CHAPTER IV

Results and Discussion

The dried stem bark of *Fissistigma polyanthoides* (DC.) Merr. was extracted with ethanol. The ethanolic extract was then chromatographed over a kieselguhr column and eluted with hexane, chloroform and methanol, respectively. The hexane fraction was further separated by vacuum liquid column chromatography and gel filtration (Sephadex LH20) to give compound FP-1. The dried leaves of *Ochna integerrima* (Lour.) Merr. were extracted with petroleum ether, ethyl acetate and methanol, respectively. The ethyl acetate extract was then separated using several chromatographic techniques to give compounds OC-1, OC-2 and OC-3. The structures of these isolated compounds were determined by analysis of spectroscopic data (UV, IR, NMR and MS) and chemical studies.

1. Structure Determination

1.1 *Fissistigma polyanthoides*

1.1.1 Structure Determination of Compound FP-1

Compound FP-1 was obtained as yellow crystals. The EIMS (Figure 5) showed a molecular ion peak at *m/z* 314 [M^+], consistent with the molecular formula $C_{17}H_{14}O_6$. The ^1H NMR spectrum (Figure 6) exhibited signals for two methoxy groups at δ 3.99 and 4.15 ppm, and five aromatic protons at δ 7.50-7.56 (3H) and 7.93-7.97 (2H). In addition, the spectrum showed two one-proton singlets at δ 6.68 and 12.24 ppm, which

could be assigned to H-3 and 5-OH, respectively. The ^{13}C NMR and DEPT spectra (Figures 7 and 8) showed the presence of two methyl, six methine and nine quaternary carbons. FP-1 was identified as 5,8-dihydroxy-6,7-dimethoxyflavone **(83)**, a flavone previously isolated from *Fissistigma lanuginosum* (Alias *et al.*, 1995) (Table 9).

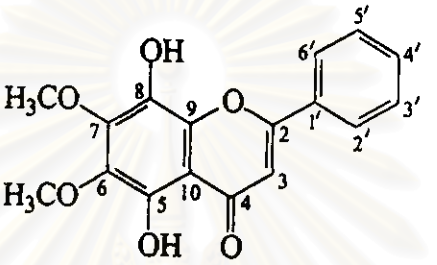

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Table 9 ^{1}H and ^{13}C NMR spectral data of compound FP-1 (in $CDCl_3$) and 5,8-dihydroxy-6,7-dimethoxyflavone (in $CDCl_3$)

position	compound FP-1		5,8-dihydroxy-6,7-dimethoxyflavone	
	δH (ppm) (multiplicity, *J* in Hz)	δC (ppm)	δH (ppm) (multiplicity, *J* in Hz)	δC (ppm)
2		164.6		164.3
3	6.68 (s)	107.5	6.70 (s)	107.3
4		183.7		183.3
5	12.24 (s) (OH)	146.4	12.30 (s) (OH)	146.0
6		136.3		136.0
6-OCH_3	3.99 (s)	61.4	3.98 (s)	61.1
7		146.6		146.4
7-OCH_3	4.15 (s)	62.1	4.15 (s)	61.9
8	5.71 (br s) (OH)	130.0	5.45 (br s) (OH)	129.7
9		140.2		139.9
10		105.5		107.3
1′		131.7		131.5
2′	7.93-7.97 (m)	129.5	7.97-7.99 (m)	129.2
3′	7.50-7.56 (m)	126.8	7.52-7.58 (m)	126.6
4′	7.50-7.56 (m)	132.4	7.52-7.58 (m)	132.1
5′	7.50-7.56 (m)	126.8	7.52-7.58 (m)	126.6
6′	7.93-7.97 (m)	129.5	7.97-7.99 (m)	129.2

1.1.2 Rivision of Structure of Compound ALK1

Compound ALK1 was an alkaloid isolated from the stem bark of *Fissistigma polyanthoides*, and its spectral data have been earlier reported (Theraratchailert, 1996). It was initially identified as 1,10-dihydroxy-2,3,9-trimethoxy-tetrahydroprotoberberine (capaurimine). The structure was revised in this study by analysis of its HMBC data and comparison of its ^{1}H NMR data with those of (-)-thaipetaline (Lavault *et al.*, 1990).

The mass spectrum of ALK1 (Figure 9) revealed a molecular peak at *m/z* 357, consistent with the molecular formula $C_{20}H_{23}NO_5$. The fragmentation pattern was indicative of a tetrahydroprotoberberine structure. The base peak, *m/z* 208, was due to a retro-Diels-Alder fission and corresponded to the ion formed by rings A and B bearing two methoxyl groups and a hydroxyl substituent. The fragment at *m/z* 149 corresponded to the D ring substituted by one methoxyl and one hydroxyl group (Scheme 1).

From the ^{1}H NMR spectrum (Figures 10-11), the substitution of C-9 and C-10 of ring D was suggested by the signals of two one-proton doublets at δ 6.80 and 6.83 (J = 8.4 Hz) due to H-11, H-12. The presence of two one-proton doublets at δ 3.58 and 4.22 (J = 15.5 Hz) due to the C-8 methylene group also supported a 9,10-substitution pattern. Inaddition, the high intensity of the peak at *m/z* 149 in the mass spectrum indicated a 9-methoxyl-10-hydroxyl-substitution. The spectrum displayed a one proton singlet at δ 6.36 in the aromatic region, suggesting that the phenolic function at ring A was at either C-1 or C-4. The study of the chemical shifts of the C-5 and C-13 methylene protons indicated a C-4 substitution. If C-1 was substituted, C-13 protons would resonate around δ 2.7 and 3.8, while C-5 protons would appear around δ 2.65 and 3.15. The HMBC correlation peak between H-1 and C-14 confirmed the 2,3,4-oxygenated

substitution of ring A. The 2- and 3-bond couplings observed between H-1 and C-2, and H-1 and C-3 placed the methoxyl groups at C-2 and C-3. Thus, ALK1 is 2,3,9-trimethoxy-4,10-dihydroxy tetrahydroprotoberberine **(84)**. This compound has been isolated from *Polyalthia stenopetala* and given the name (-)-thaipetaline. Comparison of the ^{1}H NMR data of ALK1 with those of (-)-thaipetaline did confirm their identity. Finally complete ^{13}C NMR assignments of ALK1 (Figure 12) were obtained by analysis of the HMBC spectrum (Figures13-17) (Table 10).

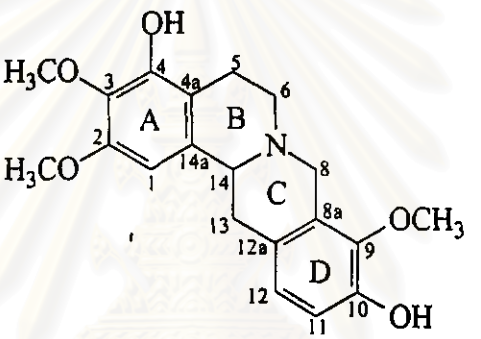

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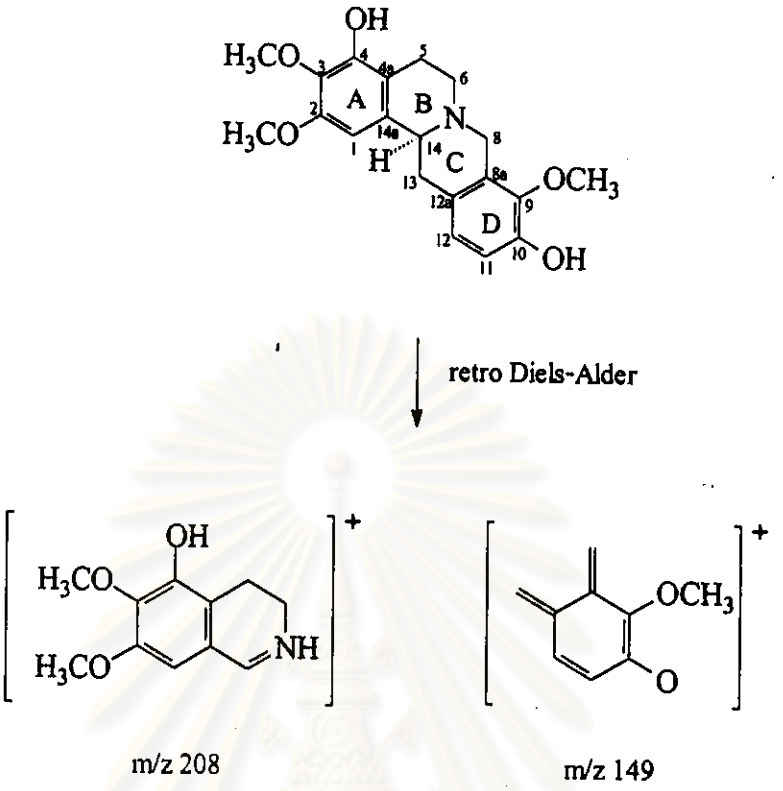
Table 10 ^{1}H **and** ^{13}C **NMR spectral data of compound ALK1 (in** $CDCl_3$**) with long-range correlations observed in HMBC spectrum and** ^{1}H **NMR data of (-)-thaipetaline (in** $CDCl_3$**)**

position	(-)-Thaipetaline	Compound ALK1		
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (correlation with proton)
1	6.38 (s)	6.36 (s)	100.6	-
2			143.2	H-1* and 2-OCH_3
3			133.7	H-1 and 3-OCH_3
4			146.4	H-5
4a			114.7	H-1, H-5*, H-6 and H-14
5	2.60, 2.83 (m)	2.59, 2.83 (m)	23.0	H-6*
6	2.84, 3.23 (m)	2.83, 3.24 (m)	51.0	H-8
8	3.58, 4.22 (d, 15.5)	3.58, 4.22 (d, 15.5)	53.9	H-6 and H-14
8a			127.9	H-8*, H-12 and H-13
9			150.5	H-8, H-11 and 9-OCH_3
10			146.5	H-11* and H-12
11	6.81 (d, 8.5)	6.80 (d, 8.4)	114.1	-
12	6.83 (d, 8.5)	6.83 (d, 8.4)	124.9	H-11*
12a			127.0	H-8, H-11, H-12*, H-13* and H-14
13	2.83, 3.23 (m)	2.83, 3.24 (m)	36.1	H-12
14	3.58 (m)	3.58 (dd, 11, 3.6)	59.6	H-1, H-6, H-8 and H-13*
14a			133.6	H-1* and H-14*
2-OCH_3	3.89 (s)	3.88 (s)	55.9	-
3-OCH_3	3.91 (s)	3.90 (s)	60.7	-
9-OCH_3	3.83 (s)	3.82 (s)	61.0	-

*Two-bond coupling



Scheme 1 Mass fragmentation Pattern of ALK1

1.2 *Ochna integerrima*

1.2.1 Structure Determination of Compound OC-1

Compound OC-1 was obtained as yellow crystals. The UV spectrum (Figure 18) showed absorption maxima at 207 (4.55), 289 (4.31) and 346 (3.57) nm. The IR spectrum (Figure 19) exhibited absorption bands at 3413 (hydroxyl group), 1636 (carbonyl group), 1445-1583 (aromatic ring) and 1101, 1176 (C-O bending) cm^{-1}. The FAB-MS (Figure 20) showed a quasi-molecular ion peak at *m/z* 535 $[M+H]^+$, consistent with the molecular formula $C_{26}H_{30}O_{12}$.

The ^1H NMR and COSY spectra (Figures 21 and 24-25) of OC-1 showed similarity to that of taxifolin (Harborne, 1994) except for the absence of the signal for H-6. In addition, signals for a γ,γ-dimethylallyl substituent [δ 3.38 (1H, m), 3.11 (1H, m), 5.17 (1H, br dd, *J*=7.0, 7.0 Hz), 1.71 (3H, s), 1.61 (3H, s)] and for a sugar moiety [δ 4.91 (1H, d, J=7.6 Hz), 3.26-3.65 (6H, m)] were present. The ^{13}C NMR resonances at δ 20.9 (CH_2), 122.5 (CH), 130.5 (C), 17.8 (CH_3) and 25.6 (CH_3) in the DEPT spectra (Figure 23) confirmed the presence of the isopentenyl unit. The sugar moiety was identified as glucose from its ^{13}C NMR signals (Figure 22) at δ 100.1 (C-1″), 77.3(C-2″), 76.6 (C-3″), 69.6 (C-4″), 77.1 (C-5″) and 60.6 (C-6″), and it was located at C-7 because of the NOESY correlation of H-1″ with H-8 (Figures 28-29). This was also substantiated by the HMBC correlation between H-1″ and C-7 (Figure 33). Therefore, the isoprene unit was placed at C-6. This was corroborated by the HMBC correlations between H_2-9 and C-5 (Figure 33). Thus, OC-1 was identified as 6-γ,γ-dimethylallyl taxifolin 7-*O*-β-D-glucoside (**85**). Complete ^{13}C NMR assignments were obtained by examination of the HETCOR and HMBC spectra (Figures 26-27 and 30-33) (Table 11). OC-1 is a new flavonoid glycoside.

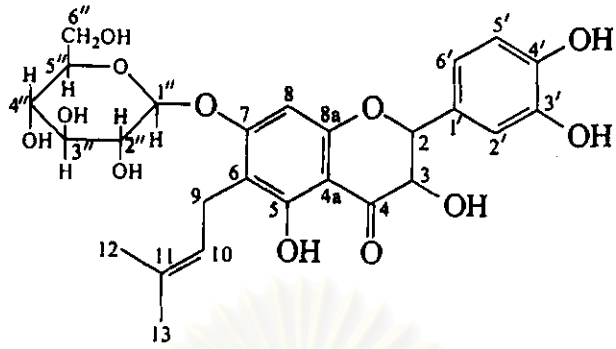


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Table 11 ^{1}H **and** ^{13}C **NMR spectral data of compound OC-1 (in DMSO-d_6) with long-range correlations observed in HMBC spectrum**

position	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	HMBC (correlation with proton)
2	5.01 (d, 11.3)	83.5	H-3*, H-2′ and H-6′
3	4.55 (d, 11.3)	72.0	H-2*
4		199.2	H-2 and H-3*
4a		101.9	H-8
5	12.05 (s) (OH)	159.7	H-9
6		109.9	H-8 and H_2-9*
7		163.3	H-8*, H-9 and H-1″
8	6.21 (s)	94.2	-
8a		160.7	H-8*
9	3.11 (m)	21.1	H-10*
	3.38 (m)		-
10	5.17 (br dd, 7.0,7.0)	122.7	H-9*, H-12 and H-13
11		130.6	H-9, H-12* and H-13*
12	1.71 (s)	17.9	H-10
13	1.61 (s)	25.8	H-10
1′		128.1	H-2*, H-3, H-5′ and H-6′*
2′	6.89 (s)	115.6	H-6′
3′		145.2[a]	H-2′* and H-5′
4′		146.1[a]	H-2′ and H-6′
5′	6.75 (s)	115.4	H-6′*

Table 11 ^{1}H **and** ^{13}C **NMR spectral data of compound OC-1 (in DMSO-d_6) with long-range correlations observed in HMBC spectrum (continued)**

position	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	HMBC (correlation with proton)
6′	6.75 (s)	119.7	H-2 and H-5′*
1″	4.91 (d, 7.6)	100.3	H-2″*, H-3″ and H-5″
2″	3.26 (m)	73.5	H-3″* and H-4″
3″	3.28 (m)	76.8	H-2″* and H-4″*
4″	3.15 (m)	69.8	H-2″ and H-6″
5″	3.40 (m)	77.3	H-3″, H-4″* and H-6″*
6″	3.43 (m)	60.8	-
	3.65 (dd, 11.3, 1.8)		

*Two-bond coupling

[a]Assignments are interchangeable

1.2.2 Structure Determination of Compound OC-2

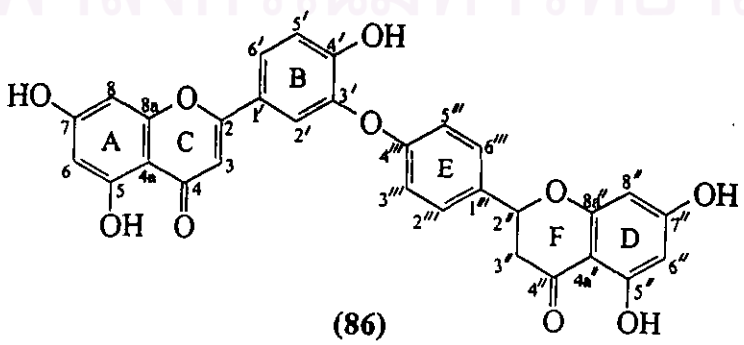
Compound OC-2 was obtained as a yellow powder. The UV spectrum (Figure 34) showed absorption maxima at 209 (4.78), 289 (4.43), 334 (4.33) nm. The IR spectrum (Figure 35) exhibited absorption bands at 3250 (hydroxyl group), 1648 (carbonyl group), 1435-1616 (aromatic ring) and 1161 (C-O bending) cm^{-1}. The FAB-MS (Figure 36) showed a quasi-molecular ion peak at *m/z* 541 $[M+H]^+$, consistent with the molecular formula $C_{30}H_{20}O_{10}$. Examination of ^1H NMR spectrum suggested that it was a biflavonoid. It was identified as 2″,3″-dihydroochnaflavone **(86)**, which is a new structure.

The ^1H NMR spectrum of OC-2 (Figure 37) showed two hydroxyl resonances appearing in the downfield region at δ 12.90 (H-5) and δ 12.11 (H-5″). The spectrum showed a one proton singlet at δ 6.8 (H-3) and three double doublets at δ 5.53 (J = 12.8, 3.1 Hz, H-2″), δ 3.26 (J = 17.1, 12.8 Hz, H-3″) and δ 2.73 (J = 17.1, 3.1 Hz, H-3″). Two meta-coupled doublets appearing at δ 6.18 and 6.47 (J = 2.1 Hz) could be assigned to H-6 and H-8 and two doublets at δ 5.88 (d, J = 2.2 Hz) and δ 5.89 (d, J = 2.2 Hz) could be assigned to H-6″ and H-8″. The signals at δ 7.13 (d, J = 8.5 Hz), 7.79 (d, J = 2.5 Hz) and 7.85 (dd, J = 8.5, 2.5 Hz) corresponded to H-5′, H-2′ and H-6′, respectively. The spectrum also showed the presence of two sets of A_2B_2-type doublets (J = 8.7 Hz) at δ 7.46 (2H) and 6.91 (2H) which could be assigned to the H-2‴ and H-6‴, and H-3‴ and H-5‴. Couplings among these protons were observed in a ^1H-^1H COSY experiment (Figures 40-41).

The ^{13}C NMR spectrum (Figure 38) showed 28 peaks. From the molecular formula, the structure should possess 30 carbon atoms. This suggested that there must be 2 pairs of carbons with equivalent chemical shift at δ 116.2 and 128.8 which could be assigned to C-3‴, C-5‴ and C-2‴, C-6‴. The signals for two carbonyl groups showed

at δ 182.1 and 196.5 ppm. The spectra of DEPT-90 and DEPT-135 (Figure 39) presented thirteen methine carbons, one methylene carbons and sixteen quaternary carbons, all of which were subsequently assigned by analysis of the HETCOR spectrum (Figures 42-43). Comparison of the above spectral data with the previously reported values of the biflavonoids from *Ochna obtusata* (Rao *et al.*, 1997) hinted that compound OC-2 could be a flavanone-flavone based biflavonoid.

From the ^{13}C NMR spectrum, the C-3′ (δ 142.8), C-4′ (δ 154.0) and C-4‴ (δ 158.5) signals appeared in the more downfield region than other carbons on the same ring, suggesting that C-4′ was substituted with a hydroxyl group, and that C-3′ and C-4‴ formed C-O-C linkage between the flavanone-flavone. The structure was confirmed by a HMBC experiment. The HMBC spectrum (Figures 44-48) showed correlations of C-3′ (δ 142.8) with H-2′ (δ 7.79) and H-5′ (δ 7.13), C-4′ (δ 154.0) with H-2′ (δ 7.79) and H-6′ (δ 7.84) and C-4‴ (δ 158.5) with H-2‴ and H-6‴ (δ 7.46), H-3‴ and H-5‴ (δ 6.91), suggesting that it was unsubstituted at C-2′, C-5′, C-6′, C-2‴, C-3‴, C-5‴ and C-6‴ positions. The correlations of C-2″ (δ 78.5) with C-2‴ and C-6‴ confirmed a flavanone skeleton. The long-range correlations of C-4a (δ 104.1) with H-3 (δ 6.83), H-6 (δ 6.18) and H-8 (δ 6.47) and C-4a″ (δ 102.2) with H-6″ and H-8″ confirmed the unsubstituted positions at C-3, C-6, C-8, C-6″ and C-8″. The correlations of HMBC spectrum are shown in Table 12.



(86)

OC-2 on methylation with CH_3I/Ag_2O in methanol gave a pentamethylation product (OC-2-Me, 87). The ^1H NMR spectrum of the methylation product (Figure 49) showed the signals of five methoxyl groups at δ 3.79 (s, 3H, 7″-OCH_3), δ 3.88 (s, 3H, 5″-OCH_3), δ 3.89 (s, 3H, 7-OCH_3), δ 3.90 (s, 3H, 4′-OCH_3), δ 3.93 (s, 3H, 5-OCH_3). The other protons appeared at δ 2.78 (dd, 1H, J = 17.1, 3.1 Hz, H-3″), δ 3.04 (dd, 1H, J = 17.1, 12.8 Hz, H-3″), δ 5.38 (dd, 1H, J = 12.8, 3.1 Hz, H-2″), δ 6.07 (d, 1H, J = 2.2 Hz, H-6″), δ 6.14 (d, 1H, J = 2.2 Hz, H-8″), δ 6.34 (d, 1H, J = 2.1 Hz, H-6), δ 6.51 (d, 1H, J = 2.1 Hz, H-8), δ 6.55 (s, 1H, H-3), δ 6.92 (d, 2H, J = 8.9 Hz, H-3‴ and H-5‴), δ 7.10 (d, 1H, J = 8.5 Hz, H-5′), δ 7.40 (d, 2H, J = 8.9 Hz, H-2‴ and H-6‴), δ 7.52 (d, 1H, J = 2.5 Hz, H-2′), δ 7.71 (dd, 1H, J = 8.5, 2.5 Hz, H-6′). The EIMS (Figure 50) exhibited a molecular ion peak at *m/z* 610, consistent with the molecular formula $C_{35}H_{30}O_{10}$. In a NOESY experiment (Figure 51), NOEs were observed between 5-OCH_3 and H-6, 7-OCH_3 and H-6, H-8, 4′-OCH_3 and H-5′, 5″-OCH_3 and H-6″, and 7″-OCH_3 and H-6″, H-8″, confirming the structure of OC-2 as 2″,3″-dihydroochnaflavone.

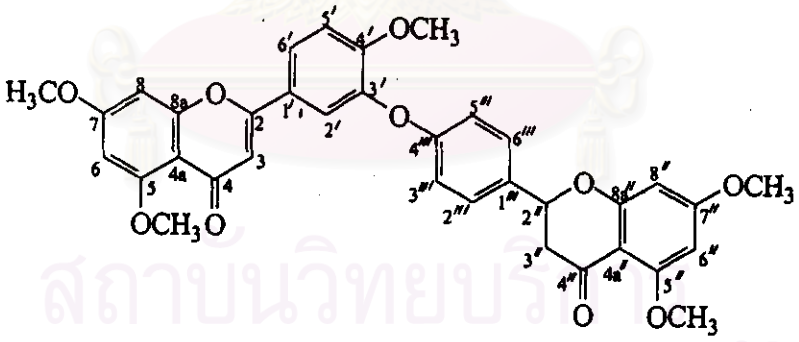


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Table 12 ^{1}H **and** ^{13}C **NMR spectral data of compound OC-2 (in DMSO-d_6) with long-range correlations observed in HMBC spectrum**

position	δ_H (ppm) (multiplicity, J in Hz)	δC (ppm)	HMBC (correlation with proton)
2		163.3	H-3*, H-2′ and H-6′
3	6.83 (s)	104.0	-
4		182.2	H-3*
4a		104.2	H-3, H-6 and H-8
5	12.90 (s) (OH)	161.9	H-6*
6	6.18 (d, 2.1)	99.4	H-8
7		164.7	H-6* and H-8*
8	6.47 (d, 2.1)	94.6	H-6
8a		157.8	H-8*
1′		122.7	H-3 and H-5′
2′	7.79 (d, 2.5)	121.5	H-6′
3′		142.8	H-2′* and H-5′
4′		154.0	H-2′ and H-6′
5′	7.13 (d, 8.5)	118.3	-
6′	7.84 (dd, 8.5, 2.5)	125.3	H-2′
2″	5.53 (dd, 12.8, 3.1)	78.5	H-3″*, H-2‴ and H-6‴
3″	2.73 (dd, 17.1, 3.1) 3.26 (dd, 17.1, 12.8)	42.4	-
4″		196.5	H-3″*
4a″		102.2	H-6″ and H-8″
5″	12.11 (s) (OH)	164.0	H-6″*

Table 12 ^{1}H and ^{13}C NMR spectral data of compound OC-2 (in DMSO-d_6) with long-range correlations observed in HMBC spectrum (continued)

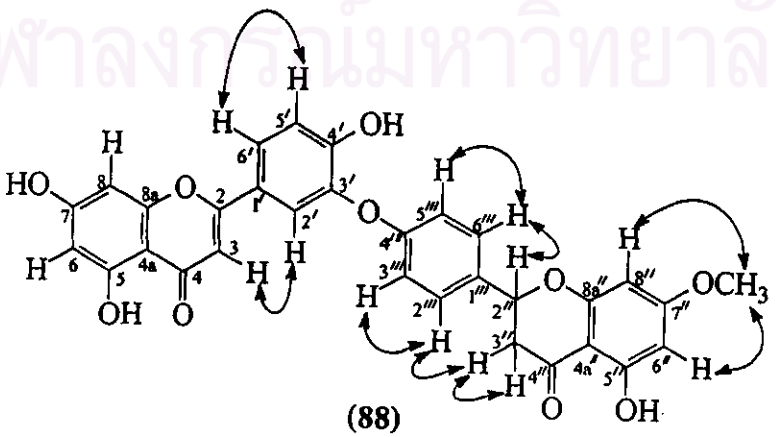
Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (correlation with proton)
6″	5.88 (d, 2.2)	96.4	H-8″
7″		167.2	H-6″* and H-8″*
8″	5.89 (d, 2.2)	95.5	H-6″
8a″		163.3	H-6″ and H-8″*
1‴		132.8	H-2″*, H-3‴ and H-5‴
2‴	7.46 (d, 8.9)	128.8	H-2″ and H-6‴
3‴	6.91 (d, 8.9)	116.2	H-5‴
4‴		158.5	H-3‴*, H-5‴*, H-2‴ and H-6‴
5‴	6.91 (d, 8.9)	116.2	H-3‴
6‴	7.46 (d, 8.9)	128.8	H-2″ and H-2‴

*Two-bond coupling

1.2.3 Structure Determination of Compound OC-3

Compound OC-3 was obtained as a yellow powder. The UV spectrum (Figure 52) showed absorption maxima at 211 (4.85), 288 (4.56), 333 (4.45) nm. The IR spectrum (Figure 53) exhibited absorption bands at 3401 (hydroxyl group), 1651 (carbonyl group), 1437-1619 (aromatic ring) and 1161 (C-O bending) cm^{-1}. The FAB-MS (Figure 54) showed a quasi-molecular ion peak at *m/z* 555 $[M+H]^+$, consistent with the molecular formula $C_{31}H_{22}O_{10}$. Compound OC-3 was identified as 2″,3″-dihydroochnaflavone 7″-*O*-methyl ether (**88**), a new natural product.

The ^1H NMR spectrum (Figure 55), the ^{13}C NMR spectrum (Figure 56), the DEPT-90 and DEPT-135 spectra (Figure 57), the ^1H-^1H COSY spectrum (Figures 58-59) and the HETCOR spectrum (Figures 60-61) showed close similarity to those OC-2, except for the presence of a methoxyl group at δ_H 3.76 (3H, s) and δ_C 56.7 ppm. The H-6″ and H-8″ protons appeared as two doublets at δ 6.09 (J = 1.8 Hz) and δ 6.12 (J = 1.8 Hz) respectively. From the NOESY spectrum (Figures 62-63), 7″-OCH_3 (δ 3.76) showed NOE interactions with H-6″ and H-8″. The other NOE interactions observed in the spectrum are summerized below.



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The COLOC spectrum (Figures 64-65) showed correlation of C-7″ (δ 168.3) with 7″-OCH_3 (δ_H 3.76), H-6″ (δ 6.12) and H-8″ (δ 6.09) confirming the position of the methoxyl group at C-7″ on ring D. All of the NMR signals for protons and carbons could be assigned from the long-range couplings displayed in the COLOC spectrum, as shown in Table 13. On methylation with CH_3I/Ag_2O in *N,N*-dimethyl formamide (DMF) (Figure 66), OC-3 gave OC-3-Me **(87)** a product identical with OC-2-Me **(87)**, thereby confirming the structure of OC-3 as 2″,3″-dihydroochnaflavone 7″-*O*-methyl ether.

Table 13 ^{1}H **and** ^{13}C **NMR spectral data of compound OC-3 (in DMSO-d_6) with long-range correlations observed in COLOC spectrum**

Position	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	COLOC (correlation with proton)
2		163.6	H-2′ and H-6′
3	6.84 (s)	104.4	-
4		182.6	H-3*
4a		104.6	H-8 and 5-OH
5	12.91 (s) (OH)	162.3	H-6*
6	6.19 (d, 1.5)	99.8	-
7		165.1	H-8*
8	6.47 (d, 1.5)	94.9	-
8a		158.2	H-8*
1′		123.1	H-3 and H-5′
2′	7.81 (d, 1.5)	121.8	-
3′		143.1	H-2′* and H-5′
4′		154.3	H-2′
5′	7.14 (d, 8.4)	118.7	-
6′	7.86 (dd, 8.4, 1.5)	125.7	H-2′
2″	5.58 (dd, 12.6, 2.4)	79.1	H-3″*
3″	2.78 (dd, 17.1, 2.4)	42.9	-
	3.33 (dd, 16.8, 12.6)		
4″		197.5	H-3″*
4a″		103.5	5″-OH, H-6″ and H-8″
5″	12.10 (s) (OH)	164.0	H-6″*

Table 13 ^{1}H and ^{13}C NMR spectral data of compound OC-3 (in DMSO-d_6) with long-range correlations observed in COLOC spectrum (continued)

Position	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	COLOC (correlation with proton)
6″	6.09 (d, 1.8)	95.6	H-8″
7″		168.3	7″-OCH_3, H-6″* and H-8″*
7″-OCH_3	3.79 (s)	56.8	-
8″	6.11 (d, 1.8)	94.7	H-6″
8a″		163.6	H-8″*
1‴		133.0	H-3‴ and H-5‴
2‴	7.49 (d, 8.5)	129.2	H-6‴
3‴	6.93 (d, 8.5)	116.6	H-5‴
4‴		158.9	-
5‴	6.93 (d, 8.5)	116.6	H-3‴
6‴	7.49 (d, 8.5)	129.2	H-2‴

* Two-bond coupling

2. Tyrosinase inhibitory activity of pure compounds

Tyrosinase is an enzyme which has been found in mammals and humans. It is involved in the biosynthesis of the skin pigment melanin. Several tyrosinase inhibitors are used for hyperpigmentation treatment. A number of flavonoids have been reported to posses tyrosinase inhibitory activity (Sritularak, 1998).

In this study, the tyrosinase inhibitory activity of each isolate was determined by the modified dopachrome method with L-DOPA as the substrate (Sritularak, 1998). The results indicated that FP-1, OC-1, OC-2 and OC-3 had no activity on tyrosinase at the concentration of 30 μg/ml.