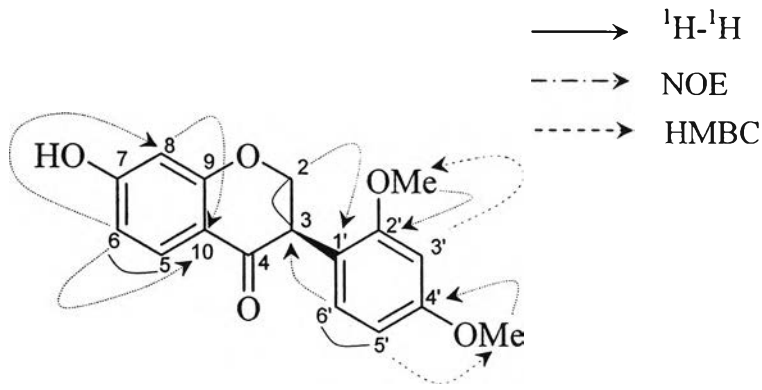


Figure 8 NOE, ^{1}H-^{1}H COSY and HMBC correlations of compound DP6

Table 27 NMR Spectral data of compound DP6 (in DMSO-d_6) and sativanone (in acetone-d_6)

Position	Compound DP6		Sativanone		HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	(correlation with ^{1}H)
2a	4.14(dd,11,6)	70.3	4.52(dd,11.8,6)	71.76	-
2b	4.41(dd,11,6)		4.41(dd,11.8,9.2)		
3	4.50(t,6)	46.6	3.82(dd,9.2,6)	48.04	-
4	-	190.2	-	191.56	H-2 ,H-5
5	7.67(d,9)	128.9	7.77(d,9.5)	131.53	-
6	6.51(d,9,2)	110.5	6.38-6.57(m)	103.38	H-8 ,7-OH
7	-	164.2	-	165.10	H-5 , 7-OH*
8	6.32(d,2)	102.3	6.38-6.57(m)	99.67	H-6, 5-OH
9	-	163.2	-	164.74	H-2, H-5
10	-	114.0	-	115.78	H-8, H-6
1'	-	116.1	-	117.37	H-2
2'	-	158.1	-	159.50	2'-OMe
3'	6.57(d,2)	98.8	6.38-6.57(m)	111.16	-
4'	-	159.9	-	161.45	H-6', 4'-OMe
5'	6.47(dd,9,2)	104.9	6.38-6.57(m)	105.69	-
6'	6.97(d,9)	130.5	7.21(d,9.5)	130.01	-
2'-OMe	3.71(s)	55.6	3.78(s)	55.58	-
4'-OMe	3.75(s)	55.2	3.78(s)	55.95	-
7-OH	10.5(s)		8.16(s)		

* Across two bonds correlations

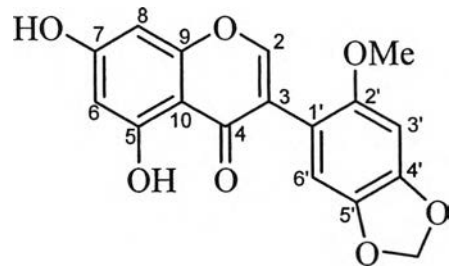
1.23 Structure Determination of Compound DP7

Compound DP7 was obtained as an amorphous powder. A molecular formula of $C_{17}H_{12}O_7$ was established from the HRFABMS which exhibited molecular ion $[M]^+$ peak at *m/z* 328.0557 (calcd for 328.0583) as showed in Figure 102. The UV maximum absorptions were observed at 299, 260 and 225 (sh) nm

The ^{1}H NMR spectrum of DP7 in acetone-d_6 (Table 28 and Figure 103) showed singlet signals at δ 8.03 (1H, s, H-2) and 12.95 (1H, s, 5-OH) suggesting the compound as 5-hydroxy isoflavone. Besides of *meta*-coupled proton signals at δ 6.27 (1H, d, J = 2 Hz, H-6) and 6.40 (1H, d, J = 2 Hz, H-8), two singlet protons at δ 6.76 and 6.84 (each 1H, H-3′ and 6′) were observed as aromatic proton signals. Furthermore, the presence of methylenedioxy group [δ 5.99 (2H, s)] and a methoxyl group [δ 3.74 (3H, s)] were observed.

The fragment ions in the mass spectral of DP7 suggested dihydroxylated ring A (*m/z* 153). The methoxyl group was assigned to the C-2′ from the NOE observed at δ H-3′ [δ 6.76 (1H, s)] by irradiation at the δ 3.74 (2′-OC<u>H</u>$_3$) in the difference NOE spectrum, as shown in Figure 104. Thus, the methylenedioxy should be located at C-4′ and C-5′. In the ^{13}C NMR spectrum (Figure 105), the chemical shift values (δ 141.9, δ 154.3 and δ 149.5) of the sp^2 carbon atoms in B-ring require oxygenation to occur on adjacent carbons at C-2′, C-4′ and C-5′.

Based on the above spectral evidence, DP7 was confirmed as a new isoflavone, 5-7-dihydroxy-2′-methoxy-4′,5′-methylenedioxyisoflavone and was named khrinone D **[424]**.



[424]

Table 28 NMR Spectral data of compound DP7 (in acetone-d_6)

Position	Compound DP7	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.03(s)	156.0
3	-	119.7
4	-	181.3
5	-	163.8
6	6.27(d,2)	100.0
7	-	165.5
8	6.40(d,2)	94.6
9	-	159.1
10	-	106.0
1'	-	110.5
2'	-	141.9
3'	6.76(s)	102.4
4'	-	154.3
5'	-	149.5
6'	6.84(s)	115.9
2'-OMe	3.74(s)	57.2
OCH_2O	5.99(s)	96.2
5-OH	12.95(s)	-

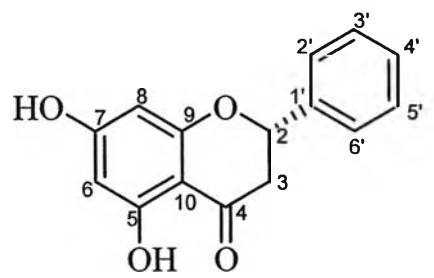
1.24 Structure Determination of Compound DP8

Compound DP8, $[\alpha]_D^{25}$ – 48.3° was obtained as an amorphous powder. The FABMS spectrum (Figure 106) showed a molecular ion $[M+H]^+$ peak at *m/z* 257, corresponding to $C_{15}H_{13}O_4$.

The ^1H NMR signals of DP8 (Table 29 and Figure 107) observed at δ 2.79 (dd, J = 17,3 Hz, H-3eq), 3.21 (dd, J = 17,13 Hz, H-3ax), 5.57 (d, J = 13,3 Hz, H-2) and 12.1(br.s) with ^{13}C NMR signals at δ 78.3 (C-2), 42.0 (C-3) and 195.7 (C-4) were characteristic of a 5-hydroxyflavanone. The correlation between H-2 and H-3 was observed in the ^1H-^1H COSY spectrum (Figure 109).

The *meta*-coupled protons at δ 5.88 (d, J = 2 Hz, H-6) and 5.91 (d, J = 2 Hz, H-8) and ^{13}C NMR signals of oxygenated carbon at δ 163.4 and 167.0 indicated 5, 7-dihydroxy A-ring. The fragment ion at *m/z* 153 and 103 resulting from *retro*-Diels-Alder cleavage of ring C supported the presence of dihydroxylated A ring and unsubstitued B ring. In order to confirm all protons and carbons signals of DP8, the HMQC and HMBC experiments were performed (Figure 110-111). Its ^1H and ^{13}C NMR data are in good agreement with the literature values (Komoda, 1989).

The absolute configuration at C-2 of this compound was then determined as 2(*S*) by the negative Cotton effect ($[\theta]_{283}$ –49300) in the CD spectrum. Hence, compound DP8 was identified as 5,7-dihydroxyflavanone (pinocembrin) **[174]**. This compound was isolated from *Dalbergia stevensonii*, *Populus nigar* and *Melodorum fruticosum* (Donnelly *et al.*, 1973; Komoda, 1989 and Jung *et al.*, 1990).



[174]

Table 29 NMR Spectral data of compound DP8 (in DMSO-d_6) and pinocembrin (in DMSO-d_6)

Position	Compound DP8		Pinocembrin		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	5.57(dd,13,3)	78.3	5.58(dd,12.5,3)	78.3	H-2', H-3*
3a	2.79(dd,17,3)	42.0	2.78(dd,17.1,3)	42.1	-
3b	3.21(dd,17,13)		3.24(dd,17.1,12.5)		
4	-	195.7	-	195.7	H-3*
5	-	163.4	-	163.2	H-6*
6	5.88(d,2)	95.9	5.92(d,2)	95.9	H-8, 5-OH
7	-	167.0	-	166.5	H-6*
8	5.91(d,2)	95.1	5.95(d,2)	95.0	-
9	-	162.6	-	162.7	-
10	-	101.6	-	101.8	H-6, 5-OH
1'	-	138.7	-	138.6	H-3, H-3', H-2*
2'	7.51(dd,8,2)	126.5	7.52(d,6.6)	126.5	H-2 ,H-4'
3'	7.42(tt,8,2)	128.4	7.30-7.50(m)	128.5	H-5'
4'	7.37(tt,8,2)	128.4	7.30-7.50(m)	128.5	H-2'
5'	7.42(tt,8,2)	128.4	7.30-7.50(m)	128.5	H-3'
6'	7.51(dd,8,2)	126.5	7.52(d,6.6)	126.5	-
5-OH	12.1(br.s)	-	-	-	-

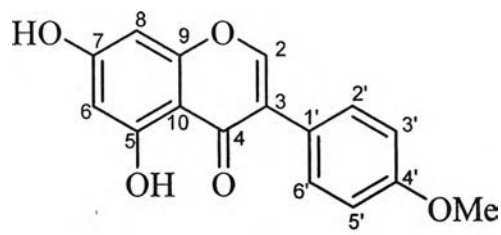
* Across two bonds correlations

1.25 Structure Determination of Compound DP9

Compound DP9, an amorphous powder, was analyzed for $C_{16}H_{13}O_5$ from its $[M+H]^+$ peak at *m/z* 285 in the FABMS spectrum (Figure 112).

The ^1H NMR spectrum of DP9 in DMSO- d_6 (Table 30) showed singlet signals at δ 8.30 (1H, s) and 12.90 (1H, s) suggesting the compound as 5-hydroxy isoflavone. Besides of *meta*-coupled signals at δ 6.22 (1H, d, J = 2 Hz, H-6) and 6.38 (1H, d, J = 2 Hz, H-8), *ortho* coupled A_2X_2-type protons at δ 7.48 and 6.99 (each 2H, d, J = 8 Hz) assignable to H-2′,6′ and H-3′,5′, respectively were observed in addition to a methoxyl proton signal at δ 3.76 (3H, s). NOE observed at δH-3′,5′ [δ 6.99 (1H, s)] by irradiation at the -OCH_3 (δ 3.76) suggested 4′-OCH_3 in the B-ring (Figure 114). The 5, 7-dihydroxy flavanone skeleton was supported by the fragment ion at *m/z* 153 in the FABMS.

By comparing the spectral data with those reported, the compound was defined as 5,7-dihydroxy-4′-methoxyisoflavone, trivially known as biochanin A **[95]**. It was isolated from several plants such as *Swartzia polyphylla* (Osawa *et al.*, 1992) and *Pueraria lobata* (Jun *et al.*, 2003).



[95]

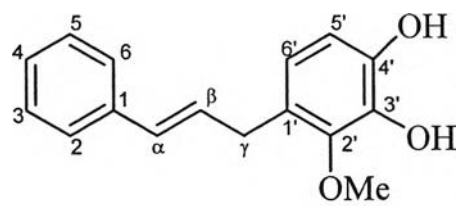
Table 30 NMR Spectral data of compound DP9 (in DMSO-d_6) and biochanin A (in DMSO-d_6)

Position	Compound DP9		Biochanin A	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.30(s)	154.1	8.34(s)	154.4
3	-	122.9	-	123.1
4	-	180.0	-	180.3
5	-	157.5	-	157.8
6	6.22(d,2)	99.0	6.23(d,2)	99.2
7	-	164.4	-	162.2
8	6.38(d,2)	93.7	6.38(d,2)	93.8
9	-	161.9	-	161.9
10	-	104.3	-	104.7
1'	-	121.9	-	122.2
2'	7.48(d,8)	130.1	7.49(d,8.7)	130.3
3'	6.99(d,8)	113.6	6.99(d,8.7)	113.9
4'	-	159.1	-	159.3
5'	6.99(d,8)	113.6	6.99(d,8.7)	113.9
6'	7.48(d,8)	130.1	7.49(d,8.7)	130.3
4'-OMe	3.76(s)	55.1	3.79(s)	55.3
5-OH	12.90(s)	-	12.91(s)	-

1.26 Structure Determination of Compound DP10

Compound DP10 was obtained as an amorphous powder. The FABMS spectrum (Figure 116) showed it $[M]^+$ ion peak at *m/z* 256 suggesting the molecular formula of $C_{16}H_{16}O_3$.

In the ^1H NMR spectrum in acetone-d_6 (Table 31 and Figure 117), the aromatic protons coupled with AA′ BB′C system δ 7.37 (2H, dd, J = 8,2 Hz, H-2, H-6), 7.27 (2H, tt, J = 8,2 Hz, H-3,H-5) and 7.17 (1H, tt, J = 8,2 Hz, H-4) were observed with *ortho*-coupled AB-type protons at δ 6.55 (1H, d, J = 8 Hz, H-5′) and 6.58 (1H, d, J = 8 Hz, H-6′) suggesting the presence of mono-substituted and 1,2,3,4-tetrasubstituted benzene ring in the molecule. Furthermore ABX_2-type proton signals at δ 6.43 (1H, d, J = 16 Hz, H-α), 6.37 (1H, dt, J = 16,6 Hz, H-β) and 3.44 (2H, d, J = 6 Hz, H-γ) revealed the partial structure [(*E*)-CH = CH-CH_2-]. These results, coupled with NOE observed at H-γ by irradiation at the OCH_3 (δ 3.80) defined the structure as (*E*)-1-(3, 4-dihydroxy-2-methoxybenzyl)-2-phenylene (hydroxyobtustyrene) **[355]**. The MS fragment at *m/z* 139 due to the cleavage C-1′-C-γ bond suggested the structure and its spectral data are in good agreement with published data, which isolated from heartwood of *Dalbergia odorifera* (Goda *et al.*, 1992).



[355]

Table 31 NMR Spectral data of compound DP10 (in acetone-d_6) and hydroxyobtustyrene (in acetone-d_6)

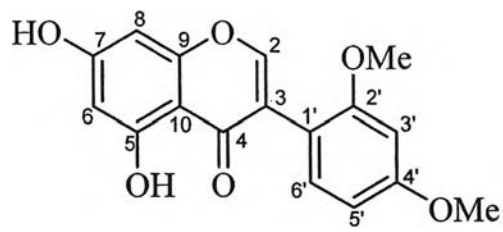
Position	Compound DP10		Hydroxyobtustyrene
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
1	-	139.0	-
2	7.37(dd,8,2)	126.8	7.00-7.40(m)
3	7.27(tt,8,2)	129.3	7.00-7.40(m)
4	7.17(tt,8,2)	127.7	7.00-7.40(m)
5	7.27(tt,8,2)	129.3	7.00-7.40(m)
6	7.37(dd,8,2)	126.8	7.00-7.40(m)
α	6.43(d,16)	131.1	6.36(m)
β	6.37(dt,16,6)	130.7	6.36(m)
γ	3.44(d,6)	33.5	3.44(m)
1'	-	125.2	-
2'	-	145.6	-
3'	-	138.7	-
4'	-	147.3	-
5'	6.55(d,8)	111.6	6.50(s)
6'	6.58(d,8)	120.4	6.50(s)
2'-OMe	3.80(s)	60.8	3.79(s)

1.27 Structure Determination of Compound DP11

Compound DP11 was obtained as an amorphous powder and a molecular ion $[M+H]^+$ in the FABMS spectrum at *m/z* 315 (Figure 120) corresponding to the molecular formula of $C_{17}H_{15}O_6$

The ^1H and ^{13}C NMR spectra in acetone-d_6 (Table 32 and Figure 121-123) showed characteristic signals for 5,7-dihydroxyisoflavone skeleton and similar signals pattern to that of DP9, except for an addition of a methoxyl signal. And the ABX pattern signals at δ 6.63 (1H, d, J = 2 Hz, H-3′), 6.57 (1H, dd, J = 8,2 Hz, H-5′) and 7.21 (1H, d, J = 8 Hz, H-6′) were observed. In the FABMS, the fragment ion at *m/z* 163 resulting from *retro*-Diels-Alder cleavage suggested dimethoxylated ring B. In the difference NOE spectrum of DP11, NOEs were observed at δ H-3′ [δ 6.63 (1H, d, J = 2 Hz)] by irradiation at the δ 3.77 (2′-OC<u>H</u>$_3$), and at H-3′ [δ 6.63 (1H, d, J = 2 Hz)], H-5′ [δ 6.57 (1H, dd, J = 9,2 Hz)] by irradiation at the δ 3.83 (4′-OC<u>H</u>$_3$).

The result substantiated the structure of DP11 as 5,7-dihydroxy-2′,4′-dimethoxyisoflavone (2′-methoxybiochanin A) **[425]**, which was found in family Myristicaceae (Reynaud *et al.*, 2005).



[425]

Table 32 NMR Spectral data of compound DP11 (in acetone-d_6)

Position	Compound DP11	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.00(s)	155.7
3	-	121.7
4	-	181.5
5	-	163.8
6	6.27(d,2)	99.9
7	-	165.1
8	6.40(d,2)	94.5
9	-	159.1
10	-	108.6
1'	-	113.3
2'	-	159.8
3'	6.63(d,2)	99.5
4'	-	162.4
5'	6.57(dd,8,2)	105.6
6'	7.21(d,8)	133.1
2'-OMe	3.77(s)	56.1[a]
4'-OMe	3.83(s)	55.7[a]
5-OH	12.99	-

[a] interchangeable within column

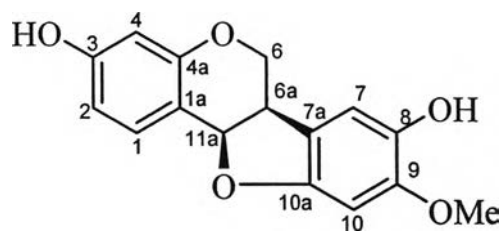
1.28 Structure Determination of Compound DP12

Compound DP12, $[\alpha]_D^{25}$ – 174.4° was obtained as an amorphous. The molecular formula was determined as $C_{16}H_{14}O_5$ by FABMS of its $[M]^+$ ion at *m/z* 286.

The ^1H NMR spectrum of compound DP12 exhibited characteristic four-spin system for pterocarpan at δ 3.53 (1H, t, J = 11 Hz, H-6), 4.18 (1H, dd, J = 11,5 Hz, H-6), 3.44 (1H, ddd, J = 11,7,5 Hz, H-6a) and 5.38 (1H, d, J = 7 Hz, H-11a). Besides of singlet signals at δ 6.76 (1H, H-7) and 6.45 (1H, H-10), three ABX-type protons at δ 7.25 (1H, d, J = 8 Hz, H-1), 6.47 (1H, dd, J = 8,2 Hz, H-2) and 6.29 (1H, d, J = 2 Hz, H-4) were observed in addition to a methoxyl proton signal at δ 3.78 (3H, s).

The absolute configuration at C-6a and C-11a of DP12 were determined as 6a*R* and 11a*R* by a negative Cotton effect ($[\theta]_{234}$ −73800) and a positive Cotton effect ($[\theta]_{296}$ 12200) in the CD spectrum.

Collectively, the above data suggested DP12 as (6a*R*, 11a*R*)-(−)-3,8-dihydroxy-9-methoxylpterocarpan **[426]**. This compound has been found in *Pterocarpus soyauxii* heartwood (Harborne, 1993).



[426]

Table 33 NMR Spectral data of compound DP12 (in MeOH-d_4)

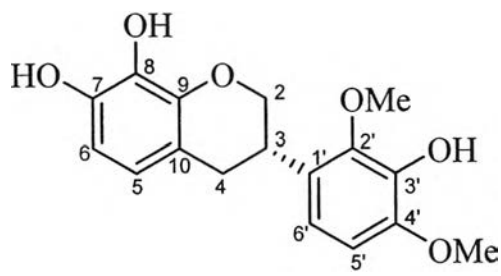
Position	Compound DP12	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
1a	-	113.2
1	7.25(d,8)	133.1
2	6.47(dd,8,2)	110.7
3	-	160.0
4	6.29(d,2)	104.1
4a		158.0
6	3.53(t,11) 4.18(dd,11,5)	67.5
6a	3.44(ddd,11,7,5)	41.7
7a	-	119.5
7	6.76(s)	112.5
8	-	141.7
9	-	149.5
10	6.45(s)	96.4
10a		154.1
11a	5.38(d,7)	79.5
9-OMe	3.78(s)	56.7

1.29 Structure Determination of Compound DP13

Compound DP13, $[\alpha]_D^{25}$ +16.3°, an amorphous powder, exhibited a molecular ion $[M]^+$ peak at *m/z* 318 in the FABMS, corresponding to $C_{17}H_{18}O_6$.

The ^1H and ^{13}C NMR spectra of DP13 in MeOH-d_4 demonstrated signals similar to those of DP2, 7,3′-dihydroxy-2′,4′-dimethoxyisoflavan, except for the additional hydroxyl group in A ring. The *ortho*-coupled aromatic protons at δ 6.41 (1H, d, J = 8 Hz, H-5) and 6.35 (1H, d, J = 8 Hz, H-6) suggested the presence of 7,8-dihydroxy groups in A-ring.

The absolute configuration at C-3 of DP13 was determined as 3(*S*) by a positive Cotton effect ($[\theta]_{238}$ 6400) in the CD spectrum. Comparing the above spectral data with the recorded data, DP13 was identified as (3*S*)-(+)-7,8,3′-trihydroxy-2′,4′-dimethoxyisoflavan ((3*S*)-(+)-8-demethylduartin) **[427]**. It was first isolated from *Dalbergia ecastophylum* vine wood (Donnelly *et al.*, 1973).



[427]

Table 34 NMR Spectral data of compound DP13 (in MeOH-d_4) and 8-demethylduartin (in $CDCl_3$)

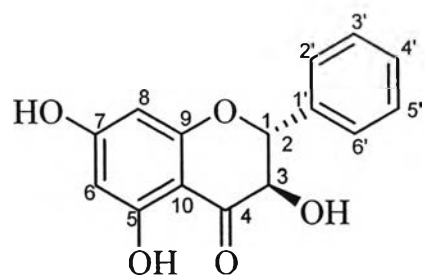
Position	Compound DP13		8- Demethylduartin
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
2	3.94(t,11)	71.7	4.35-4.45(m)
	4.28(ddd,11,3.5,2)		4.35-4.45(m)
3	3.44(tdd,11,5,3.5)	33.2	4.35-4.45(m)
4	2.78(ddd,16,5,2)	32.5	2.8(d(further splitting),7.6)
	2.89(dd,16,11)		2.8(d(further splitting),7.6)
5	6.41(d,8)	120.2	6.97(d,9)
6	6.35(d,8)	109.1	6.72(d,9)
7	-	144.8	-
8	-	134.2	-
9	-	144.2	-
10	-	115.7	-
1'	-	128.6	-
2'	-	149.1	-
3'	-	140.7	-
4'	-	147.3	-
5'	6.67(d,8)	108.5	6.72(d,9)
6'	6.58(d,8)	117.8	6.97(d,9)
2'-OMe	3.81(s)	56.7	3.84(s)
4'-OMe	3.83(s)	61.3	3.84(s)

1.30 Structure Determination of Compound DP14

Compound DP 14, $[\alpha]_D^{25}$ +12.4°, was obtained as a colorless amorphous solid. The FABMS spectrum revealed a molecular ion $[M+H]^+$ peak at *m/z* 273, consistent to the molecular formula $C_{15}H_{13}O_5$.

The ^1H NMR spectrum in acetone-d_6 of DP14 showed similar patterns with that of DP8 with *meta*-coupled doublets and mono-substituted aromatic ring system as aromatic proton signals. And the typical AB-coupled methine protons observed at δ 5.18 (1H, d, J = 12 Hz, H-2) and 4.66 (1H, d, J = 12 Hz, H-3) suggested a *trans*-dihydroflavonol skeleton.

The absolute configuration at C-2 of DP14 was determined as 2(*R*) by a negative Cotton effect ($[\theta]_{288}$ −37800) in the CD spectrum (Gaffield, 1970), and thus DP14 was identified as (2*R*,3*R*)-(+)-3,5,7-trihydroxyflavanone ((2*R*,3*R*)-(+)-pinobanksin) **[428]**. It was isolated from *Populus nigra* (Komoda, 1989) and *Bulbophyllum odoratissimum* (Majumder and Sen, 1991) and the above NMR spectral data were in good agreement with published values.



[428]

Table 35 NMR Spectral data of compound DP14 (in acetone-d_6) and pinobanksin (in DMSO-d_6)

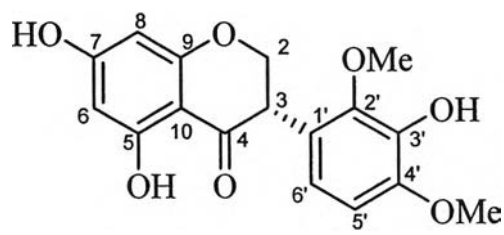
Position	Compound DP14		Pinobanksin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	5.18 (d,12)	84.4	5.18 (d,11.2)	82.8
3	4.66 (d,12)	73.2	4.61 (d,11.2)	71.5
4	-	197.9	-	197.4
5	-	165.1	-	163.3
6	6.01 (d,2)	96.1	5.88 (d,2)	96.1
7	-	167.9	-	167.0
8	5.98 (d,2)	96.1	5.92 (d,2)	95.1
9	-	164.1	-	162.4
10	-	100.2	-	100.3
1'	-	138.3	-	137.2
2'	7.59 (dd,8,2)	128.8	7.52 (d,6.1)	128.0
3'	7.42 (t,8)	129.6	7.30-7.50 (m)	128.1
4'	7.43 (dd,8,2)	129.1	7.30-7.50 (m)	128.6
5'	7.42 (t,8)	129.6	7.30-7.50 (m)	128.1
6'	7.59 (dd,8,2)	128.8	7.52 (d,6.1)	128.0

1.31 Structure Determination of Compound DP15

Compound DP15, $[\alpha]_D^{25}$ – 7.9°, a colorless amorphous powder, gave $[M+H]^+$ ion peak at *m/z* 333 in FABMS suggesting the molecular formula of $C_{17}H_{17}O_7$.

The ^1H NMR spectrum of compound DP15 in acetone-d_6 revealed characteristic three spin system for isoflavanone at δ 4.43 (1H, dd, *J* =11,6 Hz, H-2), 4.53 (1H, t, *J* = 11 Hz, H-2) and 4.25 (1H, dd, *J*=11,6 Hz, H-3). The *meta*-coupled proton signals at *δ* 5.97 (H-6) and 5.96 (H-8) and *ortho*-coupled protons at *δ* 6.65 (d, *J* = 8 Hz, H-5′) and 6.69 (d, *J* = 8 Hz, H-6′) indicated the presence of 5,7-dioxygenated A-ring and 2′,3′,4′-trisubstituted B-ring. In the difference NOE spectrum of DP15, NOEs were observed at *δ* H-3 (*δ* 4.25) by irradiation at the *δ* 3.82 (2′-OC<u>H</u>$_3$), and at H-5′ (*δ* 6.65) by irradiation at the *δ* 3.83 (4′-OC<u>H</u>$_3$). Its ^1H-and ^{13}C-NMR data are in good agreement with published data (Tanaka *et al.*, 1998).

The absolute configuration at C-3 of DP15 was then determined as 3(*S*) by a negative Cotton effect ($[\theta]_{283}$ -8900) in the CD spectrum. Based on the above spectroscopic data, this compound was identified as (3*S*)-(–)-5,7,3′-trihydroxy-2′,4′-dimethoxyisoflavone ((3*S*)-(–)-secundiflorol H) **[429]**. This compound was first isolated from *Sophora secundiflora*, *S. arizonica* and *S. gypsophila* (Tanaka *et al.*, 1998).



[429]

Table 36 NMR Spectral data of compound DP15 (in acetone-d_6) and secundiflorol H (in acetone-d_6)

Position	Compound DP15		Secundiflorol H	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2a	4.43 (dd,11,6)	71.6	4.43(dd,11,6)	71.4
2b	4.53 (t, 11)		4.53(dd,11,11)	
3	4.25 (dd,11,6)	48.1	4.25(dd,11,6)	48.1
4	-	198.3	-	198.2
5	-	165.8	-	165.7
6	5.97 (d, 2)	97.0	5.95(m)	96.9
7	-	165.1	-	169.1
8	5.96 (d, 2)	95.7	5.95(m)	95.6
9	-	164.6	-	164.5
10	-	103.5	-	103.4
1'	-	122.2	-	122.1
2'	-	146.9	-	146.8
3'	-	140.3	-	140.3
4'	-	149.4	-	149.4
5'	6.65(d,8)	107.6	6.60(d,8)	107.3
6'	6.69(d,8)	120.5	6.70(d,8)	120.4
2'-OMe	3.82(s)	60.2	3.83(s)	60.1
4'-OMe	3.83(s)	56.6	3.84(s)	56.5

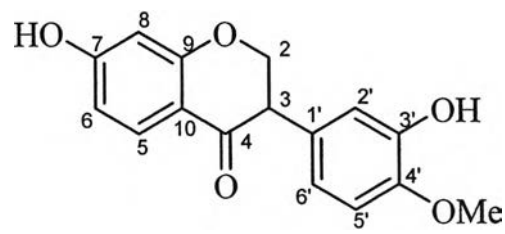
1.32 Structure Determination of Compound DP 16

Compound DP16, an amorphous powder, was analyzed for $C_{16}H_{15}O_5$ from its $[M+H]^+$ at m/z 287 in the FABMS (Figure 124).

From the ^1H NMR spectrum classified this compound as isoflavanone through the characteristic proton signals at δ 4.55 (2H, d, J = 5 Hz, H-2), 3.80 (1H, t, J = 5 Hz, H-3) with two set of ABX spin systems such as at δ 7.65 (1H, d, J = 9 Hz, H-5), 6.50 (1H, dd, J = 9,2 Hz, H-6), 6.32 (1H, d, J = 2 Hz, H-8) assignable to A-ring and at δ 6.67 (1H, d, J = 2 Hz, H-2′), 6.84 (1H, d, J = 9 Hz, H-5′), 6.64 (1H, dd, J = 9,2 Hz, H-6′) assignable to B-ring. In the difference NOE spectrum of DP3, NOEs were observed at δH-5′ (δ 6.84) by irradiation at the δ 3.73 (4′-OC<u>H</u>$_3$).

Three prominent fragment ions were observed at *m/z* 137, 150 and 135 in FABMS caused by *retro*-Diels-Alder fragmentation and demethylation, also indicating that one of the hydroxyl groups was located in the A-ring and another hydroxyl, one methoxyl group was in the B-ring.

By comparing the above spectroscopic evidence with reported data, compound DP16 was thus identified as (±)-7,3′-dihydroxy-4′-methoxyisoflavanone **[430]**. It was first reported from *Myroxylon balsamum* (De Oliveira *et al.*, 1978).



[430]

Table 37 NMR Spectral data of compound DP16 (in DMSO-d_6) and 7,3'-dihydroxy-4'-methoxyisoflavanone (in acetone-d_6)

Position	Compound DP16		7,3'-Dihydroxy-4'-methoxyisoflavanone
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
2a	4.55(d,5)	71.2	4.63(d,6)
2b	4.55(d,5)		4.63(d,6)
3	3.80(t,5)	50.1	3.76(t,6)
4	-	190.2	-
5	7.65(d,9)	128.7	7.75(d,8)
6	6.50(dd,9,2)	110.8	6.58(dd,8,2)
7	-	164.8	-
8	6.32(d,2)	102.3	6.38(d,2)
9	-	163.0	-
10	-	113.4	-
1'	-	129.0	-
2'	6.67(d,2)	115.8	6.98-6.63(m)
3'	-	146.4	-
4'	-	146.9	-
5'	6.84(d,9)	112.4	6.98-6.63(m)
6'	6.64(d,9,2)	119.1	6.98-6.63(m)
4'-OMe	3.73(s)	55.7	3.80(s)

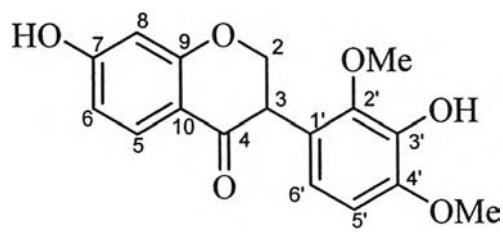
1.33 Structure Determination of Compound DP17

Compound DP17 was obtained as an amorphous powder. The molecular formula of DP17, $C_{17}H_{17}O_6$ was determined by FABMS which exhibited $[M+H]^+$ at *m/z* 317 (Figure 128).

The ^1H and ^{13}C NMR spectra of DP17 (Table 38 and Figure 129-131) resembled to those of DP15 (5,7,3′-trihydroxy-2′,4′-dimethoxyisoflavanone) and showed characteristic signals for isoflavanone. And the doublet signal at δ 7.68 (1H, d, J = 9 Hz) assignable to H-5 in the ^1H NMR indicated an ABX coupling system in A-ring. In the difference NOE spectrum of DP17, NOEs were observed at δ H-3 [δ 4.09(1H, dd, J = 11,6 Hz)] by irradiation at the δ 3.60 (2′-OC<u>H</u>$_3$), and at H-5′ [δ 6.65 (1H,d, J = 9 Hz)] by irradiation at the δ 3.76 (4′-OC<u>H</u>$_3$).

Moreover, the fragmentation ion at *m/z* 137 and 180 resulting from *retro*-Diels-Alder cleavage of ring C in the FABMS suggested the position of two methoxyls on ring B. All protons and carbons assignments were confirmed by ^1H-^1H COSY, HMQC (Figure 130-133) and HMBC experiments (Figure 134).

Hence, compound DP17 was identified as (±)-7,3′-dihydroxy-2′,4′-dimethoxyisoflavone ((±)-violanone) **[177]** from comparing the above spectral data with published data (Donnelly *et al.*, 1974). This compound has been separated from *Dalbergia oliveri*.



[177]

Table 38 Spectral data of compound DP17 (in DMSO-d_6) and violanone (in C_6D_6)

Position	Compound DP17		Violanone	HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	(correlation with ^{1}H)
2	4.50(t,11)	70.8	4.18-4.75(m)	H-3*
	4.43(dd,11,6)		4.18-4.75(m)	
3	4.09(dd,11,6)	47.3	4.18-4.75(m)	H-6', H-2*
4	-	190.5	-	-
5	7.68(d,9)	128.9	8.05(d,9)	-
6	6.52(dd,9,2)	110.6	-	H-8
7	-	164.4	-	H-5, H-6*
8	6.34(d,2)	102.4	-	H-5, H-6
9	-	163.3	-	H-2, H-5
10	-	114.0	-	H-6, H-8
1'	-	121.8	-	H-2, H-5', H-3*
2'	-	146.3	-	H-3, H-6', 2'-OMe
3'	-	139.2	-	H-5', H-6'
4'	-	148.5	-	H-6', 4'-OMe, H-5'*
5'	6.65(d ,9)	107.2	6.74(d ,9)	-
6'	6.53(d,9)	119.1	7.03(d ,9)	H-5*
2'-OMe	3.60(s)	59.5	3.80(s)	
4'-OMe	3.76(s)	56.0	3.82(s)	

* Across two bonds correlations

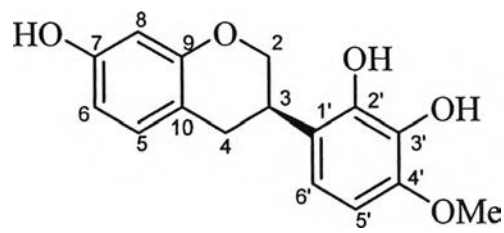
1.34 Structure Determination of Compound DP18

Compound DP18, $[\alpha]_D^{25}$ + 4.1°, a colorless solid, gave $[M+H]^+$ ion peak at *m/z* 289 in the FABMS (Figure 135), corresponding to $C_{16}H_{17}O_5$. ^1H and ^{13}C NMR spectra of DP18 showed a very similar signal pattern to those of DP1 and suggested the structure as isoflavan.

In the difference NOE spectrum, NOE was observed between the methoxyl protons (δ 3.80) and H-5′ (δ 6.47). All protons and carbons were assigned as shown in Table 39. Moreover, the fragment ions in the mass spectrum of DP4 supported monohydroxylated ring A (*m/z* 123) and dihydroxy-monomethoxylated ring B (*m/z* 165).

The absolute configuration at C-3 of DP18 was determined as 3(*R*) by a negative Cotton effect ($[\theta]_{232}$ -8600) and a positive Cotton effect ($[\theta]_{280}$ 1900) in the CD spectrum.

By comparing the spectral data with the reported data, compound DP18 was determined to be (3*R*)-(+)-7,2′,3′-trihydroxy-4′-methoxyisoflavan, known as (3*R*)-(+)-arizonicanol A **[431]**. It has been first isolated from *Sophora secundiflora*, *S. arizonica* and *S. gypsophila* (Tanaka *et al.*, 1998).



[431]

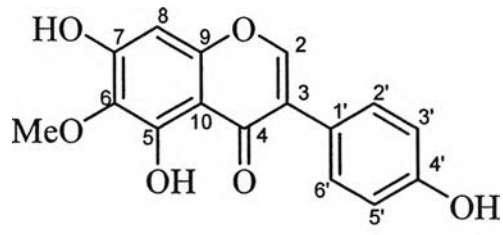
Table 39 NMR Spectral data of compound DP18 (in acetone-d_6) and arizonicanol A (in acetone-d_6)

Position	Compound DP18		Arizonicanol A	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2a	3.97 (t,10)	70.5	3.98 (dd,11,11)	70.4
2b	4.25 (dd,10,2)		4.26 (br d,11)	
3	3.50 (m)	33.1	3.50 (m)	33.0
4a	2.88 (m)	31.1	2.80 (br dd,14,3)	31.0
4b	2.88 (m)		2.97 (dd,14,11)	
5	6.88 (d,9)	131.0	6.89 (d,8)	131.0
6	6.35 (dd,9,2)	108.8	6.36 (dd,8,2)	108.7
7	-	157.6	-	157.5
8	6.28 (d,2)	103.7	6.28 (d,2)	103.7
9	-	156.1	-	156.1
10	-	114.3	-	114.3
1'	-	121.9	-	121.8
2'	-	144.4	-	144.3
3'	-	134.4	-	134.2
4'	-	147.8	-	147.7
5'	6.47 (d,9)	104.0	6.47 (d,8)	103.6
6'	6.62 (d,9)	117.8	6.62 (br d,8)	117.8
4'-OMe	3.80 (s)	56.4	3.80 (s)	56.3

1.35 Structure Determination of Compound DP19

Compound DP19 was obtained as an amorphous powder. Its molecular formula of $C_{16}H_{13}O_6$ was established by FABMS spectrum which revealed the $[M+H]^+$ peak at *m/z* 301 as shown in Figure 138.

The ^1H and ^{13}C NMR spectra in acetone-d_6 (Figure 139 and 140) were identical to BC1 in this study. Thus, compound DP19 was identified as tectorigenin **[14]**. All protons and carbons were assigned and compared with recorded data (Oh *et al.*, 2001)



[14]

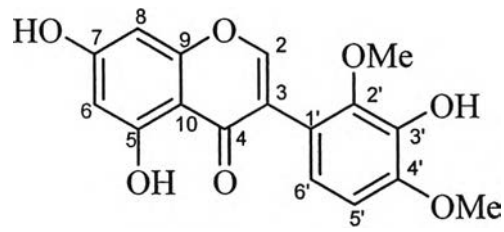
Table 40 NMR Spectral data of compound DP19 (in acetone-d_6) and tectorigenin (in acetone-d_6)

Position	Compound DP19		Tectorigenin	
	^1H(*mult.*, *J* in Hz)	^{13}C	^1H(*mult.*, *J* in Hz)	^{13}C
2	8.14 (s)	154.5	8.18 (s)	154.5
3	-	123.5	-	123.5
4	-	182.1	-	182.1
5	-	154.3	-	154.3
6	-	132.2	-	132.2
7	-	157.8	-	157.8
8	6.64 (s)	94.4	6.50 (s)	94.4
9	-	154.4		154.4
10	-	106.6	-	106.5
1'	-	123.1	-	123.0
2'	7.44 (d,8)	131.2	7.46 (d,8.5)	131.2
3'	6.89 (d,8)	116.0	6.91 (d,8.5)	116.0
4'	-	158.4	-	158.4
5'	6.89 (d,8)	116.0	6.91 (d,8.5)	116.0
6'	7.44 (d,8)	131.2	7.46 (d,8.5)	131.2
6-OMe	3.87 (s)	60.7	3.88 (s)	60.7
5-OH	13.2 (s)	-	13.20 (s)	-

1.36 Structure Determination of Compound DP20

Compound DP20 was obtained as an amorphous powder. The molecular ion $[M]^+$ peak appeared at *m/z* 330.0746 in the HRFABMS, corresponding a molecular formula of $C_{17}H_{14}O_7$ (calcd 330.0740). The UV spectrum showed the maximum absorption bands at 330, 292 and 259 (sh) nm.

The ^1H NMR spectral data of DP20 in acetone- d_6 (Table 41) showed a singlet signals at δ 7.98 (1H, s) and 12.95 (1H, s) indicating the compound as 5-hydroxy isoflavone. The *meta*-coupled proton signals at δ 6.28 (H-6) and 6.40 (H-8) suggested the presence of 5,7-dioxygenated A-ring, and the fragment ion at *m/z* 153 in the FABMS spectrum supported the partial structure. In addition, the position of one hydroxyl (3′-OH) and two methoxyls (2′,4′-OCH_3) were confirmed by the HMQC and the HMBC technique as shown in Figure 145 and 146. All data are consistent with the structure, and DP20 was thus assigned as a new compound, 5,7,3′-trihydroxy-2′,4′-dimethoxyisoflavone and was given trivial name khrinone C **[432]**.



[432]

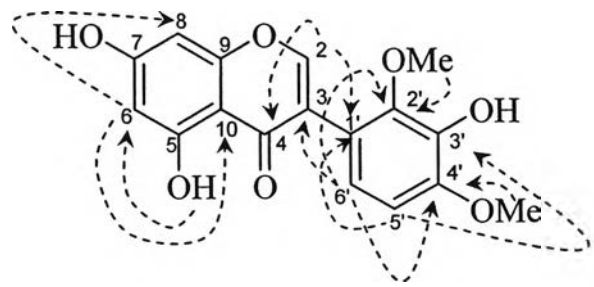


Figure 9 HMBC correlation of compound DP20

Table 41 NMR Spectral data of compound DP20 (in acetone-d_6)

Position	Compound DP20		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	7.98 (s)	155.3	-
3	-	118.6[a]	H-6', H-2*
4	-	181.6	H-2
5	-	163.8	5-OH* , H-6*
6	6.28 (d,2)	99.9	H-8 , 5-OH
7	-	165.0	-
8	6.40 (d,2)	94.6	H-6 ,H-8*
9	-	159.1	H-2 ,H-8*
10	-	106.1	H-6 ,H-8 , 5-OH
1'	-	122.3[a]	H-2 ,H-5'
2'	-	147.2	H-6', 2'-OMe
3'	-	140.3	H-5'
4'	-	150.0	H-6', 4'-OMe
5'	6.76 (s)	107.4	-
6'	6.76 (s)	121.9	-
2'-OMe	3.77(s)	60.4	-
4'-OMe	3.87(s)	56.6	-
5-OH	12.97(s)	-	-

* Across two bonds correlations

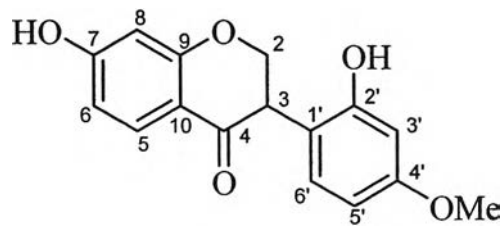
[a] interchangeable within column

1.37 Structure Determination of Compound DP21

Compound DP21 was obtained as a colorless amorphous powder. Its FABMS presented a molecular ion $[M+H]^+$ at *m/z* 287, corresponding to the molecular formula $C_{16}H_{15}O_5$. The ^1H NMR spectrum of compound DP21 in acetone-d_6 (Table 42 and Figure 148) revealed characteristic three spin system for isoflavanone at δ 4.53 (1H, dd, J =11, 5 Hz, H-2a), 4.67 (1H, t, J = 11, 5 Hz, H-2b) and 4.14 (1H, dd, J = 5 Hz, H-3). And two set of the ABX coupled protons were observed such as δ 6.40 (1H, d, J = 2 Hz, H-8), 6.57 (1H, dd, J = 9, 2 Hz, H-6) and 7.77 (d, J = 9 Hz, H-5) assignable to A-ring and δ 6.48 (1H, d, J = 2 Hz, H-3′), 6.39 (1H, dd, J = 9,2 Hz, H-5′) and 7.20 (1H, d, J = 9 Hz, H-6′) assignable to B-ring.

In the difference NOE spectrum of DP21, NOEs were observed at H-3′ [δ 6.48 (1H, d, J = 2 Hz)] and H-5′ [δ 6.39 (1H, dd, J = 9,2 Hz)] by irradiation at the δ 3.72 (4′-OC<u>H</u>$_3$). Prominent FABMS fragment ions at *m/z* 136 and 151 derived from A-ring with monohydroxyl group and B-ring with hydroxyl, methoxyl groups, respectively, supported the structure.

The structure of DP21 were proposed to be (±)-7,2′-dihydroxy-4′-methoxyisoflavanone, trivially known as vestitone **[176]**, based on the above spectral data and reported data (Macias *et al.*, 1999). This compound was found in several species of *Melilotus* (Macias *et al.*, 1999).



[176]

Table 42 Spectral data of compound DP21 (in acetone-d_6) and vestitone (in $CDCl_3$)

Position	Compound DP21		Vestitone
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)
2a	4.53 (dd,11,5)	71.6	4.79 (dd,12,5)
2b	4.67 (dd,11,5)		4.94 (dd,12,3)
3	4.14 (t,5)	47.7	3.89 (dd,5,3)
4	-	191.6	-
5	7.77 (d,9)	130.1	7.83 (d,9)
6	6.57 (dd,9,2)	111.3	6.50 (dd,9,3)
7	-	165.2	-
8	6.40 (d,2)	103.5	6.41 (d,2)
9	-	164.7	-
10	-	115.6	-
1'	-	115.9	-
2'	-	157.2	-
3'	6.48 (d,2)	102.8	6.53 (d,3)
4'	-	161.2	-
5'	6.39 (dd,9,2)	106.0	6.46 (dd,8,3)
6'	7.20 (d,9)	131.2	7.40 (d,9)
4'-OMe	3.72 (s)	55.4	3.74 (s)

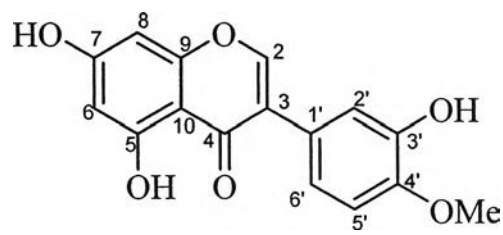
1.38 Structure Determination of Compound DP22

Compound DP22 was obtained as an amorphous powder, having the molecular formula of $C_{16}H_{13}O_6$ which was analyzed from FABMS. It showed the molecular ion $[M+H]^+$ peak at 301 (Figure 150).

The ^1H NMR spectrum of DP22 in DMSO-d_6 (Table 43 and Figure 151) showed characteristic pattern of 5-hydroxy isoflavone with a singlet proton signals at δ 8.29 (H-2) and 12.9 (5-OH). Besides of the *meta*-coupled proton signals at δ 6.21(H-6) and 6.37 (H-8), ABX pattern signals at δ 7.07 (1H, d, J = 2 Hz, H-2′), 6.96 (1H, d, J = 8 Hz, H-5′) and 6.93 (1H, dd, J = 8, 2 Hz, H-6′) were observed.

From the difference NOE spectrum (Figure 152), the methoxyl protons at δ 3.79 (3H, s) showed NOE enhancement with H-5′, indicating the 4′-OCH_3. Moreover, the mass fragment ion at *m/z* 149 suggested the presence of both a hydroxyl and a methoxyl groups in B-ring as the partial structure.

Comparing the above spectral data with those reported (Matsuura *et al.*, 1983 and Anhut *et al.*, 1984), DP22 was identified as 5,7,3′-trihydroxy-4′-methoxyisoflavone (pratensein) **[433]**. This compound has been found in several plants such as *Arachis hypogaea* (Matsuura *et al.*, 1983), *Cicer arietinum* and *C. mongoltavicum* (Jaques *et al.*, 1984).



[433]

Table 43 Spectral data of compound DP22 (in DMSO-d_6) and pratensein (in DMSO-d_6)

Position	Compound DP22		Pratensein	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.29 (s)	154.1	8.30 (s)	154.7
3	-	122.1[a]	-	123.1
4	-	180.1	-	180.4
5	-	162.0	-	162.9
6	6.21 (d,2)	98.9	6.23 (d,2)	99.6
7	-	164.2	-	161.6
8	6.37 (d,2)	93.6	6.39 (d,2)	94.5
9	-	157.5	-	157.1
10	-	104.4	-	106.1
1'	-	123.3[a]	-	122.4
2'	7.07 (d,2)	116.4	7.00 (m)	116.3
3'	-	146.1	-	146.1
4'	-	147.7	-	147.8
5'	6.96 (d,8)	112.0	7.00 (m)	112.0
6'	6.93 (dd,8,2)	119.7	7.00 (m)	119.8
4'-OMe	3.79 (s)	55.6	3.80 (s)	55.6
5-OH	12.9 (s)	-	-	-

[a] interchangeable within column

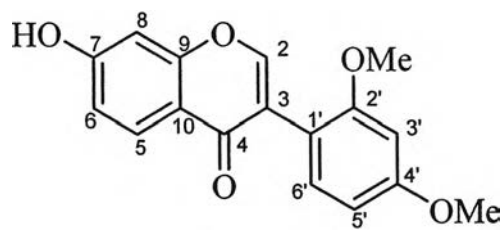
1.39 Structure Determination of Compound DP23

Compound DP23 was obtained as an amorphous powder. The FABMS showed its $[M+H]^+$ peak at 299 (Figure 154) suggesting the molecular formula of $C_{17}H_{15}O_5$.

From the ^1H NMR spectrum in acetone-d_6 (Table 44 and Figure 155) classified this compound as isoflavone through the characteristic proton signals at δ 7.98 (1H, s, H-2) with two set of ABX spin systems such as δ 8.01 (1H, d, J = 9 Hz, H-5), 6.96 (1H, dd, J = 9,2 Hz, H-6) and 6.88 (1H, d, J = 2 Hz, H-8) assignable to A-ring and δ 6.61 (1H, d, J = 2 Hz, H-3′), 6.55 (1H, dd, J = 9,2 Hz, H-5′) and 7.20 (1H, d, J = 9 Hz, H-6′) assignable to B-ring.

In the difference NOE spectrum of DP23, NOEs were observed at H-3′ (δ 6.61), H-5′ (δ 6.55) by irradiation at the δ 3.83 (4′-OC<u>H</u>$_3$), and at H-3′ (δ 6.61) by irradiation at the δ 3.75 (2′-OC<u>H</u>$_3$).

Comparing the above spectral data with the published (Jain *et al.*, 1996) DP23 was identified as 7-hydroxy-2′,4′-dimethoxyisoflavone (2′-methoxy formononetin) **[434]**. This compound was first isolated from *Eschscholtzia californica* (Jain *et al.*, 1996).



[434]

Table 44 NMR Spectral data of compound DP23 (in acetone-d_6) and 2'-methoxyformononetin (in DMSO-d_6)

Position	Compound DP23		2'- Methoxyformononetin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	7.98 (s)	154.6	8.08 (s)	153.5
3	-	123.0	-	121.3
4	-	175.4	-	174.1
5	8.01 (d,9)	128.4	7.92 (d,8.8)	126.9
6	6.96 (dd,9,2)	115.5	6.92 (dd,8.8,2.2)	114.8
7	-	163.1	-	162.1
8	6.88 (d,2)	103.3	6.85 (d,2.2)	101.9
9	-	158.9	-	157.2
10	-	118.7	-	116.4
1'	-	114.8	-	113.3
2'	-	159.7	-	158.1
3'	6.61 (d,2)	99.5	6.62 (d,2.2)	98.5
4'	-	162.1	-	160.4
5'	6.55 (dd,9,2)	105.5	6.55 (dd,8.8,2.2)	104.4
6'	7.20 (d,9)	133.2	7.13 (d,8.8)	131.8
2'-OMe	3.75 (s)[a]	55.7[a]	3.69 (s)	55.1
4'-OMe	3.83 (s)[a]	56.0[a]	3.78 (s)	55.4

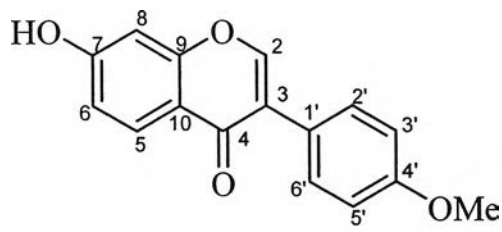
[a] interchangeable within column

1.40 Structure Determination of Compound DP24

Compound DP24, an amorphous powder, gave the molecular formula $C_{16}H_{13}O_4$ from $[M+H]^+$ peak at *m/z* 269 in the FABMS.

The signals at δ_H 8.13 assignable to H-2 and δ_C 153.4 (C-2) in the ^1H and ^{13}C-NMR spectra, respectively, were suggestive of an isoflavone type skeleton (Table 45 and Figure 158). The ^1H-NMR further revealed the presence of a methoxyl group at δ 3.81 and *ortho* coupled AA'BB'-type protons at δ 7.54 and 6.96 (each 2H, d, J = 9 Hz) assignable to H-2',6' and H-3',5' respectively. An NOE experiment (Figure 159) revealed positive enhancement of H-3' and H-5' with δ 3.81 (4'-OCH_3).

Compound DP24 was identified as 7-hydroxy-4'-methoxyisoflavone (formononetin) **[72]** by comparison of the NMR data with those reported (Jun *et al.*, 2003). Formononetin was found in many plants of the family Leguminosae (Harbone, 1993).



[72]

Table 45 NMR Spectral data of compound DP24 (in acetone-d_6) and formononetin (in DMSO-d_6)

Position	Compound DP24		Formononetin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.13 (s)	153.4	8.21 (s)	153.3
3	-	125.0	-	124.4
4	-	175.6	-	174.8
5	8.04 (d,9)	128.5	7.98 (d,8)	127.5
6	6.97 (dd,9,2)	115.7	6.93 (d,8)	115.4
7	-	163.2	-	162.8
8	6.88 (d,2)	103.2	6.87 (d,2)	102.3
9	-	160.5	-	157.6
10	-	118.7	-	116.8
1'	-	127.4	-	123.3
2'	7.54 (d,9)	131.0	7.51 (d,9)	130.2
3'	6.96 (d,9)	114.4	6.97 (d,9)	113.8
4'	-	158.9	-	159.1
5'	6.96 (d,9)	114.4	-	113.8
6'	7.54 (d,9)	131.0	-	130.2
4'-OMe	3.81 (s)	55.6	3.80 (s)	55.3

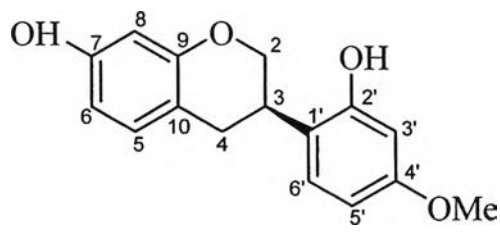
1.41 Structure Determination of Compound DP25

Compound DP25, $[\alpha]_D^{25}$ −11.2°, was obtained as colorless amorphous powder. The FABMS showed a molecular ion $[M]^+$ peak at *m/z* 272, giving the molecular formula $C_{16}H_{16}O_4$.

The ^1H NMR spectrum in acetone-d_6 of compound DP25 (Table 46 and Figure 156) was recognized as isoflavan by the characteristic complex proton signals, assignable to the CH_2-CH-CH_2 coupling system of the 3-arylchroman at δ 3.97 (1H, t, J = 10 Hz, H-2a), 4.23 (1H, ddd, J = 10,4,2 Hz, H-2b), 3.48 (1H, m, H-3), 2.79 (1H, ddd, J = 16,5,2 Hz, H-4a) and 2.95 (1H, dd, J = 16,11 Hz,H-4b). And two set of ABX spin systems were observed such as δ 6.87 (1H, d, J = 8 Hz, H-5), 6.35 (1H, dd, J = 8,2 Hz, H-6) and 6.28 (1H, d, J = 2 Hz, H-8) assignable to A-ring and δ 6.50 (1H, d, J = 2 Hz, H-3′), 6.40 (1H, dd, J = 9,2 Hz, H-5′) and 7.02 (1H, d, J = 9 Hz, H-6′) assignable to B-ring.

In the difference NOE spectrum of DP25, NOEs were observed at H-3′ (δ 6.50) and H-5′ (δ 6.40) by irradiation at the δ 3.71 (4′-OCH_3). The absolute configuration at C-3 of DP25 was then determined as 3(*R*) by a negative Cotton effect ($[\theta]_{233}$ -9700) and a positive Cotton effect ($[\theta]_{288}$ 3700) in the CD spectrum.

Based on the above data, compound DP25 was identified as (3*R*)-(−)-7, 2′-dihydroxy-4′-methoxyisoflavan ((3*R*)-(−)-vestitol) **[78]**. The ^1H and ^{13}C NMR spectra were in good agreement with reported values (Yahara *et al.*, 1989) as shown in Table 46. This compound has been isolated from several plants in genus *Cyclolobium*, *Dalbergia* and *Mechaerium* (Kurosawa *et al.*, 1978).



[78]

Table 46 NMR Spectral data of compound DP25 (in acetone-d_6) and vestitol (^{1}H NMR in acetone-d_6 and ^{13}C NMR in DMSO-d_6)

Position	Compound DP25		vestitol	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2a	3.97 (t,10)	70.5	3.79 (t,10)	70.3
2b	4.23 (ddd,10,4,2)		4.27 (dd,4,10)	
3	3.48 (m)	32.7	3.27 (m)	32.4
4a	2.79 (ddd,16,5,2)	31.1	2.87 (m)	30.8
4b	2.95 (dd,16,11)		2.87 (m)	
5	6.87 (d,8)	131.0	6.90 (d,8)	130.8
6	6.35 (dd,8,2)	108.8	6.40 (dd,2,8)	108.6
7	-	156.1	-	155.8
8	6.28 (d,2)	103.7	6.37 (d,2)	103.5
9	-	156.8	-	156.4
10	-	114.3	-	114.2
1'	-	121.0	-	120.7
2'	-	157.5	-	157.1
3'	6.50 (d,2)	102.6	6.48 (d,2)	102.4
4'	-	160.4	-	160.1
5'	6.40 (dd,9,2)	105.7	6.41 (dd,2,8)	105.5
6'	7.02 (d,9)	128.7	6.91 (d,8)	128.5
4'-OMe	3.71 (s)	55.4	3.62 (s)	55.2

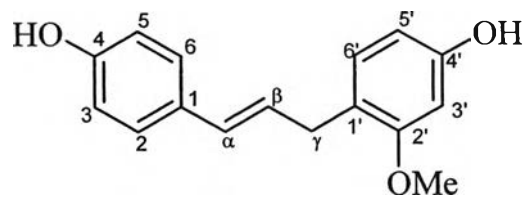
1.42 Structure Determination of Compound DP26

Compound DP26 was obtain as an amorphous powder, showing $[M]^+$ peak at *m/z* 256 in the FABMS (Figure 163), indicating the molecular formula of $C_{16}H_{16}O_3$.

In the ^1H NMR spectrum in acetone-d_6 of compound DP26 (Figure 164 and Table 47), two aromatic ring systems were recognized from 1,4-substituted aromatic proton signals at δ 6.75 (2H, d, J = 9 Hz, H-3,5), 7.20 (2H, d, J = 9 Hz, H-2, 6) and 1,2,4-trisubstituted aromatic proton signals at δ 6.45 (1H, d, J = 2 Hz, H-2′), 6.36 (1H, dd, J = 9,2 Hz, H-5′), 6.94 (1H, d, J = 9 Hz, H-6′). And one methylene group at δ 3.35 (2H, d, J = 6 Hz, H-γ) adjacent to trans olefinic group [δ 6.32 (1H, d, J = 16 Hz, H-α), 6.16 (1H, dt, J = 16,6 Hz, H-β)] was observed. The 2D NMR techniques such as the ^1H-^1H COSY, HMQC and HMBC (Figures 167-169) were performed to assign all protons and carbons, and the results are shown in Figure 10.

In the difference NOE spectrum, NOE was observed at δ H-3′ (δ 6.45) by irradiation at the δ 3.78 (2′-OC<u>H</u>$_3$). Moreover, the mass spectral fragmentation pattern of DP26 supported the structure from the signals at *m/z* 133, 123, 108 and 91 resulted from α-cleavage at C-γ and C-1′ and subsequently eliminated methyl and hydroxyl groups, respectively.

Based on the above spectral evidence and comparison of the spectral data of compound DP26 with those previously reported (Carlson *et al.*, 1982 and El-Feraly *et al.*, 1982), together with the information from the HMBC, HMQC and ^1H-^1H-COSY experiment (Figures 162-165), DP26 was identified as [*E*-1-(4-hydroxy-2-methoxybenzyl)-2-(4-hydroxyphenyl) ethylene] (xenognosin) **[435]**. This compound was isolated from gum tragacanth (El-feraly *et al.*, 1982) and *Pisum sativum* (Carlson *et al.*, 1982).



[435]

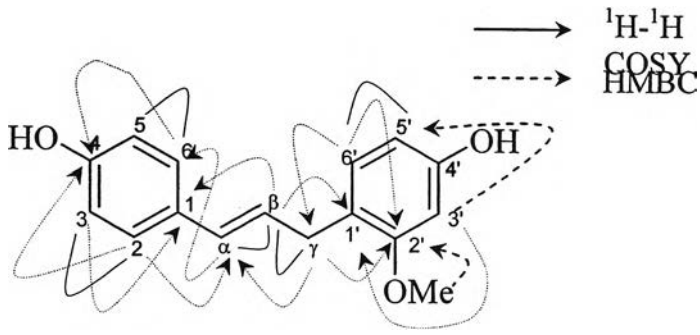


Figure 10 ^{1}H-^{1}H COSY and HMBC correlations of compound DP26

Table 47 NMR Spectral data of compound DP26 (in acetone-d_6) and xenognosin (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4)

Position	Compound DP26		Xenognosin		HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	(correlation with ^{1}H)
1	-	130.5	-	131.0	H-3, H-5, H-β
2	7.20 (d,9)	128.0	7.22 (d,9)	128.0	H-α
3	6.75 (d,9)	116.2	6.76 (d,9)	116.1	-
4	-	157.5	-	156.9	H-2, H-6, H-3*, H-5*
5	6.75 (d,9)	116.2	6.76 (d,9)	116.1	-
6	7.20(d,9)	128.0	7.22(d,9)	128.0	H-α
α	6.32(d,16)	130.6	-	130.7	H-2, H-6, H-γ, H-β*
β	6.16(dt,16,6)	127.3	-	127.5	H-α*, H-γ*
γ	3.35(d,6)	33.3	-	22.3	H-6', H-α, H-β*
1'	-	120.4	-	121.1	H-3', H-5', H-β, H-γ*
2'	-	159.1	-	159.3	H-6', 2'-OMe, H-γ
3'	6.45(d,2)	99.9	6.38-6.50(m)	99.9	H-5'
4'	-	158.0	-	157.4	H-3'*
5'	6.36(dd,9,2)	107.7	6.38-6.50(m)	107.7	H-3'
6'	6.94(d,9)	130.8	6.97(d,8)	131.0	H-β, H-γ
2'-OMe	3.78(s)	55.7	-	55.7	-

* Across two bonds correlations

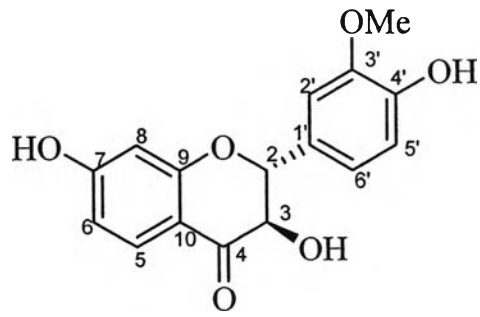
1.43 Structure Determination of Compound DP27

Compound DP27, $[\alpha]_D^{25}$ -5.0°, an amorphous powder, gave molecular ion $[M+H]^+$at *m/z* 303.0846 in the HRFAB mass spectrum, corresponding to the molecular formula $C_{16}H_{15}O_6$ (calcd for 303.0824). The UV maximal absorptions at 311, 277, 233 and 215 (sh) were observed.

The ^1H NMR spectrum in MeOH-d_4 of compound DP27 (Table 48) showed the 2,3-dihydroflavonol skeleton from the characteristic AB-coupled protons at δ 4.59 and 5.03 (d, J = 12 Hz, 1H each) as observed in DP14. Besides of a methoxyl proton signal at δ 3.88 (3H, s), two set of ABX spin systems were observed such as δ 7.72 (1H, d, J = 9 Hz, H-5), 6.62 (1H, dd, J = 9,2 Hz, H-6), 6.40 (1H, d, J = 2 Hz, H-8) assignable to A-ring and δ 7.22 (1H, d, J = 2 Hz, H-2′), 7.04 (1H, dd, J = 9,2 Hz, H-5′), 6.87 (1H, d, J = 2 Hz, H-6′) assignable to B-ring. In the difference NOE spectrum, NOE was observed at δ 7.22 (H-2′) by irradiation at the δ 3.88 (2′-OCH_3).

The absolute configuration at C-2 of DP27 was determined as 2(*R*) by a negative Cotton effect ($[\theta]_{270}$ -4760) and a positive Cotton effect ($[\theta]_{239}$ 10500) in the circular CD spectrum.

On the basis of spectroscopic studies, compound DP27 was thus confirmed as a new compound, (2*R*,3*R*)-(−)-3,7,4′-trihydroxy-3′-methoxydihydro flavonol and was given the trivial name (2*R*,3*R*)-(−)-dalparvinol A **[436]**.



[436]

Table 48 NMR Spectral data of compound DP27 (in MeOH-d_4)

Position	Compound DP27	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	4.59 (d,12)	85.2
3	5.03 (d,12)	74.0
4	-	193.2
5	7.72 (d,9)	129.9
6	6.62 (dd,9,2)	111.8
7	-	165.9
8	6.40 (d,2)	103.7
9	-	164.5
10	-	112.5
1'	-	129.8
2'	7.22 (d,2)	115.5
3'	-	148.1
4'	-	148.2
5'	7.04 (dd,9,2)	113.1
6'	6.87 (d,9)	122.2
3'-OMe	3.88 (s)	56.4

1.44 Structure Determination of Compound DP28

Compound DP28 was obtained as an amorphous powder, showed its $[M]^+$ peak at *m/z* 316.0609 in the HRFABMS (Figure 170), corresponding to the molecular formula $C_{16}H_{12}O_7$ (calcd 316.0583). The UV spectrum showed the absorption bands at 298 and 260 nm.

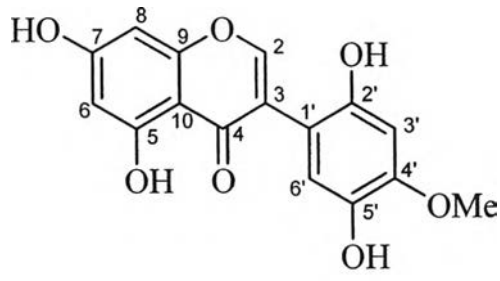
The ^1H NMR spectrum in acetone-d_6 of compound DP28 (Table 49 and Figure 171) displayed a characteristic singlet proton signal at δ 8.15 (1H, s) for the isoflavone skeleton with a chelated hydroxyl group appeared at δ 12.9. The ^1H NMR spectrum also exhibited a singlet proton signals (δ 6.49 and 6.64), a methoxyl group (δ 3.74) and *meta*-coupled aromatic protons [δ 6.21 (1H, d, J= 2 H_z, H-6) and 6.37 (1H, d, J= 2 Hz, H-8)].

In the difference NOE spectrum, NOE was observed at δ H-3′ (δ 6.49) by irradiation at the δ 3.74 ($OC\underline{H}_3$) suggesting the position at C-2′ or C-4′.

To assign all protons and carbons and confirm the position of this methoxyl group, the 2D NMR techniques such as the ^1H-^1H COSY, HMQC and HMBC were performed, and the results are shown in Figure 11.

In the HMBC, correlation from the hydroxyl proton signal at δ 8.70 (2′-OH) to carbon signals at δ 108.7 (C-1′), 101.1 (C-3′) and 148.2 (C-2′) were observed, and H-2 signal at δ 8.15 (1H, s) showed correlation with carbon signals at δ 155.6 (C-2), 119.9 (C-3), 180.3 (C-4) and 157.5 (C-9). From the above HMBC data, the hydroxyl group was concluded to be attached at C-2′ and the methoxyl position was supported to locate at C-4′ in B-ring.

By careful analysis of the obtained spectral data, compound DP28 was defined as a new isoflavone, 5,7,2′,5′-tetrahydroxy-4′-methoxyisoflavone and given the trivial name khrinone B **[437]**.



[437]

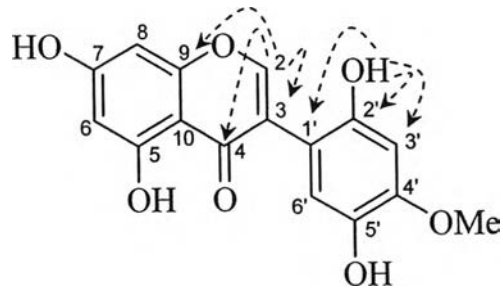


Figure 11 HMBC correlation of compound DP28

Table 49 NMR Spectral data of compound DP28 (in DMSO-d_6)

Position	Compound DP28		Compound DP28-acetate	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.15(s)	155.6	8.27(s)	154.6
3	-	119.9		121.7
4	-	180.3		172.9
5	-	161.8		150.1
6	6.21(d,2)	98.8	7.09(d,2)	114.8
7	-	164.1		154.2
8	6.37(d,2)	93.6	7.48(d,2)	109.8
9	-	157.5		157.4
10	-	104.5		114.9
1'	-	108.7		116.5
2'	-	148.2[a]		147.4
3'	6.49(s)	101.1	7.06(s)	108.4
4'	-	148.1[a]		151.5
5'	-	138.7		136.8
6'	6.64(s)	118.1	7.11(s)	125.1
4'-OMe	3.74(s)	55.5	3.79(s)	56.6
5-OH	12.9(s)	-	-	-
7-OH	10.8(s)	-	-	-
2'-OH	8.70(s)	-	-	-
$OCO\underline{C}H_3$	-	-	2.08(s)	20.5
			2.26(s)	20.8
			2.28(s)	21.0
			2.32(s)	21.1
$O\underline{C}OCH_3$				168.6
				168.6
				168.6
				169.0

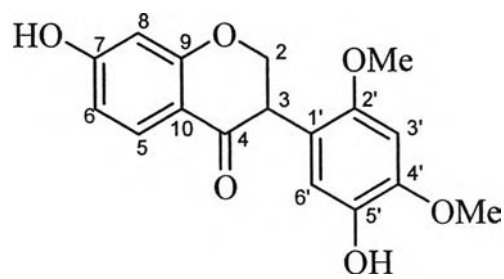
[a] interchangeable within column

1.45 Structure Determination of Compound DP29

Compound DP29, an amorphous powder, showed a molecular ion $[M]^+$peak at *m/z* 316.0947 in the HRFABMS corresponding to the molecular formula $C_{17}H_{16}O_6$ (calcd 316.0947). The UV spectrum displayed four absorption bands at 312, 278, 230 and 215 (sh) nm.

In the ^1H NMR spectrum in acetone-d_6 (Table 50 and Figure 174), characteristic three spin system for isoflavanone were observed at δ 4.54 (1H, t, J = 11 Hz, H-2), 4.44 (1H, dd, J = 11,5 Hz, H-2) and 4.13 (1H, dd, J = 11,5 Hz, H-3). Besides of two singlet signals [δ 6.58 and 6.68], three ABX-type protons [δ 7.76 (1H, d, J = 9 Hz, H-5), 6.57 (1H, dd, J = 8,2 Hz, H-6) and 6.40 (1H, d, J = 2 Hz, H-8)] assignable to A-ring were observed in addition to two methoxyl group signals [δ 3.73 and 3.75]. In the ^{13}C NMR spectrum (Table 50 and Figure 176), three oxygenated aromatic carbons appeared at δ 152.0, 148.1 and 141.3 suggested the presence of 2,4,5-trioxygenated phenyl group. In the difference NOE spectrum, NOEs were observed at H-3′ (δ 6.68) by irradiations at both of the methoxyl groups.

This compound was optically inactive. Thus, the structure of compound DP29 was concluded to be a new isoflavanone, (±)-7,5′-dihydroxy-2′,4′-dimethoxy isoflavanone and given the trivial name dalparvin **[438]**.



[438]

Table 50 NMR Spectral data of compound DP29 (in acetone-d_6)

Position	Compound DP29	
	^{1}H(*mult.*, *J* in Hz)	^{13}C
2a	4.54 (t,11)	71.9
2b	4.44 (dd,11,5)	
3	4.13 (dd,11,5)	47.9
4	-	191.1
5	7.76 (d,9)	130.0
6	6.57 (dd,9,2)	111.2
7	-	165.0
8	6.40 (d,2)	103.5
9	-	164.7
10	-	116.0
1'	-	117.5
2'	-	152.0
3'	6.68 (s)	99.4
4'	-	148.1
5'	-	141.3
6'	6.58 (s)	117.5
2'-OMe	3.73 (s)	57.0[a]
4'-OMe	3.75 (s)	56.6[a]

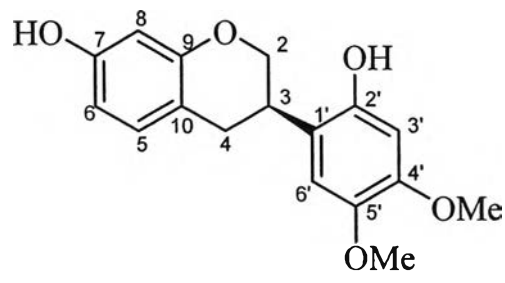
[a] interchangeable within column

1.46 Structure Determination of Compound DP30

Compound DP30, $[\alpha]_D^{25}$ – 10.0°, was obtained as an amorphous powder. It showed a molecular $[M]^+$ ion peak at *m/z* 302 in FABMS, suggesting a molecular formula of $C_{17}H_{18}O_5$.

In the ^1H NMR spectrum of compound DP30 in acetone-d_6 (Table 51), the characteristic signals assignable to H-2, 3 and 4 of the isoflavan skeleton appeared at δ 4.00 (1H, t, J = 10 Hz, H-2), 4.21 (1H, ddd, J = 10,4,2 Hz, H-2), 3.48 (1H, m, H-3) and 2.98 (2H, dd, J = 16,10 Hz, H-4). Furthermore, the ABX type signals at δ 6.89 (1H, d, J = 9 Hz, H-5), 6.35 (1H, dd, J = 9,2 Hz, H-6) and 6.27 (1H, d, J = 2 Hz, H-8) assignable to A-ring and two singlet signals at δ 6.57 and 6.77 assignable to B-ring were observed in addition to the signals due to two methoxyl groups at δ 3.70 and 3.73. By comparing its ^1H NMR data with those of DP25 (vestitol), compound DP30 was deduced to possess one additional methoxyl group linked to C-5′ of vestitol. Signals that originated from the A and C ring in the ^{13}C NMR spectrum of DP30 were coincident with those of vestitol, except that signal assignable to the C-5′ shifted 38.6 ppm to lower field, while signals due to the *ortho* (C-4′ and C-6′) and *para* (C-2′) carbons were shifted to higher field by 10.4, 14.4 and 6.5 ppm, supporting the above presumptive structure.

The configuration at C-3 of DP30 was estimated as 3(*R*) from a negative Cotton effect ($[\theta]_{237}$ –7500) in the CD spectrum. By analysis of the above spectroscopic data and comparison of its ^1H and ^{13}C NMR values with previously reported (Yahara *et al.*, 1989), DP30 was identified as (3*R*)-(–)-7,2′-dihydroxy-4′,5′-dimethoxyisoflavan (5′-methoxyvestitol) **[183]**.



[183]

Table 51 NMR Spectral data of compound DP30 (in acetone-d_6) and 5'- methoxyvestitol (^{1}H NMR in acetone-d_6 and ^{13}C NMR in DMSO-d_6)

Position	Compound DP30		5'- Methoxyvestitol	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2a	4.00 (t,10)	70.5	3.80-4.40 (m)	70.2
2b	4.21 (ddd,10,4,2)		3.80-4.40 (m)	
3	3.48 (m)	32.9	3.50 (m)	32.7
4a	2.98 (dd,16,10)	31.1	2.90 (m)	30.9
4b	2.98 (dd,16,10)		2.90 (m)	
5	6.89 (d,9)	131.0	6.88 (d,8)	130.8
6	6.35 (dd,9,2)	108.8	6.36 (dd,2,8)	108.7
7	-	157.6	-	157.2
8	6.27 (d,2)	103.7	6.30 (d,2)	103.6
9	-	156.1	-	155.9
10	-	116.0	-	114.1
1'	-	119.8	-	119.4
2'	-	151.0	-	149.9
3'	6.57 (s)	102.5	6.57 (s)	102.2
4'	-	150.0	-	149.6
5'	-	144.3	-	143.6
6'	6.77 (s)	114.3	6.76 (s)	113.9
4'-OMe	3.70 (s)[a]	56.2[a]	3.70 (s)[a]	56.0[a]
5'-OMe	3.73 (s)[a]	57.5[a]	3.73 (s)[a]	57.2[a]

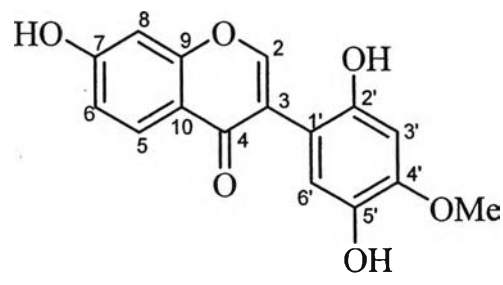
[a] interchangeable within column

1.47 Structure Determination of Compound DP31

Compound DP31 was obtained as an amorphous powder and showed molecular ion $[M]^+$ peak at *m/z* 300.0647 in the HRFABMS, giving the molecular formula $C_{16}H_{12}O_6$ (calcd 300.0634). The UV spectrum displayed three absorption bands at 300, 264 and 248 (sh) nm.

The ^1H NMR spectrum of compound DP31 in DMSO-d_6 (Figure 178 and Table 52) demonstrated this compound as 7-hydroxyisoflavone through the characteristic proton signals at δ 8.17 (1H, s, H-2) and 7.95 (1H, d, J = 8 Hz, H-5) characteristic for H-5 locating at *peri* position of carbonyl group (C-4), forming a set of ABX spin system with δ 6.94 (1H, dd, J = 8,2 Hz, H-6) and 6.86 (1H, d, J = 2 Hz, H-8). Furthermore, two singlet signals were observed at δ 6.49 and 6.66 assignable from the chemical shifts to H-3′ and H-6′, respectively, in B-ring. The proton signal observed in higher field (δ 6.49) cause by the *O*-functions in both *ortho* positions. In the difference NOE spectrum of DP31, NOEs were observed at H-3 (δ 6.49) by irradiation at the δ 3.74 (-OCH_3). The HMBC showed the similar correlations (Table 52) with DP28, that methoxyl group located at C-4′ and two hydroxyl groups located at C-2′ and C-5′ in B-ring.

Though the acetate of DP31, DP31a gave identical NMR data with the compound, 7,4′,5′-triacetyl-2′-methoxyisoflavone reported by M. Bekker, *et al.*, compound DP30 was identified as a new compound, 7,2′,5′-trihydroxy-4′-methoxyisoflavone based on the above spectroscopic evidence, and was given the trivial name khrinone A **[439]** since they concluded the position of methoxyl group only from its NOE spectrum.



[439]

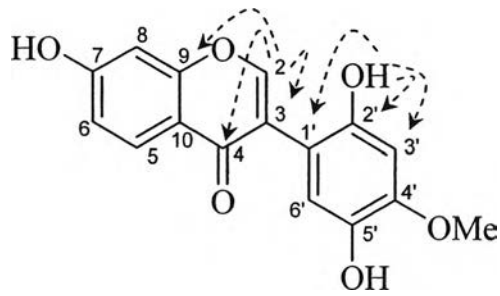


Figure 12 HMBC correlation compound DP31

Table 52 NMR Spectral data of compound DP31 (in DMSO-d_6)

Position	Compound DP31		Compound DP31-acetate		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	8.17 (s)	154.8	8.31 (s)	155.6	
3	-	121.4		120.7	H-2*
4	-	175.2		174.1	H-2
5	7.95 (d,8)	127.1	8.14 (d,8)	127.1	
6	6.94 (dd,8,2)	115.1	7.31 (dd,8,2)	120.5	
7	-	162.5		154.8	
8	6.86 (d,2)	102.0	7.55 (d,2)	112.7	
9	-	157.4		156.4	H-2
10	-	116.4		121.6	
1'	-	110.4		116.8	2'-OH,H-2
2'	-	148.1		147.2	2'-OH*
3'	6.49 (s)	101.5	7.08 (s)	108.4	2'-OH
4'	-	148.1		151.5	
5'	-	138.8		136.8	
6'	6.66 (s)	117.9	7.17 (s)	125.2	
4'-OMe	3.74 (s)	55.5	3.79 (s)	56.5	
7-OH	10.8 (s)				
2'-OH	8.70				
OCO<u>C</u>H$_3$			2.07 (s)	20.5	
			2.26 (s)	20.9	
			2.33 (s)	21.1	
O<u>C</u>OCH$_3$				168.6	
				168.6	
				168.8	

* Across two bonds correlations

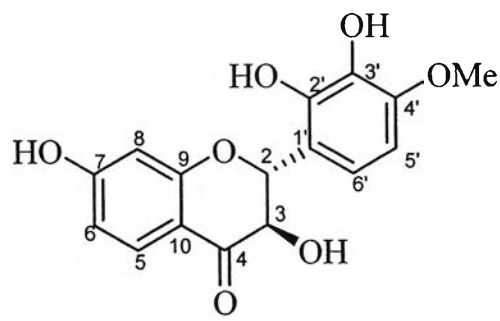
1.48 Structure Determination of Compound DP32

Compound DP32, $[\alpha]_D^{25}$ – 407.8°, was obtained as an amorphous powder. A molecular formula of $C_{16}H_{14}O_7$ was established based on the HRFABMS which exhibited molecular ion $[M]^+$ peak at *m/z* 318.0755 (calcd for 318.0740). The UV spectrum showed the absorption bands at 310, 278, 231 and 215 (sh) nm.

The ^{1}H NMR spectrum of compound DP32 in acetone-d_6 (Table 53 and Figure 183), showed the 2,3-dihydroflavonol skeleton from the characteristic AB-coupled signals at δ 4.89 and 4.19 (1H, d, J = 12Hz each) as observed in DP14. It also showed the *ortho-meta*-coupled aromatic signals at δ 7.76 (1H, d, J = 9 Hz), 6.51 (1H, dd, J = 9,2 Hz) and 6.30 (1H, d, J = 2 Hz), assignable to H-5, H-6 and H-8, respectively in A-ring. The presence of *ortho*-coupled aromatic signals at δ 6.47 (1H, d, J = 9 Hz, H-5′) and 6.91 (1H, d, J = 9 Hz, H-6′) indicated the 1,2,3,4-tetrasubstituted B-ring. In the difference NOE spectrum of DP15, NOEs were observed at H-5′ (δ 6.47) (Figure 184) by irradiation at the δ 3.81 (4′-OC<u>H</u>$_3$). All carbon atoms were assigned by the analysis of the ^{1}H-^{1}H COSY, HMQC and HMBC spectra (Figure 186-188).

The absolute configuration at C-2 of DP32 was determined as 2(*R*) by a negative Cotton effect ($[\theta]_{276}$ −10900) and a positive Cotton effect ($[\theta]_{315}$ 21900) in the circular CD spectrum.

All data are consistent with the structure of compound DP32, which was thereof assigned as a new dihydroflavonol, (2*R*,3*R*)-(−)-3,7,2′,3′-tetrahydoxy-4′-methoxydihydroflavonol (dalparvinol B) **[440]**.



[440]

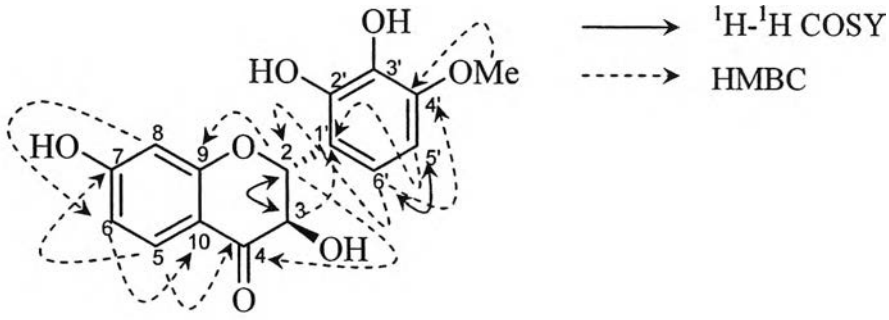


Figure 13 ^{1}H-^{1}H COSY and HMBC correlations of compound DP32

Table 53 NMR Spectral data of compound DP32 (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4)

Position	Compound DP32		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	4.89 (d,12)	75.7	H-6′
3	4.19 (d,12)	75.1	-
4	-	192.2	H-2, H-3*, H-5
5	7.76 (d,9)	130.9	-
6	6.51 (dd,9,2)	112.0	H-8
7	-	166.5	H-5
8	6.30 (d,2)	103.6	-
9	-	164.8	H-2, H-5, H-8*
10	-	114.0	H-6, H-8
1'	-	119.8	H-3 , H-5' , H-6'*
2'	-	144.5	H-5', H-6'
3'	-	135.4	H-5', H-6'
4'	-	149.9	H-6', 4'-OMe, H-5'*
5'	6.47 (d,9)	103.9	-
6'	6.90 (d,9)	118.3	-
4'-OMe	3.81 (s)	56.6	-

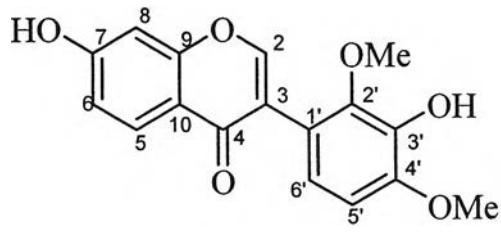
* Across two bonds correlations

1.49 Structure Determination of Compound DP33

Compound DP33 was obtained as an amorphous powder. The HRFABMS of this compound suggested a molecular formula of $C_{17}H_{15}O_6$ from its $[M+H]^+$ at *m/z* 315.0872 (calcd for 315.0869). The UV spectral data exhibited absorption band at 298, 248 and 239 (sh) nm.

The ^1H NMR spectrum of compound DP33 in acetone-d_6 (Table 54 and Figure 189) displayed the characteristics 7-hydroxyisoflavone such as δ 8.01 (1H, s, H-2) and the ABX coupled protons at δ 8.03 (d, J = 9 Hz), 6.94 (1H, dd, J = 9,2 Hz) and 6.85 (1H, d, J = 2 Hz) assignable to H-5, 6 and 8 in A-ring, respectively. Furthermore, ^1H NMR spectrum showed the AB system at δ 6.77 and 6.72 (1H, d, J = 9 Hz, each) due to H-5′ and H-6′ in B-ring, respectively. A methoxyl group at δ 3.70 was considered to be located at C-2′ as suggested from the HMBC correlation between the methoxyl protons and C-2′ (δ 147.9). Another methoxyl group at δ 3.88 was placed at C-4′, as indicated from the HMBC correlation between the methoxyl protons and C-4′ (δ 150.8). Complete ^1H and ^{13}C assignments of this compound were performed from HMQC (Figure 191) and HMBC (Figure 192) experiment, as summarized in Table 54.

Based on above spectral evidence, compound DP33 was confirmed as a new isoflavone, 7,3′-dihydroxy-2′,4′-dimethoxyisoflavone and was named khrinone E **[441]**.



[441]

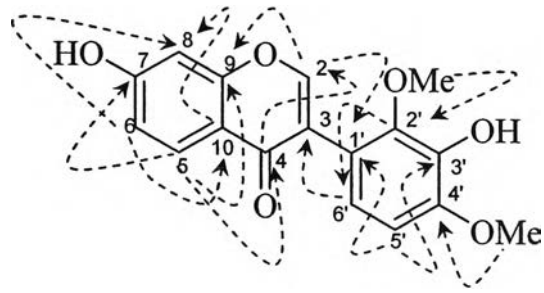


Figure 14 HMBC correlation of compound DP33

Table 54 NMR Spectral data of compound DP33 (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4)

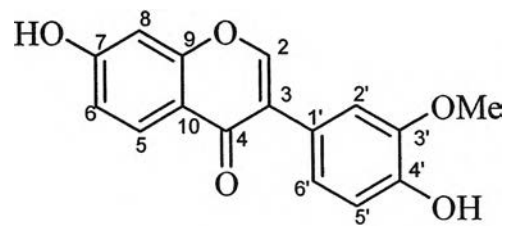
Position	Compound DP33		HMBC (correlation with ^{1}H)
	^{1}H(*mult.*, *J* in Hz)	^{13}C	
2	8.01 (s)	155.7	-
3	-	123.7	H-6' , H-2*
4	-	178.3	H-2 , H-5
5	8.03 (d,9)	128.5	-
6	6.94 (dd,9,2)	116.5	H-8
7	-	164.8	H-5, H-8*
8	6.85 (d,2)	103.4	H-6
9	-	159.9	H-2,H-5,H-8*
10	-	118.1	H-6
1'	-	120.0	H-2 , H-5'
2'	-	147.9	H-6', 2'-OMe
3'	-	140.8	H-5'
4'	-	150.8	H-6', 4'-OMe
5'	6.77 (d,9)	108.2	-
6'	6.72 (d,9)	122.3	H-2
2'-OMe	3.70 (s)	56.8	
4'-OMe	3.88 (s)	60.8	

* Across two bonds correlations

1.50 Structure Determination of Compound DP34

Compound DP34 was obtained as an amorphous powder. Its ^{1}H NMR spectrum in MeOH-d_4 (Table 55 and Figure 193) also showed the compound as 7-hydroxyisoflavone through the characteristic proton signals at δ 8.14 (1H, s, H-2) and 8.05 (1H, d, J = 9 Hz, H-5). The H-5 formed a set of ABX coupling system with δ 6.94 (1H, dd, J = 9,2 Hz, H-6) and 6.84 (1H, d, J = 2 Hz, H-8). For B-ring, the another ABX coupling pattern signals were observed at δ 7.16 (1H, d, J = 2 Hz, H-2′), 6.85 (1H, d, J = 9 Hz, H-5′), 6.95 (1H, d, J = 9,2 Hz, H-6′). The H-2′ was assigned to be lower field than H-8 because H-2′ was donated electron from only one oxygenated functional group, while H-8 was donated electron from two groups. In the case of H-6′ and H-6, H-6′ was assigned to be lower field because the donating group at *ortho* position has potent inductive effect than *para* and *meta* position. A methoxyl signal at δ 3.89 (3H, s) was observed and was confirmed its position at C-3′ according to NOE interaction with H-2′.

On the basis of the above spectroscopic studies, DP34 was identified as 7,4′-dihydroxy-3′-methoxyisoflavone (3′-methoxydaidzein) **[196]**. Its ^{1}H and ^{13}C NMR data were in good agreement with published data (Jun *et al.*, 2003). This compound has been isolated to be present widely in plants such as *Dalbergia odorifera* (Yahara *et al.*, 1989), *Pueraria lobata* (Rong *et al.*, 1998), and *Tephrosia purpurea* (Chang *et al.*, 1997).



[196]

Table 55 NMR Spectral data of compound DP34 (in MeOH-d_4) and 3'-methoxydaidzein (in DMSO-d_6)

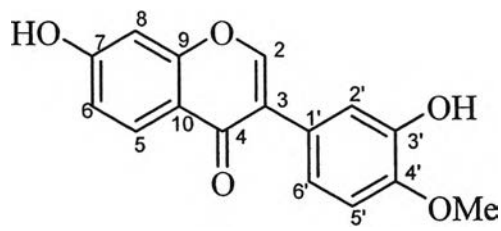
Position	Compound DP34		5'- Methoxyvestitol	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.14 (s)	154.8	8.31 (s)	152.9
3	-	124.9	-	122.9
4	-	178.2	-	175.0
5	8.05 (d,9)	128.5	7.97 (d,8.3)	127.2
6	6.94 (dd,9,2)	116.6	6.93 (dd,2.4,8.3)	115.0
7	-	164.9	-	162.5
8	6.84 (d,2)	103.3	6.85 (d,2.4)	102.0
9	-	159.8	-	157.3
10	-	118.2	-	116.6
1'	-	126.0	-	123.4
2'	7.16 (d,2)	116.2	7.16 (d,1.9)	113.3
3'	-	147.9	-	146.4
4'	-	148.8	-	147.0
5'	6.85 (d,9)	114.3	6.81 (d,8.3)	115.1
6'	6.95 (dd,9,2)	122.9	6.99 (dd,1.9,8.3)	121.5
3'-OMe	3.89 (s)	56.5	3.80 (s)	55.6

1.51 Structure Determination of Compound DP35

Compound DP35 was obtained as an amorphous powder. It showed $[M]^+$ peak at *m/z* 284 in the FABMS (Figure 196), corresponding to the molecular formula $C_{16}H_{12}O_5$.

The ^1H and ^{13}C NMR spectra in MeOH-d_4 (Table 56 and Figure 196-197) of compound DP35 was similar to those of DP34. However, the chemical shifts of the B-ring protons were different. In order to identify the attached position of the methoxyl group on B-ring, the HMBC experiment was carried out. The HMBC correlation of C-4′(δ 149.2) with H-2′ (δ 7.03), H-6′ (δ 6.94) and 4′-OC<u>H</u>$_3$ (δ 3.86) indicated that methoxyl group placed at C-4′.

On the basis of the above spectroscopic data, together with the information from ^1H-^1H COSY, HMQC and HMBC experiment from ^1H-^1H COSY, HMQC and HMBC experiments, compound DP35 was identified as 7,3′-dihydroxy-4′-methoxyisoflavone (calycosin) **[81]**. It ^1H, ^{13}C NMR data are in good agreement with reported values (Hirakura *et al.*, 1997 and Kamnaing *et al.*, 1999).



[81]

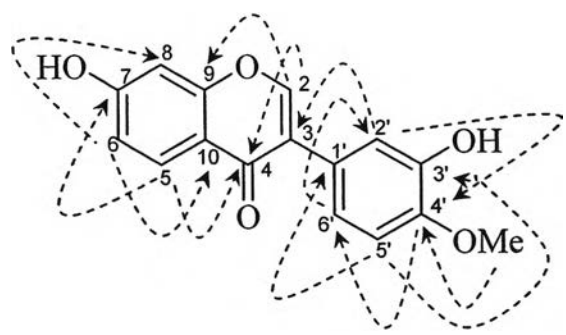


Figure 15 HMBC correlation of Compound DP35

Table 56 NMR Spectral data of compound DP35 (in MeOH-d_4) and calycosin (in DMSO-d_6)

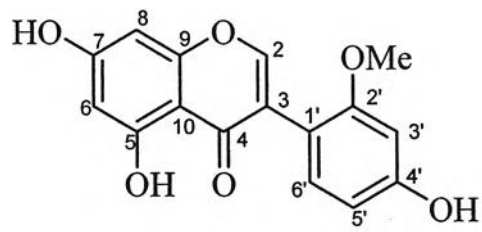
Position	Compound DP35		Calycosin		HMBC
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C	(correlation with ^{1}H)
2	8.06 (s)	154.7	8.39 (s)	153.0	-
3	-	125.8	-	123.3	H-2'
4	-	178.0	-	174.5	H-2, H-5
5	8.02 (d,9)	128.5	8.06 (d,8.8)	127.3	-
6	6.91 (dd,9,2)	116.6	7.15 (dd,2.2,8.8)	115.1	H-8
7	-	164.8	-	162.5	H-5, H-8*
8	6.81 (d,2)	103.3	7.24 (d,2.2)	102.2	H-5, H-6
9	-	159.8	-	157.3	H-2, H-5, H-8*
10	-	118.2	-	116.2	H-6, H-8
1'	-	126.4	-	124.7	H-5'
2'	7.03 (s)	117.5	6.97 (br .s)	116.4	H-6'
3'	-	147.5	-	146.0	H-5'
4'	-	149.2	-	147.5	H-2', H-6', 4'-OMe
5'	6.94 (s)	112.9	6.97 (br. s)	111.9	-
6'	6.94 (s)	121.7	7.08 (br. s)	119.7	H-2'
4'-OMe	3.86 (s)	56.6	3.80 (s)	55.6	-

* Across two bonds correlations

1.52 Structure Determination of Compound DP36

Compound DP36 was obtained as an amorphous powder. The molecular formula of $C_{16}H_{12}O_6$ was deduced from its $[M]^+$ ion at *m/z* 300 in the FABMS. Its ^1H NMR spectrum in MeOH-d_4 (Table 57 and Figure 201) showed proton signals for a typical H-2 of isoflavone nucleus at δ 7.90 (1H, s), with the *meta*-coupled signals at δ 6.21 (d, J = 2 Hz, H-6) and 6.33 (d, J = 2 Hz, H-8) and an ABX type signals at δ 6.50 (1H, d, J = 2 Hz, H-3′), 6.43 (1H, dd, J = 8,2 Hz, H-5′) and 7.04 (1H, d, J = 8 Hz, H-6′) indicated the presence of 5,7-dioxygenated A-ring and 1′,2′,4′-trisubstituted B-ring. The methoxyl group (δ 3.74) was assigned to C-2′ according to its NOE interaction with H-3′. The ^1H NMR assignments were found in accordance with those reported (Ko *et al.*, 2004).

The ^{13}C NMR spectral data of compound DP36 in MeOH-d_4 (Table 57 and Figure 203) were identical with those in the literature (Ko *et al.*, 2004). Thus, compound DP36 was identified as 5,7,4′-trihydroxy-2′-methoxyisoflavone or 2′-methoxygenistein (theralin) **[442]**, which was first isolated from *Crotalaria pallida* and *C. assamica* (Ko *et al.*, 2004).



[442]

Table 57 NMR Spectral data of compound DP36 (in MeOH-d_4) and theralin (in MeOH-d_4)

Position	Compound DP36		Theralin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	7.90 (s)	156.2	7.95 (s)	154.5
3	-	122.6	-	125.3
4	-	182.4	-	179.0
5	-	165.9	-	163.0
6	6.21 (d,2)	100.4	6.38 (d,2)	97.7
7	-	163.7	-	165.0
8	6.33 (d,2)	94.8	6.44 (d,2)	96.1
9	-	159.8	-	161.5
10	-	106.2	-	109.0
1'	-	112.2	-	112.5
2'	-	160.6	-	160.2
3'	6.50 (d,2)	100.1	6.46 (d,2)	108.3
4'	-	160.4	-	158.1
5'	6.43 (dd,8,2)	108.2	6.35 (dd,8,2)	104.7
6'	7.04 (d,8)	133.2	7.00 (d,8)	133.0
2'-OMe	3.74 (s)	56.0	3.89 (s)	56.5

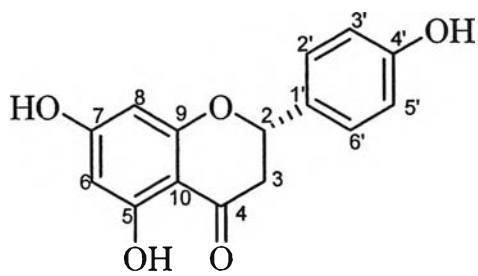
1.53 Structure Determination of Compound DP37

Compound DP37, $[\alpha]_D^{25}$ – 79.5°, was obtained as colorless amorphous powder. The FABMS spectrum exhibited a molecular ion $[M+H]^+$ peak at *m/z* 273, corresponding to molecular formula $C_{15}H_{13}O_5$.

The ^1H NMR spectrum of compound DP37 in acetone-d_6 (Table 58 and Figure 204) showed three doublet of doublet coupling of three proton of H-2 and H-3, suggested that compound DP37 was either a flavanone or an isoflavanone, however coupling constant of these three protons at δ 5.31 (1H, dd, J = 10,2 Hz, H-2), δ 2.68 (1H, dd, J = 17,2 Hz, H-3) and δ 3.08 (1H, dd, J = 17,10 Hz, H-2), clarifying that it was an flavanone skeleton. The protons in B-ring (H-2′, H-3′, H-5′ and H-6′) formed a characteristic AA′ BB′ pattern at δ 7.29 (2H, d, J = 9 Hz, H-2′, H-6′), δ 6.80 (2H, d, J = 9 Hz, H-3′ and H-5′), while the signals of H-6 and H-8 in A-ring appeared as doublets at δ 5.87 (d, J = 2 Hz) and δ 5.89 (d, J = 2 Hz), respectively.

The absolute configuration at C-2 was estimated as 2(*S*) from negative Cotton effect ($[\theta]_{235}$ –7500) and ($[\theta]_{287}$ -23300) in the CD spectrum.

By analysis of the above spectroscopic data and comparison with previously reported data (Ibrahim, 2000), compound DP37 was identified as (2*S*)-(–)-5,7,4′-trihydroxyflavanone ((2*S*)-(–)-naringenin) **[251]**, previously isolated from several plants.



[251]

Table 58 NMR Spectral data of compound DP37 (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4) and naringenin (in DMSO-d_6)

Position	Compound DP37		Naringenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	5.31 (dd,10,2)	80.4	5.43 (dd,13,4)	79.0
3a	2.68 (dd,17,2)	44.0	2.70 (dd,17,2.8)	42.6
3b	3.08 (dd,17,10)		3.20 (m)	
4	-	197.7	-	196.4
5	-	164.9[a]	-	163.7
6	5.87 (d,2)	97.1	5.88 (s)	96.4
7	-	168.3	-	166.9
8	5.89 (d,2)	96.2	5.88 (s)	95.5
9	-	165.4[a]	-	163.3
10	-	103.4	-	102.3
1'	-	131.1	-	129.4
2'	7.29 (d,9)	129.0	7.32 (d,9)	128.7
3'	6.80 (d,9)	116.3	6.79 (d,9)	115.7
4'	-	159.0	-	158.1
5'	6.80 (d,9)	116.3	6.79 (d,9)	115.7
6'	7.29 (d,9)	129.0	7.32 (d,9)	128.7

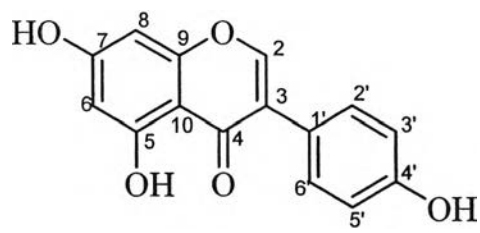
[a] interchangeable within column

1.54 Structure Determination of Compound DP38

Compound DP38, a colorless amorphous powder, showed a molecular ion $[M]^+$peak at *m/z* 270 in the FABMS corresponding to the molecular formula $C_{15}H_{10}O_5$.

The ^1H NMR spectrum of compound DP38 in MeOH-d_4 (Table 59 and Figure 206) showed a characteristic signal at δ 8.00 (1H, s) ascribable to H-2 of isoflavone. Moreover, signals belong to an aromatic AA′BB′ system at δ 7.34 and 6.83 (2H, d, J = 8 Hz, each) were assignable to H-2′, 6′ and H-3′, 5′, respectively in B-ring. The two aromatic signals at δ 6.20 (br.s) and 6.31 (br.s) indicated trioxygenated A-ring. The ^{13}C NMR spectrum (Table 59 and Figure 203) also supported the presence of oxygenated carbons at δ 163.8 (C-5), 165.9 (C-7) and 158.8 (C-4′).

According to the comparison above results with the previously reported (Kinjo *et al.*, 1987), compound DP38 was verified to be 5,7,4′-trihydroxyisoflavone (genistein) **[3]**. This compound was commonly found in many plants of the family Leguminosae (Harborne, 1993).



[3]

Table 59 NMR Spectral data of compound DP38 (in MeOH-d_4) and genistein (in DMSO-d_6)

Position	Compound DP38		Genistein	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.00 (s)	154.7	8.31 (s)	153.8
3	-	124.7	-	122.2
4	-	182.2	-	180.1
5	-	163.8	-	162.0
6	6.20 (br.s)	100.1	6.38 (d,2)	98.9
7	-	165.9	-	164.3
8	6.31 (br.s)	94.8	6.22 (d,2)	93.6
9	-	159.7	-	157.5
10	-	106.3	-	104.4
1'	-	123.3	-	121.1
2'	7.34 (d,8)	131.4	7.37 (d,8)	130.0
3'	6.83 (d,8)	116.3	6.81 (d,8)	115.0
4'	-	158.8	-	157.4
5'	6.83 (d,8)	116.3	6.81 (d,8)	115.0
6'	7.34 (d,8)	131.4	7.37 (d,8)	130.0

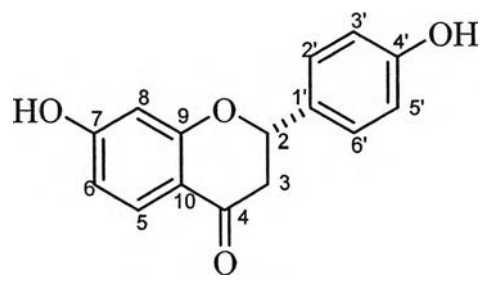
1.55 Structure Determination of Compound DP39

Compound DP39, $[\alpha]_D^{25}$ – 14.5°, was obtained as an amorphous powder. The FABMS spectrum showed a molecular ion $[M]^+$ peak at *m/z* 256, corresponding to molecular formula $C_{15}H_{12}O_4$.

The ^1H NMR spectrum in acetone-d_6 (Table 60 and Figure 208) of compound DP39 revealed the characteristic three spin system for a flavanone at δ 5.33 (1H, dd, J = 13, 2 Hz, H-2), 2.66 (1H, dd, J = 17,2 Hz, H-3a) and 3.00 (1H, dd, J = 17,13 Hz, H-3b).

Furthermore, the ABX types at δ 7.69 (1H, d, J = 8 Hz, H-5), 6.45 (1H, dd, J = 8,2 Hz, H-6) and 6.30 (1H, d, J = 2 Hz, H-8) assignable to A-ring and the AA′BB′type protons at δ 6.81 (d, J = 8 Hz, H-3′,5′) and 7.29 (d, J = 8 Hz, H-2′,6′) assignable to B-ring were observed. By comparison of ^1H NMR spectral data of compound DP39 with those of DP37, the absence of chelated hydroxyl group at C-5 was observed. Its ^{13}C NMR data (Table 60 and Figure 209) showed 13 signals for 15 carbon atoms.

Therefore, compound DP39 was identified as (2*S*)-(–)-7,4′-dihydroxyflavanone ((2*S*)-(-)-liquiritigenin) **[84]**, based on the above spectral evidence and reported data (Kong *et al.*, 2000).



[84]

Table 60 NMR Spectral data of compound DP39 (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4) and liquiritigenin (in DMSO-d_6)

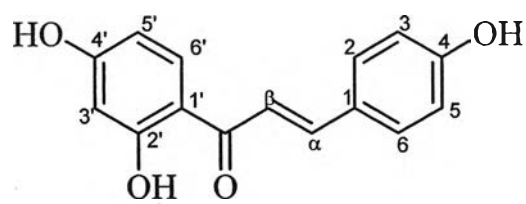
Position	Compound DP39		Liquiritigenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	5.33 (dd,13,2)	80.9	5.45 (dd,13.2,2.3)	81.3
3a	2.66 (dd,17,2)	44.9	2.67 (dd,16.9,2.3)	45.1
3b	3.00 (dd,17,13)		3.05 (dd,16.9,13.3)	
4	-	193.4	-	192.7
5	7.69 (d,8)	129.8	7.73 (d,8.2)	129.1
6	6.45 (d,8,2)	112.7	6.58 (dd,8.2,2.1)	110.3
7	-	168.9	-	165.4
8	6.30 (d,2)	104.1	6.42 (d,2.1)	104.7
9	-	165.7	-	166.1
10	-	114.2	-	115.9
1'	-	131.5	-	130.9
2'	7.29 (d,8)	128.9	7.41 (d,8.3)	129.1
3'	6.81 (d,8)	116.3	6.90 (d,8.3)	116.4
4'	-	158.9	-	159.0
5'	6.81 (d,8)	116.3	6.90 (d,8.3)	117.0
6'	7.29 (d,8)	128.9	7.41 (d,8.3)	128.6

1.56 Structure Determination of Compound DP40

Compound DP40 was obtained as yellow powder. It showed $[M]^+$ peak at *m/z* 256 in the FABMS, corresponding to the molecular formula $C_{15}H_{12}O_4$.

The ^1H NMR spectrum of compound DP40 in acetone-d_6 (Table 61 and Figure 210) showed a pair of doublet signals (J = 16 Hz, each) at δ 7.72 and δ 7.81 assignable to the *trans* olefinic protons for H-α and β. The aromatic protons showed an ABX coupling system at δ 6.36 (1H, d, J = 2 Hz), 6.45 (1H, dd, J = 9,2 Hz) and 8.09 (1H, d, J = 9 Hz) which were assigned to H-3′, 5′ and 6′, respectively. The other aromatic protons exhibited an AA′BB′ coupling system at δ 7.71 (2H, d, J = 9 Hz) and 6.92 (2H, d, J = 9 Hz) which were assigned to H-2, 6and H-3, 5, respectively. The ^{13}C NMR spectrum (Table 61 and Figure 211) displayed 13 signals for 15 carbon atoms were observed.

By comparing its NMR data with those reported (Kong *et al.*, 2000), compound DP40 was identified as 4,2′,4′-trihydroxychalcone (isoliquiritigenin) **[83]**, first isolated from *Dahlia variabilis* as a natural occurring substance (Smith and Swain, 1953).



[83]

Table 61 NMR Spectral data of compound DP40 (^{1}H NMR in acetone-d_6 and ^{13}C NMR in MeOH-d_4) and isoliquiritigenin (in DMSO-d_6)

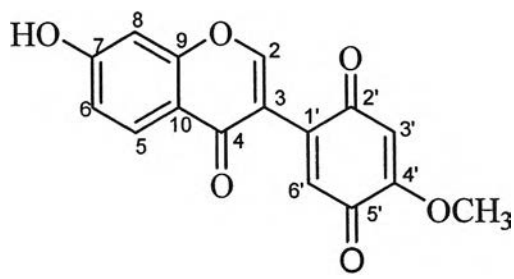
Position	Compound DP40		Isoliquiritigenin	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
1	-	128.1	-	127.1
2	7.71 (d,9)	132.2	7.76 (br.d,8.4)	131.7
3	6.92 (d,9)	117.3	6.95 (d,8.4)	116.7
4	-	161.5	-	161.0
5	6.92 (d,9)	117.3	6.95 (d,8.4)	116.7
6	7.71 (d,9)	132.2	7.76 (br.d,8.4)	131.7
α	7.72 (d,16)	118.9	7.79 (d,15.5)	118.2
β	7.81 (d,16)	145.6	7.86 (br.d,15.5)	145.1
γ	-	193.4	-	192.5
1'	-	115.0	-	116.8
2'	-	168.1	-	167.6
3'	6.36 (d,2)	104.3	6.38 (d,2.1)	103.7
4'	-	166.1	-	165.6
5'	6.45 (dd,9,2)	109.2	6.48 (dd,8.2,2.1)	108.7
6'	8.09 (d,9)	133.7	8.15 (d,8.2)	133.3
4-OH	9.22 (br.s)	-	9.15 (br.s)	-
2'-OH	13.60 (s)	-	13.66 (s)	-
4'-OH	9.22 (br.s)	-	9.15 (br.s)	-

1.57 Structure Determination of Compound DP41

Compound DP41 was obtained as an amorphous powder. The FABMS exhibited molecular ion $[M]^+$ peak at *m/z* 298, corresponding to the molecular formula of $C_{16}H_{10}O_6$. The UV spectrum showed maximum absorptions at 297, 249 nm.

The ^1H NMR spectrum of compound DP41 in DMSO-d_6 (Table 62 and Figure 212) showed the characteristic signal due to H-2 of isoflavone at δ 8.32 (1H, s). The presence of an ABX coupling pattern at δ 7.92 (1H, d, J = 8 Hz), 6.95 (1H, dd, J = 8,2 Hz) and 6.89 (1H, d, J = 2 Hz) were assigned to H-5, H-6 and H-8 respectively, in A-ring. For B-ring two singlet signals at δ 6.22 (H-3′) and 7.05 (H-6′) were observed. The signal at δ 3.82 (3H, s) were attributed to the methoxyl group which showed NOE enhancement at δ 6.22 (H-3′). In the ^{13}C NMR spectrum, three carbonyl signals at δ 173.4, 185.3 and 181.5 were assignable to C-4 on ring C, and C-2′ and C-5′ in ring B as *para*-quinone.

All protons and carbons were assigned completely from 2D techniques such as ^1H-^1H COSY, HMQC and HMBC experiments. According to the comparing of its ^1H and ^{13}C NMR data with those reported previously (Brown *et al.*, 1974 and Yahara *et al.*, 1989), compound DP41 was identified as 7-hydroxy-4′-methoxyisoflavone-quinone (bowdichione) **[443]**. This compound was first found in *Bowdichia nitida* (Brown *et al.*, 1974).



[443]

Table 62 NMR Spectral data of compound DP41 (in DMSO-d_6) and bowdichione (in DMSO-d_6)

Position	Compound DP41		Bowdichione	
	^{1}H(*mult.*, *J* in Hz)	^{13}C	^{1}H(*mult.*, *J* in Hz)	^{13}C
2	8.32 (s)	156.6	8.32 (s)	156.7
3	-	116.6	-	116.9
4	-	173.4	-	173.7
5	7.92 (d,8)	127.1	7.91 (d,8)	133.0
6	6.95 (dd,8,2)	115.6	6.80-7.06 (m)	115.8
7	-	163.0	-	158.6
8	6.89 (d,2)	102.3	6.80-7.06 (m)	102.5
9	-	157.1	-	157.4
10	-	116.1	-	116.2
1'	-	138.9	-	139.3
2'	-	185.3	-	185.5
3'	6.22 (s)	108.0	6.22 (s)	108.1
4'	-	158.3	-	163.2
5'	-	181.5	-	181.8
6'	7.05 (s)	132.8	7.06 (s)	127.4
4'-OMe	3.82 (s)	56.4		56.4
7-OH	10.9 (s)			

2. Estrogenic Activities

Phytoestrogens comprise a vast variety of structurally diverse compounds; include isoflavones, lignans, stilbenes. Other less investigated compounds include the prenylflavonoids, flavones, flavans, isoflavans and phytosterol ester (Mueller, 2002). The estrogenic activity of many chemical depends on the ability of compound to interact with the estrogen receptor (ER). The estrogen receptors, ER-α and ER-β, mediate the effects of endogenous estrogen, *i.e.* regulation of reproductive function, cell proliferation and differentiation. Both receptors have a distinct tissue distribution and play a distinct role in physiology. The ER is a ligand inducible transcription factor which binds to specific regulatory sequences in target genes to regulate processes in functions of hormone-dependent cells. Thus, plant-derived chemicals that showed estrogenic activity have received much attention for prevention of post menopausal symptoms such as osteoporosis, cardiovascular disease and Alzheimer's disease.

In this study, the estrogenic activity of each pure compound was evaluated by measuring the cell proliferation and luciferase reporter gene assay on MCF-7 (ER-α positive) and T47D (ER-α and ER-β positive). Both cell systems for detection of the biological activities of the constituents of the rhizomes of *B. chinensis* and heartwood of *D. parviflora* were compared.

2.1 Cell Proliferation

2.1.1 Cell Proliferation effect of Compounds from *B. chinensis*

The cell proliferation were tested with increasing concentration ranging from 10 nM to 100 μM, and their EqE_{10} and EqE_{100} values were determined for the required concentrations against cell proliferation equivalent to 10 and 100 pM of estradiol (E2) treatment, respectively (Table 63).

Effect on MCF-7 cell proliferation of isoflavones, tectorigenin **[14]**, irilin D **[412]**, and tectoridin **[13]** were found to have stimulatory activities (EqE_{10}: 0.3 μM, 5.6 μM, 0.02 μM, respectively) against the cell proliferation at low concentrations and showed high potencies, as effective as 100 pM of E2 (EqE_{100}: 1.0 μM, 12.7 μM, and 0.08 μM, respectively). Tectoridin **[13]** showed higher activity compared to genistein at a concentration of 1 μM, whereas cytotoxicity of the

compound was observed at a concentration of 100 μM. Although irisflorentin **[7]** iristectorin B **[413]**, iristectorin A **[414]** were observed to enhance cell proliferation (EqE_{10}: 50.3 μM, 77.3 μM, and 26.1 μM, respectively), their EqE_{100} values could not be assessed since their stimulatory activities were not high enough, even at a concentration of 100 μM. The remaining compounds did not show any significant activities against the cell line at these concentrations, and their EqE_{10} values could not be evaluated.

The effect of isoflavones on breast cancer cell proliferation was tested in T47D cell (Table 63). Almost all compounds showed cytotoxicities at concentrations of 10 μM or above, whereas they exhibited maximum effects on MCF-7 cell proliferation at those concentrations. Tectorigenin **[14]** and tectoridin **[13]** stimulated T47D cell proliferation (EqE_{10}: 0.04 μM and 0.2 μM, respectively). Iristectorin B **[413]** and iristectorin A **[414]** showed significant activity against this cell line at concentrations of less than 1 μM, but their EqE_{10} values could not be evaluated as a result of their low activity. Other compounds had no effect on cell proliferation over a concentration range of 1 nM to 10 μM.

The stimulatory effects of other phenolic compounds on MCF-7 and T47D cell proliferation were examined. Treatment of these cells with belamphenone **[419]** and iriflophenone **[418]** resulted in an increase of cell proliferation in a concentration-dependent manner (EqE_{10} against MCF-7: 0.8 μM for belamphenone **[419]** and 0.7 μM for iriflophenone **[418]**, against T47D: 0.09 μM for belamphenone **[419]** and 4.9 μM for iriflophenone **[418]**). Belamphenone **[419]** showed the highest activity, and it was the only compound of the six to afford a EqE_{100} value against both cell lines. Resveratrol **[57]** showed activity against these two cell line (EqE_{10} against MCF-7: 1.6 μM, EqE_{100} against T47D: 0.03 μM), whereas cytotoxicity of this compound were observed at, or above, concentrations of 10 μM. Belalloside A **[420]** and belalloside B **[421]** had no effect on both cell proliferations over a concentration range of 1 nM to 100 μM.

Table 63 Cell proliferation stimulation activities of isolated compound from *Belamcanda chinensis* against MCF-7 and T47D cells.

Compound	MCF-7						T47D					
	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)
Genistein[3]	+++	+++	+++	+++	0.003	0.013	+	++	++	+++	0.001	0.009
Tectoriginin[14]	+++	+++	+	-	0.3	1.0	+++	++	+	-	0.04	0.5
Irisflorentin[7]	+	-			50.3	ND	-	-	-	-	ND	ND
Irigenin[5]	+	-			ND	ND	-	-	-	-	ND	ND
Irilin D[412]	+++	++	-	-	5.6	12.7	+	-	-	-	ND	ND
Tectoridin[13]	+++	+++	+	+	0.02	0.08	++	++	+	+	0.2	ND
Iristectorin B[413]	++	+			77.3	ND	+	+	+	+	ND	ND
Iristectorin A[414]	++	+			26.1	ND	+	-	-	-	ND	ND
Iridin[8]	+	-			ND	ND	+	+	+	+	ND	ND
Hispiduloside[415]	+	-			ND	ND	+	+	+	+	ND	ND
Jaceoside[416]	-	-			ND	ND	-	+	+	+	ND	ND
Androsin[417]	+	-			ND	ND	-	-	-	-	ND	ND
Iriflophenone[418]	+++	+	+	+	0.7	6.8	++	+	+	+	4.9	ND
Belamphenone[419]	+++	++	++	+	0.8	12.8	+++	+++	++	+	0.09	37.1
Belalloside A[420]	+	-			ND	ND	-	-	-	-	ND	ND
Belalloside B[421]	-	+			ND	ND	-	-	-	-	ND	ND
Resveratrol[57]	-	+	+	++	1.6	ND	++	++	+	+	0.03	ND

+++ : > 100 pM of estradiol (E_2), ++ : > 10 pM of E_2, + : > 1 pM of E_2, - : < 1 pM of E_2.
EqE_{10} and EqE_{100} : $E_2$10 pM, 100 pM equivalent cell proliferation activity of the concentration of test compound : (μM)
ND : not determine

2.1.2 Cell Proliferation effect of Compounds from *D. parviflora*

The cell proliferation were tested with increasing concentration ranging from 10 nM to 10 μM and their EqE_{10} and EqE_{100} value were determined for the required concentrations against cell proliferation equivalent to 10 and 100 pM of E2 treatment, respectively (Table 64).

5′-Methoxyvestitol **[183]** was the only compound of the six isoflavans that showed stimulatory activities on MCF-7 cell (EqE_{10}: 1.06 μM and EqE_{100}: 4.16 μM). Including it showed activity against T47D cell (EqE_{10}: 0.02 μM). Mucronulatol **[77]** and vestitol **[78]** exhibited activity against T47D (EqE_{10}: 1.62 μM, 0.02 μM, respectively). Vestitol showed high potency against T47D (EqE_{100}: 0.17 μM). The other compounds did not show any significant activities against the cell lines, therefore their EqE_{10} and EqE_{100} were unable to calculate.

Almost all isoflavanones showed activity against both cell lines in a concentration-dependent manner. The EqE_{10} value against MCF-7 of 3′-methoxyviolanone **[197]**, onogenin **[252]**, sativanone **[175]**, secundiflorol H **[429]**, violanone **[177]**, vestitone **[176]**, dalparvin **[438]** were 6.09 μM, 4.24 μM, 2.73 μM, 2.85 μM, 2.05 μM, 6.09 μM and 2.07 μM, respectively, and EqE_{100} value were 9.41 μM for onogenin **[252]**, 8.56 μM for sativanone **[175]**, 9.44 for violanone **[177]** and 4.73 μM for dalparvin **[438]**. The stimulatory activity against T47D cell showed higher response than MCF-7 cell. The EqE_{10} value of 3′-methoxyviolanone **[197]**, onogenin **[252]**, sativanone **[175]**, secundiflorol H **[429]**, violanone **[177]**, vestitone **[176]** and dalparvin **[438]** were 6.12 μM, 0.98 μM, 1.98 μM, 0.54 μM, 2.14 μM, 4.13 μM and 0.57 μM, respectively, and EqE_{100} value were 8.09 μM for onogenin **[252]**, 9.22 μM for sativanone **[175]**, 5.66 μM for secundiflorol H **[429]**, 8.90 μM for violanone **[177]** and 11.60 μM for dalparvin **[438]**, respectively.

Many compounds of isoflavones also showed stimulatory effect on MCF-7 and T47D cell proliferation. Genistein **[3]** is well known phytoestrogen, showed the highest activity against both cell lines (EqE_{10} against MCF-7: 0.01 μM, EqE_{10} against T47D: <0.01 μM; EqE_{100} against MCF-7: 0.11 μM, EqE_{100} against T47D: 0.08 μM). However cytotoxicity of this compound was observed at, or above, concentrations of 10 μM. Khrinone D **[424]**, biochanin A **[95]** 2′-methoxybiochanin

A **[425]**, tectorigenin **[14]**, khrinone C **[432]** pratensein **[433]** and 2′-methoxy formononetin **[434]** showed high activity against MCF-7 and T47D cell lines in the range of EqE_{10} against MCF-7 0.28-7.59 μM, EqE_{100} 2.66-6.73 μM, EqE_{10} against T47D <0.01-6.40 μM. Especially, khrinone D **[424]**, biochanin A **[95]**, 2′-methoxyformononetin **[434]** and genistein showed highest activity against T47D cell (EqE_{10}: <0.01 μM) and biochanin A, 2′-methoxybiochanin A showed potency in the range of EqE_{100} at 0.46 and 1.7 μM, respectively. Moreover, flavanone, naringenin **[251]** and liquiritigenin **[84]** cell proliferation against both cell lines in a concentration-dependent manner (EqE_{10} against MCF-7: 0.18 μM and 0.14 μM, respectively, EqE_{100} against MCF-7: 0.66 μM and 0.65 μM, respectively, EqE_{10} against T47D: 0.09 μM and 0.64 μM, respectively, EqE_{100} against T47D: 1.57 μM for naringenin **[251]**. Dihydroflavonols had no effect on both cell proliferations, except dalparvinol A **[436]** exhibited EqE_{10} value against T47D at 5.7 μM. A pterocarocarpan, (6a, 11a)-3,8-dihydroxy-9-methoxypterocarpan **[426]** showed high stimulatory effect only on MCF-7 cell (EqE_{10}: 0.72 μM) but the cytotoxicity of this compound was observed at concentration of 10 μM. A chalcone, isoliquiritigenin **[83]** also exhibited the high activity against two cell lines (EqE_{10} against MCF-7: 1.10 μM, EqE_{10} against MCF-7: 3.97 μM, EqE_{10} against T47D: 1.00 μM). Moreover, cinnamylphenols, xenognosin **[435]** showed high stimulatory effect against both cell line (EqE_{10} against MCF-7: 0.34 μM, EqE_{10} against T47D: 0.67 μM). The results are summarized in Table 64.

Table 64 Cell proliferation of stimulation of compounds from *Dalbergia parviflora* against MCF-7 and T47D cells

Compound	MCF-7						T47D					
	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)
Isoflavans												
Khriol A[422]	-	-	-	-	ND	ND	-	+	+	+	ND	ND
Mucronulatol[77]	-	+	-	-	ND	ND	++	+	+	-	1.62	ND
8-Demethylduartin [427]	-	-	-	-	ND	ND	+	+	+	+	ND	ND
Arizonicanol A[431]	-	-	-	-	ND	ND	+	+	-	+	ND	ND
Vesitol[78]	+	+	-	+	ND	ND	+++	+++	++	++	0.02	0.17
5'-Methoxy violanone [183]	+++	+	-	+	1.06	4.16	++	++	++	+	0.02	ND
Isoflavanones												
3'-Methoxy violanone [197]	++	+	-	-	6.09	ND	++	+	+	+	6.12	ND
Onogenin [252]	+++	+	-	-	4.24	9.41	+++	++	++	+	0.98	8.09
Sativanone [175]	+++	++	-	-	2.73	8.56	+++	+	+	+	1.98	9.22
Secundiflorol H [429]	++	+	+	-	2.85	ND	+++	++	+	-	0.54	5.66
7,3'-Dihydroxy-4'-methoxyisoflavanone [430]	+	-	-	-	ND	ND	+	-	+	+	ND	ND
Violanone[177]	+++	++	+	+	2.05	9.44	+++	+	+	+	2.14	8.90
Vestitone[176]	++	+	-	-	6.09	ND	++	+	+	+	4.13	ND
Dalparvin[438]	+++	-	-	-	2.07	4.73	+++	++	+	+	0.57	11.6
Isoflavones												
7-Demethylrobus-tigenin[423]	-	-	-	-	ND	ND	++	++	+	+	0.12	ND
Khrinone D[424]	+++	+	-	-	1.82	4.21	++	++	++	++	<0.01	ND
Biochanin A[95]	+++	++	+	+	0.33	3.77	+++	+++	+++	++	<0.01	0.46
2'-Methoxy-biochanin A[425]	++	+	+	+	3.18	ND	++	++	+	+	0.36	ND
Khrinone C[432]	++	+	+	+	3.72	ND	++	+	+	+	3.03	ND
Pratensein[433]	++	+	-	+	7.59	ND	++	-	-	-	6.40	ND
2'-Methoxy-formononetin[434]	+++	++	+	-	0.28	5.24	+++	+++	++	++	<0.01	1.71
Formononetin[72]	+	+	+	+	ND	ND	++	++	++	+	0.03	ND

Table 64 (Continued)

Compound	MCF-7						T47D					
	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)	10 μM	1 μM	0.1 μM	0.01 μM	EqE_{10} (μM)	EqE_{100} (μM)
Khrinone B[437]	+	+	+	-	ND	ND	+	+	+	+	ND	ND
Khrinone A[439]	+	-	-	-	ND	ND	+	+	+	+	ND	ND
Khrinone E[441]	++	+	-	-	4.7	ND	+	+	+	-	ND	ND
3'-Methoxy-daidzein[196]	++	+	-	-	4.7	ND	+	+	+	-	ND	ND
Calycosin[81]	+	+	-	-	ND	ND	++	+	-	+	4.13	ND
Theralin[442]	++	+	-	-	0.60	ND	++	++	+	+	1.73	ND
Genistein[3]	-	+++	+++	++	0.01	0.11	++	++	+++	++	<0.01	0.08
Bowdichione[443]	+++	++	++	++	ND	6.73	+	+	+	-	ND	ND
Flavanones												
Pinocembrin[174]	+	+	-	-	ND	ND	++	+	+	+	7.6	ND
Naringenin[251]	+++	+++	+	+	0.18	0.66	+++	++	+	+	0.09	1.57
Liquiritigenin[84]	+++	+++	++	+	0.14	0.65	+	++	+	+	0.64	ND
Flavonols												
Pinobanksin[428]	+	+	-	-	ND	ND	+	+	+	+	ND	ND
Dalparvinol A[436]	+	+	+	+	ND	ND	++	+	+	+	5.7	ND
Dalparvinol B[440]	-	-	-	-	ND	ND	+	+	+	-	ND	ND
Pterocarpan												
(6a,11a)-3,8-di hydroxy-9-methoxy pterocarpan[426]	-	++	+	-	0.72	ND	-	-	-	+	ND	ND
Chalcone												
Isoliquiritigenin[83]	+++	++	+	+	1.10	3.97	+	++	+	-	1.00	ND
Cinnamylphenols												
Hydroxyobtustyrene [355]	-	+	+	+	ND	ND	+	+	+	+	ND	ND
Xenognosin [435]	-	++	+	+	0.34	ND	-	++	+	+	0.67	ND

+++ : > 100 pM of estradiol (E_2), ++ : > 10 pM of E_2, + : > 1 pM of E_2, -: < 1 pM of E_2.
EqE_{10} and EqE_{100} : $E_2$10 pM, 100 pM equivalent cell proliferation activity of the concentration of test compound : (μM)
ND : not determine

2.2 Luciferase Recepter Gene Assay

2.2.1 Luciferase Recepter Gene effect of compounds from *B. chinensis*

The luciferase reporter assay were tested at the concentrations ranging from 10 nM to 100 μM and their EqE_{10} and EqE_{100} values were determined for the required concentrations in an increase in the luciferase expression equivalent to 10 and 100 pM of E2 treatment, respectively (Table 65). Tectoridin **[13]**, tectorigenin **[14]**, irisflorentin **[7]**, irilin D **[412]** and resveratrol **[57]** were selected to evaluated the luciferase reporter assay and were observed to have luciferase inducing activity in both MCF-7/Luc and T47D/Luc cells. Their EqE_{10} against MCF-7/Luc cells were ranging from 4.25-9.36 μM and EqE_{100} against MCF-7/Luc cells were ranging from 7.75-18.79 μM. No activity against T47D/Luc cells was observed.

Table 65 Luciferase Activities of isolated compounds from *Belacanda chinensis* against transfected MCF-7 and T47D

Compound	Transfected MCF-7		Transfected T47D	
	EqE_{10} (μM)	EqE_{100} (μM)	EqE_{10} (μM)	EqE_{100} (μM)
Irisflorentin[7]	9.18	ND	ND	ND
Irilin D[412]	4.25	ND	ND	ND
Tectoridin [13]	4.28	8.22	ND	ND
Resveratrol [57]	9.36	ND	ND	ND

EqE_{10} and EqE_{100} : E_2 10 pM, 100 pM equivalent cell proliferation activity at the concentration of test compounds (μM)
ND : not determine

2.2.2 Luciferase Recepter Gene Effect of Compounds from *D. parviflora*

The luciferase reporter assay were tested with increasing concentration ranging from 10 nM to 100 μM and their EqE_{10} and EqE_{100} values were determined for the required concentrations against cell proliferation equivalent to 10 and 100 pM of estradiol (E2) treatment, respectively

In isoflavans group, only mucronulatol **[77]** was observed to have luciferase including activity in both MCF-7/Luc (EqE_{10} against MCF-7/Luc: 0.86 μM, EqE_{100} against MCF-7/Luc: 1.72 μM) whereas, arizonicanol A **[431]** have luciferase inducing activity only T47D/Luc cells (EqE_{10}: 1.32 μM and EqE_{100}: 3.71 μM). All compounds of isoflavanones, 3′-methoxyviolanone **[197]**, onogenin **[252]**, sativanone **[175]**, secundiflorol H **[429]**, violanone **[177]**, vestitone **[176]**, dalparvin **[438]** and 7,3′-dihydroxy-4′-methoxyisoflavanol **[430]** showed luciferase inducing activity in T47D/Luc cells in the rang of EqE_{10} 0.61-7.33 μM. Onogenin **[252]**, sativanone **[175]** and violanone **[177]** showed moderate luciferase inducing activity in the range of EqE_{100} 5.55, 1.08 and 1.10 μM. For 7,3′-dihydroxy-4′-methoxy isoflavanol **[430]** and vestitone **[176]** showed lower luciferase inducing activity than other compound, since their cell proliferation activity also showed low activity. Secundiflorol H **[429]** and dalparvin **[438]** exhibited high activity in MCF-7/Luc cells with EqE_{10} 0.58 μM, 0.66 μM, EqE_{100} 2.72 μM and 1.62 μM, respectively. The other compounds were not determined. Almost all isoflavones showed luciferase inducing activity. The highest increase in induction of luciferase in MCF-7/Luc cells was observed with EqE_{10} and/or EqE_{100} <0.01 μM of formononetin **[72]**, genistein **[3]**, khrinone D **[424]**, theralin **[442]**. The other compound was observed with EqE_{10} ranging from 0.60-5.81 μM and EqE_{100} ranging from 0.44-7.79 μM. The highest increase in induction of luciferase in T47D/Luc cells were biochanin A **[95]**, formononetin **[72]**, genistein **[3]**. It showed that formononetin **[72]** and genistein **[3]** have highest activity in both cells. The other isoflavones were observed with EqE_{10} ranging from 0.20-8.84 μM and EqE_{100} ranging from 0.55-8.40 μM. In addition, liquiritigenin **[84]**, isoliquiritigenin **[83]** and (6a, 11a)-3,8-dihydroxypterocarpan **[426]** showed highest activity in MCF-7/Luc cells at EqE_{10} and/or EqE_{100} <0.01 μM,

moreover liquiritigenin **[84]**, naringenin **[251]** and xenognosin **[435]** also showed highest activity in T47D/Luc cells. The results are summarized in Table 66.

Table 66 Luciferase Activities of isolated compounds from *Dalbergia pariflora* against transfected MCF-7 and T47D cells.

Compound	MCF-7		T47D	
	EqE_{10} (μM)	EqE_{100} (μM)	EqE_{10} (μM)	EqE_{100} (μM)
Isoflavans				
Khriol A**[422]**	ND	ND	ND	ND
Mucronulatol**[77]**	0.86	1.72	ND	ND
8-Demethylduartin **[427]**	ND	ND	ND	ND
Arizonicanol A**[431]**	ND	ND	1.32	3.71
Vesitol**[78]**	ND	ND	ND	ND
5′-Methoxy violanone **[183]**	ND	ND	ND	ND
Isoflavanones				
3′-Methoxy violanone **[197]**	ND	ND	3.30	ND
Onogenin **[252]**	ND	ND	1.92	5.55
Sativanone **[175]**	ND	ND	0.70	1.08
Secundiflorol H **[429]**	0.58	2.72	1.30	ND
7,3′-Dihydroxy-4′-methoxyisoflavanone **[430]**	ND	ND	5.99	ND
Violanone**[177]**	ND	ND	0.61	1.10
Vestitone**[176]**	ND	ND	7.33	ND
Dalparvin**[438]**	0.66	1.62	2.56	ND
Isoflavones				
7-Demethylrobus-tigenin**[423]**	5.41	7.79	6.27	ND
Khrinone D**[424]**	<0.01	0.58	5.27	ND
Biochanin A**[95]**	ND	ND	<0.01	<0.01
2′-Methoxy-biochanin A**[425]**	ND	ND	1.78	3.11
Tectorigenin **[14]**	ND	ND	1.39	2.23
Khrinone C**[432]**	ND	ND	0.20	1.99
Pratensein**[433]**	ND	ND	ND	ND

Table 66 (Continue)

Compound	MCF-7		T47D	
	EqE_{10} (μM)	EqE100 (μM)	EqE_{10} (μM)	EqE_{100} (μM)
2′-Methoxy-formononetin[434]	ND	ND	1.81	6.24
Formononetin[72]	<0.01	<0.01	<0.01	<0.01
Khrinone B[437]	ND	ND	ND	ND
Khrinone A[439]	ND	ND	5.64	8.40
Khrinone E[441]	5.81	ND	ND	ND
3′-Methoxy-daidzein[196]	1.07	2.20	1.18	5.84
Calycosin[81]	0.60	4.67	8.84	ND
Theralin[442]	<0.01	0.44	ND	ND
Genistein[3]	<0.01	<0.01	<0.01	0.55
Bowdichione[443]	0.66	0.88	ND	ND
Flavanones				
Pinocembrin[174]	ND	ND	ND	ND
Naringenin[251]	1.65	2.63	<0.01	5.83
Liquiritigenin[84]	<0.01	<0.01	<0.01	5.88
Flavonols				
Pinobanksin[428]	2.42	5.77	ND	ND
Dalparvinol A[436]	ND	ND	0.32	ND
Dalparvinol B[440]	1.23	3.44	ND	ND
Pterocarpan				
(6a,11a)-3,8-di hydroxy-9-methoxy pterocarpan[426]	<0.01	<0.01	ND	ND
Chalcone				
Isoliquiritigenin[83]	ND	<0.01	ND	ND
Cinnamylphenols				
Hydroxyobtustyrene [355]	ND	ND	ND	ND
Xenognosin [435]	ND	ND	<0.01	5.71

EqE_{10} and EqE_{100} : E_210 pM, 100 pM equivalent cell proliferation activity of the concentration of test compound : (μM)
ND : not determine