CHAPTER IV

RESULTS AND DISCUSSION

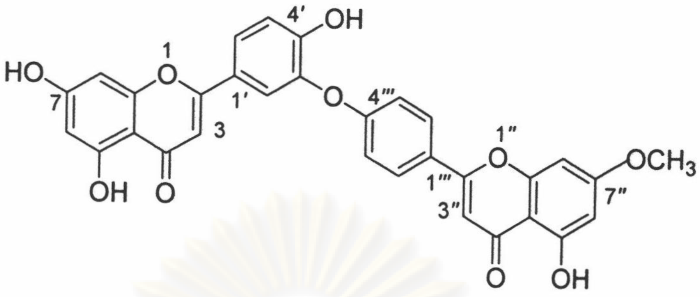
Five extracts prepared from *O. integerrima*, including the ethyl acetate extract of the dried leaves, and the methanol extracts of the stem bark, the stem wood, the root bark and the root wood, were investigated, using several chromatographic techniques. A total of nineteen compounds were obtained. The structures of these compounds were determined based on their UV, IR, MS and NMR data. The DPPH radical scavenging activities of nine compounds were evaluated.

1. Structure Determination of Isolated Compounds

1.1 Structure Determination of Compound 47

Compound **47** was obtained as yellow crystals. The FABMS (Figure 5) showed a quasimolecular ion $[M+H]^+$ at *m/z* 553, consistent with the molecular formula $C_{31}H_{21}O_{10}$. The UV spectrum (Figure 6) exhibited absorption bands at 332, 287, 270, 246 and 211 nm. The ^1H NMR spectrum (Figure 7) displayed two singlet proton signals at δ 6.81 and 6.88 ppm due to H-3 and H-3″, two sets of meta coupling type protons at δ 6.16 and 6.45 ppm (*J*= 2.1, each), and at δ 6.33 and 6.65 ppm (*J*= 2.0, each), corresponding to H-6 and H-8, and H-6″ and H-8″. These proton signals hinted that compound **47** was composed of two flavone moieties. The ^{13}C NMR spectrum (Figure 8) displayed 31 carbon signals, including two flavone carbonyl carbons, twenty-eight *sp*^{2} carbons, supporting the biflavone structure of compound **47**. Compound **47** was identified as 7″-*O*-methyl ochnaflavone (**47**) by comparing its ^1H and ^{13}C NMR data with reported values (Kamil *et al.*, 1983). All of the ^1H and ^{13}C NMR data (Table 21) were completely assigned for the first time, using ^1H-^1H COSY

(Figure 9), HMQC (Figure10), HMBC (Figures 11-12) and NOE difference (Figure 13) experiments.



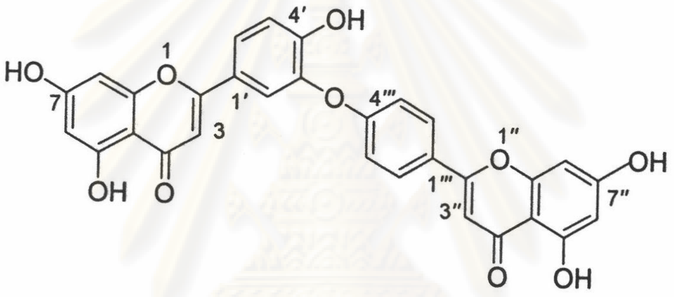
(47)

Table 21 The ^{1}H and ^{13}C NMR data of compound 47 in DMSO–d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2		163.1	2″		163.8
3	6.81 (1H, *s*)	104.0	3″	6.88 (1H, *s*)	104.7
4		182.2	4″		182.4
4a		104.3	4a″		105.3
5		161.9	5″		161.7
6	6.16 (1H, *d*, 2.1)	99.4	6″	6.33 (1H, *d*, 2.0)	98.6
7		164.8	7″		165.7
8	6.45 (1H, *d*, 2.1)	94.6	8″	6.65 (1H, *d*, 2.0)	93.2
8a		157.8	8a″		157.7
1′		122.7	1‴		124.8
2′	7.85 (1H, *d*, 5.8)	125.8	2‴, 6‴	8.03 (1H, *d*, 7.9)	128.9
3′		142.1	3‴, 5‴	7.02 (1H, *d*, 8.8)	116.6
4′		153.9	4‴		161.4
5′	7.14 (1H, *d*, 8.2)	118.5	5-OH	12.87 (1H, *s*)	
6′	7.86 (1H, *d*, 5.8)	121.7	5″-OH	12.83 (1H, *s*)	
			7″-OCH$_3$	3.95 (1H, *s*)	56.5

1.2 Structure Determination of Compound 4

Compound **4** was obtained as a yellow solid. The FABMS (Figure14) exhibited an $[M+H]^+$ ion at *m/z* 539, corresponding to $C_{30}H_{19}O_{10}$. The UV absorptions appeared (Figure 15) at λ_{max} 332, 284, 270, 246 and 211 nm. This compound was determined as ochnaflavone (**4**) (Okigawa and Kuwano, 1973). The ^1H NMR (Figure 16) and ^{13}C NMR (Figure 17) data showed close similarity to those of compound **47** except for the absence of the methoxy group. The present work completely assigned the ^1H and ^{13}C NMR data of this compound (Table 22) by HMQC (Figure 18) and HMBC (Figures 19-20) experiments.



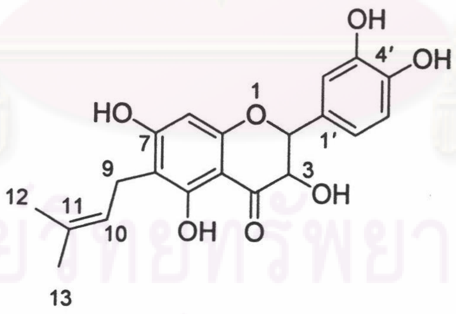
(4)

Table 22 The ^{1}H and ^{13}C NMR data of compound 4 in DMSO–d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2		163.2	2″		163.6
3	6.85 (1H, *s*)	104.1	3″	6.85 (1H, *s*)	104.3
4		182.2	4″		182.3
4a		104.3	4a″		104.3
5		161.9	5″		162.0
6	6.17 (1H, *d*, 1.8)	99.5	6″	6.18 (1H, *d*, 1.8)	99.4
7		164.8	7″		164.9
8	6.48 (1H, *d*, 1.8)	94.7	8″	6.48 (1H, *d*, 1.8)	94.6
8a		157.9	8a″		157.8
1′		122.7	1‴		124.9
2′	7.89 (1H, *m*)	125.8	2‴, 6‴	8.03 (1H, *d*, 7.0)	128.9
3′		142.2	3‴, 5‴	7.02 (1H, *d*, 8.8)	116.6
4′		154.1	4‴		161.3
5′	7.15 (1H, *d*, 8.2)	118.5			
6′	7.88 (1H, *m*)	121.8			

1.3 Structure Determination of Compound 170

Compound **170** was obtained as a yellow solid. The UV absorption bands (Figure 21) appeared at λ_{max} 295, 254 and 204 nm. The IR spectrum displayed (Figure 22) absorption bands at 3337 (OH stretching), 1632 (conjugated C=O stretching) and 1112 (C-O stretching) cm^{-1}. The FABMS spectrum (Figure 23) exhibited a quasimolecular ion peak $[M+H]^+$ at *m/z* 373, consistent to the molecular formula $C_{20}H_{21}O_7$. This compound was identified as 3,3′,4′,5,7-pentahydroxy-6-prenylflavanone (Buckingham, 2001). The ^1H NMR spectrum (Figure 24) showed the typical AB coupled protons at δ 4.87 and 4.48 (J=11.5 Hz, each) due to H-2 and H-3 of a dihydroflavonol, respectively. The olefinic proton at δ 5.18, methylene protons at δ 3.20, two methyl group protons at δ 1.74 and δ 1.64 were assigned to H-10, CH_2-9, CH_3-12 and CH_3-13, respectively. The ^{13}C NMR spectrum (Figure 25) showed twenty carbon signals, corresponding to a dihydroflavonol with a prenyl moiety. The ^1H and ^{13}C NMR assignments were studied using ^1H-^1H COSY (Figure 26), HMQC (Figures 27-28) and HMBC (Figures 29-30) experiments as shown in Table 23.



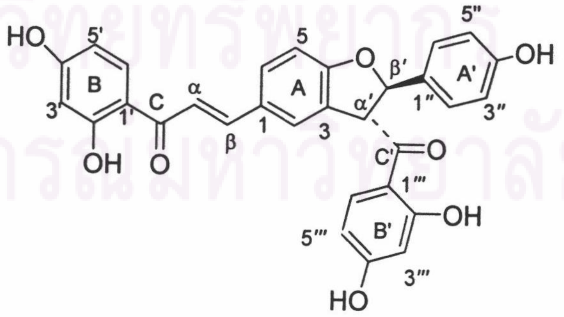
(170)

Table 23 The ^{1}H and ^{13}C NMR data of compound 170 in MeOH-d_4

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	4.87 (1H, *d*, 11.5)	85.9
3	4.48 (1H, *d*, 11.5)	74.6
4		199.2
4a		102.4
5		110.8
6		163.0
7		167.1
8	5.90 (1H, *s*)	96.3
8a		163.0
9	3.20 (2H, *d*, 11.5)	22.7
10	5.18 (1H, *t*, 0.9, 0.9)	124.5
11		132.5
1′		130.8
2′	6.95 (1H, *d*, 2.0)	116.7
3′		147.1
4′		147.9
5′	6.79 (1H, *d*, 12.0)	116.9
6′	6.83 (1H, *d*, 12.0)	121.7
12-CH_3	1.74 (3H, *d*, 1.3)	18.6
13-CH_3	1.64 (3H, *d*, 1.3)	26.7

1.4 Structure Determination of Compound 1

Compound **1** was obtained as a yellow solid. The UV spectrum (Figure 31) displayed absorptions at 370, 391, 286, 254 and 203 nm. The FABMS spectrum (Figure 32) showed a quasimolecular ion peak at *m/z* 511, suggesting a molecular formula of $C_{30}H_{23}O_8$. The ^{13}C NMR spectrum (Figure 34) provided thirty carbon signals, including two carbonyl carbons, twenty-six sp^2 carbons and two aliphatic carbons. Compound **1** was identified as lophirone C (**1**) (Ghogomu Tih, *et al.*, 1989). The ^{1}H NMR spectrum (Figure 33) showed a pair of doublet signals (*J*=15.5 Hz, each) at δ 7.71 and 7.80 ppm assignable to the *trans* olefinic protons H-α and H-β. The aromatic ring protons showed an ABX coupling system at δ 6.35 (*J*=2.3 Hz), 6.49 (*J*=2.3, 8.9 Hz) and 8.00 (*J*=8.9 Hz) which were assigned to H-3′, H-5′ and H-6′, respectively. The other ABX coupled aromatic signals at δ 7.63 (*J*=1.6 Hz), 7.00 (*J*=8.6 Hz) and 7.83 (*J*=1.6, 8.6 Hz) were assigned to H-2, H-5 and H-6. The proton signals at δ 6.42 (*J*=2.6 Hz), 6.56 (*J*=2.6, 8.9 Hz) and 7.98 (*J*=8.9 Hz) ppm were assigned to H-3‴, H-5‴ and H-6‴, respectively. The H-2″(6″) and H-3″(5″) aromatic protons were observed at δ 7.32 (*J*=2.0, 6.6 Hz) and 6.87 (*J*=2.0, 6.6 Hz). The complete ^{1}H and ^{13}C NMR assignments of compound **1** were obtained by ^{1}H-^{1}H COSY (Figure 35), HMQC (Figures 36-37) and HMBC (Figures 38-41) experiments.



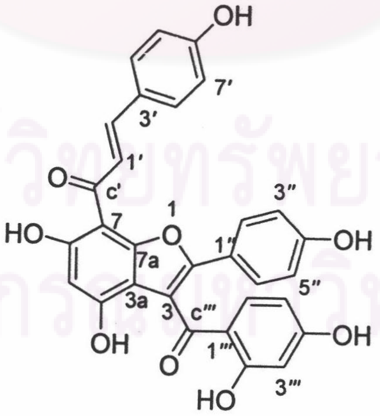
(1)

Table 24 The ^{1}H and ^{13}C NMR data of compound 1 in acetone-d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
1		128.3	1″		131.1
2	7.63 (1H, *d*, 1.6)	126.2	2″, 6″	7.32 (2H, *dd*, 2.0, 6.6)	127.9
3		128.3	3″, 5″	6.87 (2H, *dd*, 2.0, 6.6)	115.5
4		162.3	4″		157.9
5	7.00 (1H, *d*, 8.6)	110.1	1‴		113.0
6	7.83 (1H, *dd*, 8.6, 1.6)	131.2	2‴		166.0
α	7.71 (1H, *d*, 15.5)	118.6	3‴	6.42 (1H, *d*, 2.6)	103.0
β	7.80 (1H, *d*, 15.5)	143.9	4‴		162.3
CO (c)		191.8	5‴	6.56 (1H, *d*, 2.6, 8.9)	108.8
1′		113.6	6‴	7.98 (1H, *d*, 8.9)	133.9
2′		166.4			
3′	6.35 (1H, *d*, 2.3)	102.8			
4′		164.8			
5′	6.49 (1H, *dd*, 2.3, 8.9)	107.9			
6′	8.00 (1H, *d*, 8.9)	132.4			
α′	undetected	undetected			
β′	6.20 (1H, *s*)	87.7			
CO (c′)		200.4			

1.5 Structure Determination of Compound 171

Compound **171** was obtained as an orange amorphous solid. The UV spectrum (Figure 42) showed absorptions at 359, 342, 299, 259 and 201 nm. The IR spectrum (Figure 43) exhibited absorption bands at 2926 (CH-stretching), 1741 (C=O stretching) and 807 (aromatic out of plane bending) cm^{-1}. The FABMS spectrum (Figure 44) revealed the $[M+H]^+$ at *m/z* 525, corresponding to the molecular formula $C_{30}H_{21}O_9$. The ^{13}C NMR spectrum (Figure 46) exhibited thirty carbon signals: two carbonyl carbons, twenty-eight *sp*^{2} carbons. Compound **171** were identified as 3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone (**171**) (Marston, *et al.*, 1988). The ^1H NMR spectrum (Figure 45) showed the *trans* coupled olefinic H-1′ and H-2′ protons at δ 7.91 and 8.26 ppm (*J*=15.2 Hz). The AA′BB′ type doublets (*J*=8.8 Hz, each) at δ 7.72 and 6.97 belonged to H-2″, 6″ and H-3″, 5″. The other AA′BB′ type protons (*J*=8.4 Hz, each) at δ 7.61 and 6.91 were assigned to H-4′, 8′ and H-5′, 7′. The aromatic protons showing an ABX coupling system at δ 6.35 (*J*=2.4 Hz), 6.21 (*J*=2.4, 9.2 Hz) and 7.43 (*J*=9.2 Hz) ppm, were assigned to H-3‴, 5‴ and H-6‴.



(171)

Table 25 The ^{1}H and ^{13}C NMR data of compound 171 in acetone–d_6

Position	Compound171		3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone *	
	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2		153.0		150.0
3		113.8		112.1
3a		112.1		111.2
4		166.6		165.6
5	6.23 (1H, *s*)	99.8	6.25 (1H, *s*)	98.4
6		159.5		153.7
7		102.3		101.0
7a		154.9		153.7
1′	7.91 (1H, *d*, 15.2)	123.0	7.85 (1H, *d*, 15.1)	121.6
2′	8.26 (1H, *d*, 15.2)	144.9	8.15 (1H, *d*, 15.1)	144.1
3′		127.8		125.8
4′, 8′	7.61 (2H, *d*, 8.4)	131.5	7.69 (2H, *d*, 8.0)	130.7
5′, 7′	6.91 (2H, *d*, 8.4)	117.0	6.89 (2H, *d*, 8.5)	116.4
6′		161.2		160.4
CO (c′)		190.3		188.9
1″		121.7		119.7
2″, 6″	7.72 (2H, *d*, 8.8)	129.2	7.50 (2H, *d*, 8.0)	127.2
3″, 5″	6.97 (2H, *d*, 8.8)	116.8	6.85 (2H, *d*, 8.0)	116.0
4″		159.5		158.4

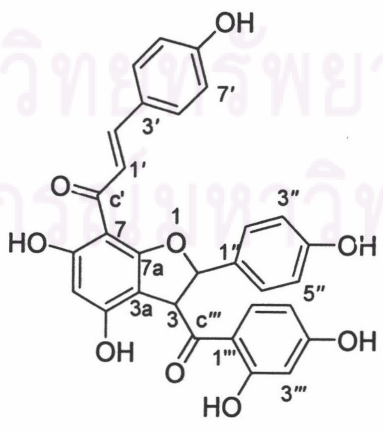
Table 25 The ^{1}H and ^{13}C NMR data of compound 171 in acetone–d_6 (continued)

Position	**Compound171**		**3-(2,4-dihydroxybenzoyl)-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone ***	
	^{1}H δ (ppm), *J* (Hz)	**^{13}C δ (ppm)**	**^{1}H δ (ppm), *J* (Hz)**	**^{13}C δ (ppm)**
1‴		115.4		114.2
2‴		166.6		165.6
3‴	6.35 (1H, *d*, 2.4)	103.4	6.33 (1H, *d*, 2.4)	102.4
4‴		167.7		164.5
5‴	6.21 (1H, *dd*, 2.4, 9.2)	109.3	6.26 (1H, *dd*, 2.4, 8.8)	108.8
6‴	7.43 (1H, *d*, 9.2)	136.7	7.32 (1H, *d*, 8.8)	135.3
CO (c‴)		196.8		195.0

* DMSO-d_6

1.6 Structure Determination of Compound 172

Compound **172** was obtained as a yellow solid. The UV spectrum (Figure 47) showed absorption bands at 368, 285, 261 and 202 nm. The IR spectrum (Figure 48) displayed absorption bands at 3647 (OH-stretching), 3110 (CH-stretching), 1625 (C=O stretching) and 979 (aromatic out of plane bending) cm^{-1}. The FABMS (Figure 49) exhibited the quasimolecular ion peak $[M+H]^+$ at *m/z* 527, consistent with $C_{30}H_{23}O_9$. The ^{13}C NMR spectrum (Figure 51) included thirty carbon signals, comprising two carbonyl carbons, two methine carbons and twenty six *sp*^{2} carbons. The ^1H (Figure 50) and ^{13}C NMR (Figure 51) spectra resembled those of compound **171**, except that a pair of the *trans* coupling methine protons (*J*=5.6 Hz) at δ 6.07 and 5.32 ppm, C-2 (δ 90.1 ppm) and C-3 (δ 54.3 ppm) were aliphatic. These data suggested that compound **172** was a dihydro derivative of compound **171**. Compound **172** was characterized as 3-(2,4-dihydroxybenzoyl)-2,3-dihydro-4,6-dihydroxy-2-(4-hydroxyphenyl)-1-benzofuran-7-yl 2-(4-hydroxyphenyl) ethenyl ketone **(172)** (Marston, *et al.*, 1988). The ^1H and ^{13}C assignments of this compound were studied using ^1H-^1H COSY (Figure 52), HMQC (Figures 53-54) and HMBC (Figures 55-57) techniques as shown in Table 26.



(172)

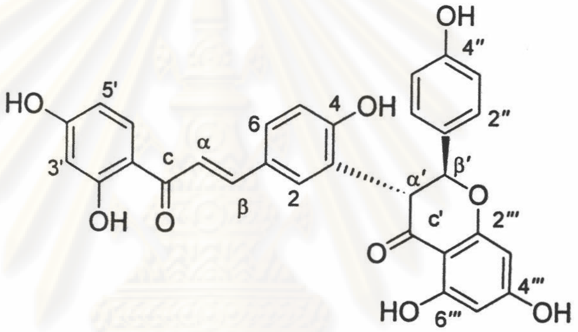
Table 26 The ^{1}H and ^{13}C NMR data of compound 172 in acetone–d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	6.07 (1H, *d*, 5.6)	90.1	1″		131.4
3	5.32 (1H, *d*, 5.6)	54.3	2″, 6″	7.37 (2H, *d*, 8.2)	127.5
3a		105.1	3″, 5″	6.90 (2H, *d*, 6.6)	115.7
4		167.3	4″		158.0
5	5.96 (1H, *s*)	96.4	1‴		113.1
6		167.8	2‴		166.4
7		101.4	3‴	6.37 (1H, *d*, 2.3)	103.2
7a		163.3	4‴		165.5
1′	8.02 (1H, *d*, 15.5)	122.6	5‴	6.38 (1H, *dd*, 2.3, 8.7)	108.0
2′	7.80 (1H, *d*, 15.5)	143.4	6‴	7.77 (1H, *d*, 8.7)	133.4
3′		127.0	CO (c‴)		202.1
4′, 8′	7.49 (2H, *d*, 8.2)	130.5			
5′, 7′	6.85 (2H, *d*, 8.6)	116.0			
6′		160.0			
CO (c′)		190.6			

1.7 Structure Determination of Compound 173

Compound **173** was obtained as a yellow solid. The HRFABMS showed the $[M+H]^+$ at *m/z* 527.1362 (calcd 527.1342) consistent with the molecular formula, $C_{30}H_{23}O_9$. The UV spectrum (Figure 58) showed absorption bands at 374, 292 and 203 nm. The IR spectrum (Figure 59) exhibited absorption bands at 3330 (OH stretching), 1628 (C=O stretching), 1161 (C-O stretching) and 825 (aromatic out of plane bending) cm^{-1}. Its ^{13}C NMR spectrum (Figure 61) exhibited 30 signals which were comprised by two carbonyls, two aliphatic methines, and twenty six olefinic and aromatic carbons, suggesting a biflavonoid structure. In the ^{1}H NMR spectrum (Figures 62-63) of **173**, the typical olefinic protons of a chalcone structure at δ 7.67 and 7.72 (*J*=15.2 Hz, each) and the characteristic aliphatic protons of a flavanone skeleton at δ 4.66 and 5.90 (*J*=12.0 Hz, each) indicated a biflavonoid skeleton of the chalcone-flavanone type for **173**. Examination of the ^{1}H NMR properties of **173** in comparison with those of known biflavonoids containing a chalcone-flavanone structure revealed some similarities between **173** and lophirone B, a biflavonoid obtained from *Lophira lanceolata* (Ghogomu Tih, *et al.*, 1989), except that in **173** the H-5‴ signal appeared as a doublet at δ 6.01 (*J* = 2.0 Hz), and the H-6‴ resonance was not evident, being replaced by a chelated phenolic proton at δ 12.27 (Table 27). In support of this, the ^{13}C NMR spectrum of **173** displayed a significant downfield shift for C-6‴ (δ 34.7 ppm) and expected upfield shifts for C-1‴ (δ 13.0 ppm) and C-5‴ (δ 15.2 ppm), as compared with their counterparts in lophirone B. The presence of an OH-6‴ substituent was confirmed by the long-range couplings with C-1‴ and C-5‴, and the C-3 to C-α′ interflavonoid linkage was ascertained by the correlations of H-2 to C-α′, and H-β′ to C-3 in the HMBC spectrum (Figures 66-70). Thus, **173** is the OH-6‴ derivative of lophirone B. With regard to the stereochemistry of C-α′ and C-β′ carbons on the pyrone ring of the flavanone unit, the large vicinal coupling constant (*J*=12.0 Hz) between H-α′ and H-β′ was suggestive of a *trans* relative configuration. The absolute configuration was then determined by comparing the CD spectrum

(Figure 71) of **173** with that of (2*S*,3*R*)-4′,5,7-tri-*O*- methylnaringenin(3β,3′)-α, 2′,4,4′,6′-pentamethoxychalcone (Bekker, Brandt and Ferreira, 1996). It was observed that the CD data of **173** resembled those of (2*S*,3*R*)-4′,5,7-tri-*O*-methylnaringenin-(3β,3′)-α,2′,4,4′,6′-pentamethoxychalcone ($[\theta]_{326.1}$ = +3.2 x 10^3, ($[\theta]_{275}$ = -3.6 x 10^{-3}), exhibiting a positive Cotton effect at 323.1 nm ($[\theta]_{323.1}$ = +5.5 x 10^3) and a negative sign at 288.2 nm ($[\theta]_{288.2}$ = -3.7 x 10^4). Therefore, the absolute configuration of compound **173** was assigned α′*R*, β′*S*. Compound **173** was characterized as 6‴-hydroxylophirone B (**173**). Its structure was hitherto unknown. The ^{1}H and ^{13}C assignments are shown in Table 27.



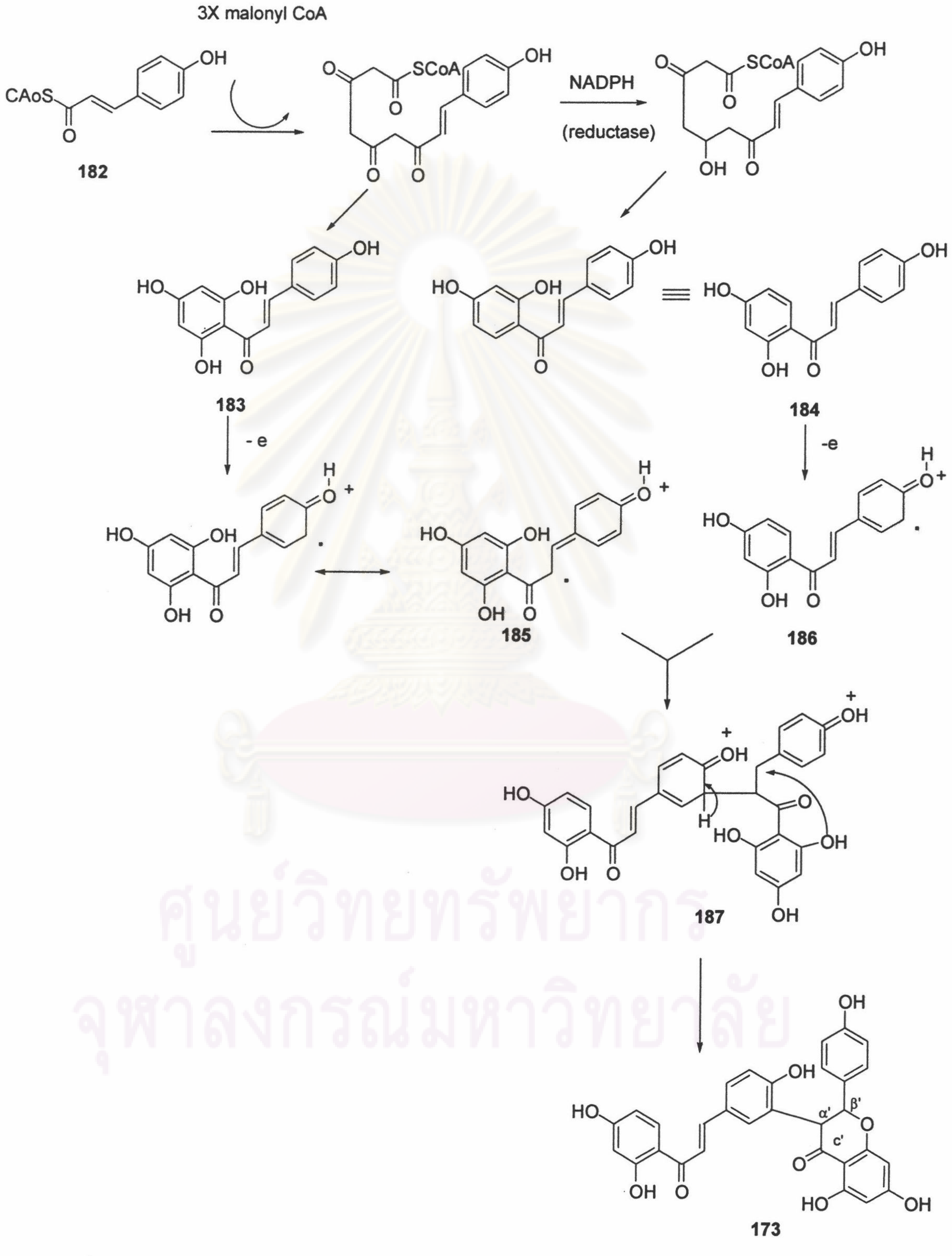
(173)

Table 27 The ^{1}H and ^{13}C NMR data of compound 173 in acetone–d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
1		126.8	α′	4.66 (1H, *d*, 12.0)	54.3
2	7.55 (1H, *br s*)	133.4	β′	5.90 (1H, *d*, 12.0)	82.5
3		123.1	CO (c′)		197.0
4		157.9	1″		129.1
5	6.86 (1H, *d*, 8.9)	115.8	2″, 6″	7.29 (2H, *dd*, 2.0, 6.6)	129.2
6	7.56 (1H, *m*)	129.8	3″, 5″	6.74 (2H, *dd*, 2.0, 6.6)	115.0
α	7.67 (1H, *d*, 15.2)	117.6	4″		157.7
β	7.72 (1H, *d*, 15.2)	144.2	1‴		102.2
CO (c)		191.9	2‴		163.5
1′		113.7	3‴	6.00 (1H, *d*, 2.0)	95.0
2′		166.8	4‴		166.4
3′	6.36 (1H, *d*, 2.3)	103.0	5‴	6.01 (1H, *d*, 2.0)	96.2
4′		164.8	6‴		164.8
5′	6.45 (1H, *dd*, 2.3, 8.9)	107.9	OH-2′	13.60 (1H, *s*)	
6′	8.01 (1H, *d*, 8.9)	132.4	OH-6‴	12.27 (1H, *s*)	

The proposed biogenetic pathway of 6‴-hydroxylophirone B (173)

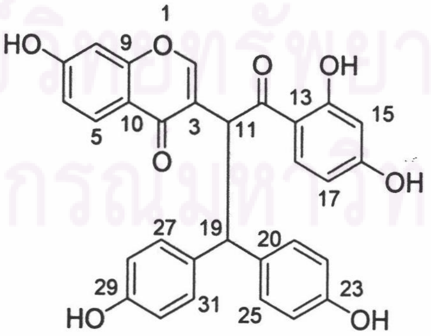
The starter units are 4-hydroxy cinnamoyl CoA (**182**) from the shikimate pathway and the chain extension unit, using three molecules of malonyl CoA from the polyketide pathway. The poly β keto chain could be folded using chalcone synthase enzyme by claisen-like reaction and generated naringenin chalcones (**183**). In the other way, the action of a reductase enzyme concomitant with the chalcone synthase gives isoliquiritigenin (**184**) (Dewick, 2002). One electron oxidation of chalcones **183** and **184** yields intermediates **185** and **186**. Then subsequent regioselective dimerization gives **187**. Structure **187** cyclizes to form a chromanone ring of compound **173** (Shimamura, *et al*., 1996).



Scheme 2 The proposed biogenetic pathway of 6‴-hydroxylophirone B (**173**)

1.8 Structure Determination of Compound 27

Compound **27** was obtained as a white solid. The UV absorption spectrum (Figure 72) showed absorption bands at 286, 263, 234, 227 and 202 nm. The IR spectrum (Figure 73) exhibited OH stretching at 3170 cm^{-1}, C=O stretching at 1627 cm^{-1} and C-O stertching at 1199 cm^{-1}. The FABMS (Figure 74) displayed the $[M+H]^+$ at *m/z* 511, suggesting the molecular formula $C_{30}H_{23}O_8$. The ^1H and ^{13}C NMR data of compound were in agreement with the known compound lophirone A (**27**) (Ghogomu, *et al.*, 1987). The ^{13}C NMR spectrum (Figure 77) exhibited thirty carbon signals, including two carbonyl carbons, two methine carbons and twenty six *sp*^{2} carbons. The ^1H NMR spectrum (Figures 75-76) exhibited the *trans* coupled protons H-11 and H-19 (*J*= 12.4 Hz,each) at δ 6.09 and 4.75 ppm. The AA′BB′ coupling type protons at δ 6.61 (*J*= 8.4 Hz) and 7.21 (*J*= 8.4 Hz) ppm were assigned to H-22, H-24 and H-21, H-25. The other AA′BB′ coupling aromatic ring protons at δ 6.66 (*J*= 8.8 Hz, each) and 7.21 (*J*= 8.4 Hz, each) ppm were assigned to H-28, 30 and H-27, 31. The ABX coupling type protons at δ 7.89 (*J*= 9.2 Hz), 6.84 (*J*= 9.2, 2.4 Hz) and 6.73 (*J*= 2.4 Hz) ppm were assigned to H-5, H-6 and H-8. The trisubstitued aromatic ring protons at δ 6.15 (*J*= 2.4 Hz), 6.40 (*J*= 8.8, 2.4 Hz) and 8.30 (*J*= 8.8 Hz) ppm belonged to H-15, H-17 and H-18, respectively.



(27)

Table 28 The ^{1}H and ^{13}C NMR data of compound 27 in acetone–d_6

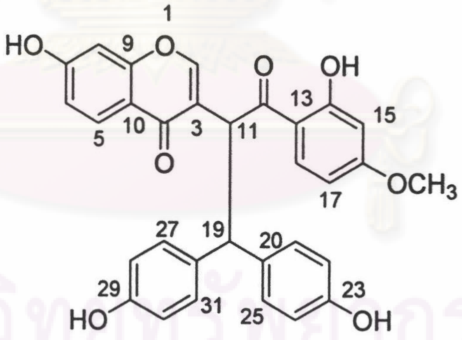
Position	Compound 27		Lophirone A	
	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	8.22 (1H, *s*)	156.2	8.27 (1H, *s*)	156.3
3		122.2		122.1
4		175.1		175.4
5	7.89 (1H, *d*, 9.2)	128.3	7.94 (1H, *d*, 8.8)	128.1
6	6.84 (1H, *dd*, 2.4, 9.2)	115.9	6.91 (1H, *dd*, 2.3, 8.8)	115.9
7		163.3		163.4
8	6.73 (1H, *d*, 2.4)	103.2	6.77 (1H, *d*, 2.3)	103.1
9		158.5		158.5
10		117.3		117.2
11	6.09 (1H, *d*, 12.4)	43.9	6.14 (1H, *d*, 12.3)	43.9
12		204.6		204.5
13		114.1		114.0
14		166.8		166.8
15	6.15 (1H, *d*, 2.4)	103.4	6.20 (1H, *d*, 2.4)	103.3
16		166.0		166.1
17	6.40 (1H, *dd*, 2.4, 8.8)	109.0	6.44 (1H, *dd*, 2.4, 9.0)	108.9
18	8.30 (1H, *d*, 8.8)	134.5	8.34 (1H, *d*, 9.0)	134.3
19	4.75 (1H, *d*, 12.4)	53.3	4.80 (1H, *d*, 12.3)	53.4
20		134.7		134.5
21	7.21 (1H, *d*, 8.4)	130.1	7.26 *m*	130.0
22	6.61 (1H, *d*, 8.4)	115.8	6.61 *m*	115.7
23		156.5		156.4
24	6.61 (1H, *d*, 8.4)	115.8	6.61 *m*	115.7
25	7.21 (1H, *d*, 8.4)	130.1	7.25 *m*	130.0

Table 28 The ^{1}H and ^{13}C NMR data of compound 27 in acetone–d_6 (continued)

Position	Compound 27		Lophirone A	
	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
26		135.7		135.6
27	7.21 (1H, *d*, 8.4)	129.5	7.25 *m*	129.4
28	6.66 (1H, *d*, 8.8)	115.9	6.65 *m*	115.9
29		156.5		156.4
30	6.66 (1H, *d*, 8.8)	115.9	6.65 *m*	115.9
31	7.21 (1H, *d* 8.4)	129.5	7.26 *m*	129.4

1.9 Structure Determination of Compound 21

Compound 21 was obtained as a white solid. The UV spectrum (Figure 78) displayed absorption bands at 284, 262, 233, 227 and 202 nm. The IR spectrum (Figure 79) exhibited OH stretching at 3141 cm^{-1}, CH stretching at 2922 cm^{-1}, C=O stretching at 1628 cm^{-1}, C-O stertching at 1199 cm^{-1} and aromatic out of plane bending at 956 cm^{-1}. The FABMS (Figure 80) revealed the $[M+H]^+$ at *m/z* 525, consistent with the molecular formula $C_{31}H_{25}O_8$. The ^1H and ^{13}C NMR spectra (Figures 81-83) were similar to those of compound **27**, except that a methoxy group was observed at δ_C 56.0 and δ_H 3.78 ppm. The ^1H NMR (Figure 84) exhibited *trans* aliphatic coupled protons, H-11 and H-19 (*J*= 12.0 Hz, each) at δ 6.10 and 4.75 ppm. The chemical shifts of C-15 (δ 101.4 ppm) was more upfield than those of compound **27**, suggesting that the electron donating methoxy group was located at C-16. Compound **21** was identified as calodenone (**21**). The ^1H and ^{13}C NMR data of compound **21** were in agreement with previously reported values (Messanga, *et al.*, 1992).



(21)

Table 29 The ^{1}H and ^{13}C NMR data of compound 21 in acetone–d_6

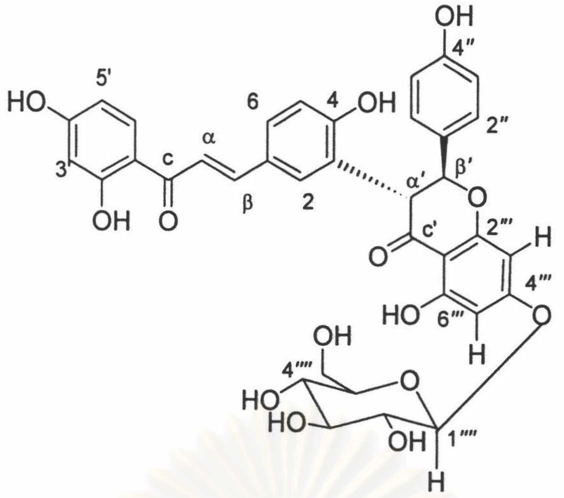
Position	Compound 21		Calodenone
	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	^{1}H δ (ppm), *J* (Hz)
2	8.23 (1H, *s*)	156.2	8.26 (1H, *s*)
3		122.0	
4		175.1	
5	7.87 (1H, *d*, 8.8)	128.2	7.91 (1H, *d*, 8.8)
6	6.87 (1H, *dd*, 2.3, 8.8)	115.9	6.88 (1H, *dd*, 2.3, 8.8)
7		163.4	
8	6.72 (1H, *d*, 2.4)	103.1	6.74 (1H, *d*, 2.3)
9		158.5	
10		117.2	
11	6.10 (1H, *d*, 12.0)	44.1	6.14 (1H, *d*, 12.3)
12		205.0	
13		114.5	
14		167.5	
15	6.25 (1H, *d*, 2.8)	101.4	6.28(1H, *d*, 2.4)
16		166.8	
17	6.46 (1H, *dd*, 2.8, 9.0)	108.2	6.48(1H, *dd*, 2.4, 8.1)
18	8.34 (1H, *d*, 9.2)	133.9	8.37 (1H, *d*, 8.1)
19	4.75 (1H, *d*, 12.0)	53.3	4.78 (1H, *d*, 12.3)
20		134.5	
21	7.21 (1H, *d*, 8.4)	130.4	7.24 *m*
22	6.56 (1H, *d*, 8.4)	115.8	6.59 *m*
23		156.5	
24	6.56 (1H, *d*, 8.8)	115.8	6.59 *m*
25	7.21 (1H, *d*, 8.4)	130.0	7.24 *m*

Table 29 The ^{1}H and ^{13}C NMR data of compound 21 in acetone–d_6 (continued)

Position	**Compound 21**		**Calodenone**
	^{1}H δ (ppm), *J* (Hz)	**^{13}C δ (ppm)**	**^{1}H δ (ppm), *J* (Hz)**
26		135.6	
27	7.21 (1H, *d*, 8.4)	129.4	7.24 *m*
28	6.60 (1H, *d*, 8.8)	115.9	6.63 *m*
29		156.5	
30	6.60 (1H, *d*, 8.8)	115.9	6.63 *m*
31	7.21 (1H, *d*, 8.4)	129.4	7.24 *m*
OCH_3	3.78 (3H, *s*)	56.0	3.77 (3H, *s*)
OH	12.66 (1H, *s*)		12.69 (1H, *s*)

1.10 Structure Determination of Compound 174

Compound **174** was isolated as a yellow solid. The UV spectrum (Figure 84) showed absorption bands at 369, 285 and 205 nm. The IR spectrum (Figure 85) exhibited OH stretching at 3343 cm^{-1}, C=O stretching at 1632 cm^{-1} and C-O stertching at 1172 cm^{-1}. The molecular formula was determined to be $C_{36}H_{33}O_{14}$ by the $[M+H]^+$ at *m/z* 689.1837 (calcd 689.1870) in the HRFABMS. The ^{1}H NMR (Figure 87) and ^{13}C NMR (Figures 88-89) spectra of **174** were reminiscent of those of **173,** with additional signals for a sugar moiety. The ^{1}H NMR signals at δ 5.11 (H-1''''), 3.47 (H-2''''), 3.47 (H-3''''), 3.55 (H-4''''), 3.62 (H-5''''), 3.70 and 3.88 (H_2-6''''), together with the coupling constant between H-1'''' and H-2'''' (*J*=7.6 Hz), indicated a glucopyranosyl unit with β-configuration. This was also corroborated by the ^{13}C NMR resonances at δ 100.3 (C-1''''), 73.6 (C-2''''), 70.2 (C-3''''), 76.8 (C-4''''), 77.1 (C-5'''') and 61.6 (C-6''''). The sugar unit was connected to the aglycon via an ether bridge linking its anomeric carbon to C-4''' of the flavanone part, as evidenced by the NOEs of H-1'''' with H-3''' and H-5''' (Figure 92), and the HMBC coupling between H-1'''' and C-4''' (Figure 93). From the above spectral data, it could be concluded that **174** is 6'''–hydroxylophirone B 4'''-*O*-β-glucoside. Compound **174** showed positive and negative CD Cotton effects (Figure 95) at wavelengths similar to those of **173** and (2*S*,3*R*)-4′,5,7-tri-*O*-methylnaringenin-(3*β*,3')-*α*,2′,4,4′,6′-pentamethoxy-chalcone (Bekker, Brandt and Ferreira, 1996) ($[\theta]_{352.6\ nm}$ +3.8 x 10^{3} and $[\theta]_{287.0\ nm}$ -2 x 10^{4}). This indicated that **174** also possessed the α'*R*, β'*S* absolute configuration. The ^{1}H and ^{13}C assignments were shown in Table 30.



(174)

Table 30 The ^{1}H and ^{13}C NMR data of compound 174 in acetone–d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
1		126.8	3′	6.36 (1H, *d*, 2.3)	103.5
2	7.55 (1H, *br s*)	133.4	4′		164.7
3		122.8	5′	6.45 (1H, *dd*, 2.3, 9.0)	107.9
4		157.8	6′	8.00 (1H, *d*, 9.0)	132.4
5	6.88 (1H, *d*, 8.2)	115.8	α′	4.70 (1H, *d*, 12.0)	53.5
6	7.56 (1H, *dd*, 2.3, 8.2)	129.8	β′	5.93 (1H, *d*, 12.0)	82.7
α	7.65 (1H, *d*, 15.2)	117.7	CO (c′)		197.6
β	7.71 (1H, *d*, 15.2)	144.1	1″		128.9
CO (c)		191.8	2″, 6″	7.30 (2H, *dd*, 2.0, 6.6)	129.2
1′		113.6	3″, 5″	6.75 (2H, *dd*, 2.0, 6.6)	115.0
2′		166.9	4″		157.8

Table 30 The ^{1}H and ^{13}C NMR data of compound 174 in acetone–d_6 (continued)

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)	Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
1‴		102.8	1⁗	5.11 (1H, *d*, 7.6)	100.3
2‴		163.2	2⁗	3.47 (1H, *dd*, 7.6, 7.9)	73.6
3‴	6.20 (1H, *d*, 2.3)	95.6	3⁗	3.47 (1H, *dd*, 7.9, 8.9)	70.2
4‴		166.4	4⁗	3.55 (1H, *dd*, 8.9, 8.9)	76.8
5‴	6.22 (1H, *d*, 2.3)	97.0	5⁗	3.62 (1H, *m*)	77.1
6‴		165.8	6⁗	3.70 (1H, *dd*, 5.3, 11.9) 3.88 (1H, *dd*, 2.6, 11.9)	61.6

1.11 Structure Determination of Compound 26

Compound **26** was obtained as a white solid. The UV spectrum (Figure 96) showed absorption bands at 271, 236 and 201 nm. The IR spectrum (Figure 97) exhibited absorption bands at 2930 (OH stretching), 1783 (C=O stretching), 1187 (C-O stretching) and 942 (aromatic out of plane bending) cm^{-1}. The FABMS (Figure 98) revealed the $[M+H]^+$ at *m/z* 313, suggesting that the molecular formula was $C_{17}H_{13}O_6$. Compound **26** was identified as 5-hydroxy-4′-methoxy-6,7-methylenedioxy isoflavone, which was previously isolated from *O. calodendron* (Messanga *et al.*, 1998). The ^1H NMR spectrum (Figure 99) showed a singlet (2H) at δ 6.11 ppm due to the methylenedioxy protons. The characteristic H-2 of isoflavone was observed at δ 7.90 ppm. The singlet proton at δ 7.90 ppm belonged to H-8. The AA′BB′ coupling protons at δ 7.47 and 6.99 ppm (*J*=8.4 Hz, each) were assigned to H-2′, 6′ and H-3′, 5′. The intermolecular hydrogen bonding protons was observed at δ 12.80 ppm. Comparison the ^1H NMR pattern of this compound with those of compound **175** indicated that methoxy group was substituted at C-4′.

(26)

Table 31 The ^{1}H NMR data of compound 26 in $CDCl_3$

Position	Compound 26	Compound 175
	^{1}H δ (ppm), *J* (Hz)	^{1}H δ (ppm), *J* (Hz)
2	7.90 (1H, *s*)	7.77 (1H, *s*)
3		
4		
4a		
5		
6		
7		
8	6.51 (1H, *s*)	6.63 (1H, *s*)
8a		
1′		
2′, 6′	7.47 (2H, *d*, 8.4)	7.47 (2H, *d*, 6.6)
3′, 5′	6.99 (2H, *d*, 8.4)	6.94 (2H, *d*, 6.6)
4′		
-OCH_2O-	6.11 (2H, *s*)	6.06 (2H, *s*)
5-OH	12.80 (1H, *s*)	
5-OCH_3		4.08 (3H, *s*)
4′-OCH_3	3.84 (3H, *s*)	3.83 (3H, *s*)

1.12 Structure Determination of Compound 58

Compound **58** was obtained as a white solid. The UV spectrum (Figure 100) exhibited absorption bands at 273, 241, 219, 212 and 202 nm. The IR spectrum (Figure 101) showed absorption bands at 2970 (OH stretching), 2355 (CH stretching), 1679 (C=O stretching) and 930 (aromatic out of plane bending) cm^{-1}. The FABMS (Figure 102) revealed the $[M+H]^+$ at *m/z* 343, suggesting the molecular formula $C_{18}H_{15}O_7$. The ^1H NMR spectrum (Figure 103) displayed the characteristic H-2 singlet proton peak at δ 7.90 ppm. The ABX type protons at δ 7.10, 6.92 (J=8.1 Hz) and 7.07 (J=8.1 Hz) ppm were assigned to H-2′, H-5′ and H-6′. A methoxy group at δ_H 3.91 ppm was considered to be located at C-3′, as suggested by the HMBC correlation between these methoxy protons and C-3′ (δ_C 149.4 ppm). The other methoxy group at δ_H 3.90 ppm was placed at C-4′, as indicated by the HMBC correlation between these methoxy protons and C-4′ (δ_C 149.9 ppm) (Figures 106-107). The methylenedioxy protons were present at δ 6.10 ppm. Compound **58** was identified as squarrosin (Rao and Gunasekar, 1989). The ^{13}C NMR assignments have been reported for the first time in this study.

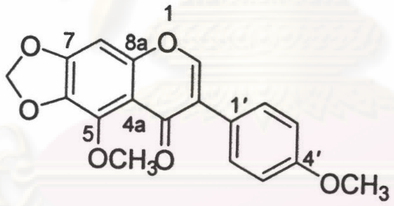
(58)

Table 32 The ^{1}H and ^{13}C NMR data of compound 58 in $CDCl_3$

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	7.90 (1H, *s*)	153.3
3		123.9
4		181.7
4a		108.8
5		143.1
6		130.0
7		154.6
8	6.49 (1H, *s*)	89.7
8a		154.0
1′		123.6
2′	7.10 (1H, *s*)	112.9
3′		149.4
4′		149.9
5′	6.92 (1H, *d*, 8.1)	111.8
6′	7.07 (1H, *d*, 8.1)	121.7
-OCH_2O-	6.10 (2H, *s*)	103.1
5-OH	12.78 (1H, *s*)	
3′-OCH_3	3.91 (3H, *s*)	56.4
4′-OCH_3	3.90 (3H, *s*)	56.4

1.13 Structure Determination of Compound 175

Compound **175** was obtained as a white solid. The UV spectrum (Figure 108) showed absorption bands at 323, 307, 264, 235 and 203 nm. The FABMS (Figure 109) displayed the $[M+H]^+$ at *m/z* 327, corresponding to $C_{18}H_{15}O_6$. The ^{13}C NMR spectrum (Figure 111) displayed eighteen carbon signals: a carbonyl carbon, two methoxy carbons, a methylene carbon and fourteen sp^2 carbons. The ^1H NMR (Figure 110) spectrum of compound **175** was similar to that of compound **26**. The presence of a methoxy group at δ 4.08 ppm and the absence of perihydroxy at δ 12.80 ppm was observed, indicating that the methoxy group was located at C-5. This was confirmed by the HMBC spectrum (Figures 113-114), showing the correlation between 5-OCH_3 (δ_H 4.08 ppm) with C-5 (δ_C 141.8 ppm). From the above data, compound **175** was identified as 5,4′-dimethoxy-6,7-methylenedioxy isoflavone (El-Emary *et al.*, 1980). This study provided the first ^{13}C NMR report for this compound.



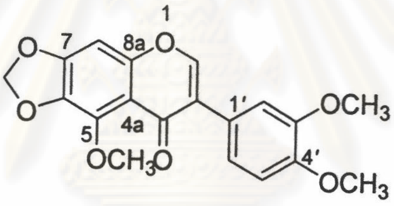
(175)

Table 33 The ^{1}H and ^{13}C NMR data of compound 175 in $CDCl_3$

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	7.77 (1H, *s*)	150.3
3		125.4
4		175.5
4a		113.9
5		141.8
6		152.9
7		135.6
8	6.63 (1H, *s*)	93.3
8a		154.8
1′		124.2
2′, 6′	7.47 (2H, *d*, 6.6)	130.4
3′, 5′	6.94 (2H, *d*, 6.6)	114.0
4′		159.6
$-OCH_2O-$	6.06 (2H, *s*)	102.2
5-OCH_3	4.08 (3H, *s*)	61.3
4′-OCH_3	3.83 (3H, *s*)	55.4

1.14 Structure Determination of Compound 60

Compound **60** was obtained as a white solid. The UV spectrum (Figure 115) showed absorption bands at 266, 240 and 203 nm. The FABMS (Figure 116) exhibited the $[M+H]^+$ at *m/z* 357, consistent to $C_{19}H_{17}O_7$. Compound **60** was identified as 5,3′,4′-trimethoxy-6,7-methylenedioxy isoflavone (Rao and Gunasekar, 1989). The ^{13}C NMR spectrum (Figure 117) displayed nineteen carbon signals: a carbonyl carbon, three methoxy carbons, a methylene carbon and fourteen *sp*^{2} carbons. The ^1H NMR spectrum (Figure 118) was similar to that of compound **58**. This compound showed a methoxy group at δ 4.08 ppm, suggesting that the methoxy group was positioned at C-5. This was confirmed by the HMBC correlation (Figures 120-121). The first ^{13}C NMR data of **60** was obtained in this study.



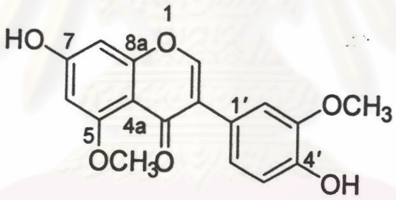
(60)

Table 34 The ^{1}H and ^{13}C NMR data of compound 60 in $CDCl_3$

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	7.80 (1H, *s*)	150.5
3		125.5
4		175.5
4a		113.9
5		141.8
6		135.6
7		152.9
8	6.63 (1H, *s*)	93.3
8a		154.8
1′		124.7
2′	7.20 (1H, *d*, 1.9)	112.9
3′		148.8
4′		149.1
5′	6.90 (1H, *d*, 8.2)	111.1
6′	7.00 (1H, *d*, 1.9, 8.2)	121.4
$-OCH_2O-$	6.10 (2H, *s*)	102.3
5-OCH_3	4.08 (3H, *s*)	61.3
3′-OCH_3	3.90 (3H, *s*)	56.0
4′-OCH_3	3.92 (3H, *s*)	56.1

1.15 Structure Determination of Compound 176

Compound **176** was obtained as a white solid. The UV spectrum (Figure 122) showed absorption bands at 257, 232 and 203 nm. The FABMS (Figure 123) revealed the $[M+H]^+$ at *m/z* 315, corresponding to $C_{17}H_{15}O_6$. The ^{13}C NMR spectrum (Figure 125) displayed seventeen carbons, including a carbonyl carbon, two methoxy carbons and fourteen sp^2 carbons. The ^1H NMR spectrum (Figure 124) showed a characteristic H-2 of isoflavone at δ 8.06 ppm. The meta coupled protons at δ 6.36 and 6.37, (*J*=2 Hz, each) were assigned to H-6 and H-8, respectively. The ABX coupling type protons at δ 7.07 (*J*=2 Hz), 6.76 (*J*=8.2 Hz) and 7.00 (*J*=2, 8.2 Hz) belonged to H-2′, H-5′ and H-6′. From the NOE difference spectrum (Figure 129), 3′–OCH_3 (δ_H 3.77 ppm) showed NOE enhancements with H-2′ (δ_H 7.07 ppm). This compound was identified as gerontoisoflavone A (**176**), (Chang *et al.*, 1995).



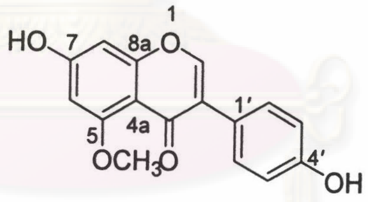
(176)

Table 35 The ^{1}H and ^{13}C NMR data of compound 176 in DMSO-d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	8.06 (1H, *s*)	150.7
3		124.7
4		173.8
4a		107.8
5		161.2
6	6.36 (1H, *d*, 2.0)	96.5
7		162.5
8	6.37 (1H, *d*, 2.0)	94.8
8a		159.1
1′		123.3
2′	7.07 (1H, *d*, 2.0)	113.5
3′		147.0
4′		146.3
5′	6.76 (1H, *d*, 8.2)	115.1
6′	7.00 (1H, *dd*, 8.2, 2.0)	121.6
5-OCH_3	3.78 (3H, *s*)	55.9
3′-OCH_3	3.77 (3H, *s*)	55.7

1.16 Structure Determination of Compound 177

Compound **177** was obtained as a white solid. The UV spectrum (Figure 130) exhibited absorption bands at 256, 227 and 202 nm. The FABMS (Figure 131) displayed the $[M+H]^+$ at *m/z* 285, corresponding to $C_{16}H_{13}O_5$. The ^{13}C NMR spectrum (Figure 133) showed sixteen carbons, including a carbonyl carbon, a methoxy carbon and fourteen sp^2 carbons. The ^1H NMR spectrum (Figure 132) revealed a characteristic H-2 of isoflavone at δ 8.03 ppm. The AA′BB′ coupled protons at δ 7.29 and 6.77, (*J*=6.6 Hz, each) belonged to H-2′, 6′ and H-3′, 5′. The meta coupled protons at δ 6.36 and 6.38, (*J*=2 Hz, each) were assigned to H-6 and H-8, respectively. The perihydroxy proton was absent, suggesting that a OCH_3 group was present at C-5 (δ_C 161.7 ppm). All protons and carbons were assigned by analysis of the HMQC (Figure 134) and HMBC spectra (Figures 135-136). Compound **177** was identified as the known compound 4′,7-dihydroxy 5-methoxy isoflavone **(177)**, (Sekizaki *et al.*, 1988).



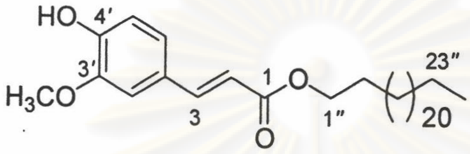
(177)

Table 36 The ^{1}H and ^{13}C NMR data of compound 177 in DMSO-d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	8.03 (1H, *s*)	150.8
3		125.2
4		174.3
4a		108.2
5		161.7
6	6.36 (1H, *d*, 2.0)	97.2
7		163.9
8	6.38 (1H, *d*, 2.0)	95.4
8a		159.7
1′		123.4
2′, 6′	7.29 (2H, *d*, 6.6)	130.7
3′, 5′	6.77 (1H, *d*, 6.6)	115.3
4′		157.5
5-OCH_3	3.78 (3H, *s*)	56.4

1.17 Structure Determination of Compound 178

Compound **178** was obtained as a white powder. The FABMS (Figure 137) showed the $[M+H]^+$ at *m/z* 531, suggesting the molecular formula $C_{34}H_{59}O_4$. The UV spectrum (Figure 138) exhibited absorption bands at 218, 234 and 324 nm. Compound **178** was identified as *trans* tetracocyl ferulate (**178**) (Tezuka, Ueda and Kikuchi, 1989). The ^1H and ^{13}C NMR assignments were summarized in Table 37.



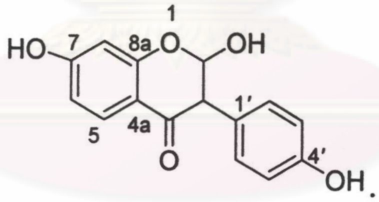
(178)

Table 37 The ^1H and ^{13}C NMR data of compound 178 in $CDCl_3$

Position	**^1H δ (ppm), *J* (Hz)**	**^{13}C δ (ppm)**
1		167.1
2	6.27 (1H, *d*, 15.9)	114.6
3	7.59 (1H, *d*, 15.9)	144.4
1′		127.0
2′	7.02 (1H, *s*)	109.2
3′		146.6
4′		147.7
5′	6.90 (1H, *d*, 8.1)	115.6
6′	7.05 (1H, *d*, 8.1)	122.9
1″	4.17 (2H, *d*, 6.9)	64.6
2″-23″	1.64 (2H, *d*, 6.9) 0.84-0.88 (42H, *m*)	26.1, 27.3, 28.9, 29.4 29.7, 29.8, 32.0
24″	0.85 (3H, *t*, 6.9,)	14.3
3′-OCH_3	3.90 (3H, *s*)	55.9
4′-OH	5.82 (1H, *s*)	

1.18 Structure Determination of Compound 179

Compound 179 was obtained as a white solid. The UV spectrum showed absorptions (Figure 145) at λ_{max} 220, 258 and 295 nm. The FABMS (Figure 146) exhibited the $[M+H]^+$ at *m/z* 273, consistent to the molecular formula $C_{15}H_{13}O_5$. This compound was identified as 2,7,4′-trihydroxy isoflavone which is an intermediate in daidzein biosynthesis (Hashim *et al.*, 1990). The ^{1}H NMR spectrum (Figure 147) exhibited the dihydroisoflavonol protons H-2 and H-3 at δ 4.70 and 5.42 (*J*=5.1 Hz, each). The protons at δ 7.55 (*J*=9.0 Hz), 6.01 (*J*=9.0, 1.5 Hz) and 6.10 (*J*=1.5 Hz) were assigned to H-5, H-6 and H-8, respectively. The protons at δ 7.40 (*J*=8.1 Hz) and 6.82 (*J*=7.8 Hz) were assigned to H-2′, 6′ and H-3′, 5′, respectively. The ^{13}C NMR spectrum (Figure 148) showed fifteen carbon signals, including a pair of methine carbons, a carbonyl carbon and twelve sp^2 carbons. The present work provided complete ^{13}C NMR assisgnment by analysis of the HMQC (Figure 149) and HMBC spectra (Figures 150-151). The ^{1}H and ^{13}C assignments are shown in Table 38.



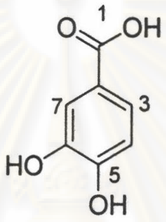
(179)

Table 38 The ^{1}H and ^{13}C NMR data of compound 179 in acetone-d_6

Position	^{1}H δ (ppm), *J* (Hz)	^{13}C δ (ppm)
2	4.70 (1H, *d*, 5.1)	60.2
3	5.42 (1H, *d*, 5.1)	85.5
4		203.4
4a		115.4
5	7.55 (1H, *d*, 9.0)	134.6
6	6.01 (1H, *d*, 9.0, 1.5)	109.5
7		166.7
8	6.10 (1H, *d*, 1.5)	104.3
8a		104.3
1′		133.1
2′, 6′	7.40 (2H, *d*, 8.1)	129.9
3′, 5′	6.82 (1H, *d*, 7.8)	116.9
4′		159.1

1.19 Structure Determination of Compound 180

Compound **180** was obtained as a white solid. The EIMS (Figure 152) exhibited an $[M]^+$ ion at m/z 154, corresponding to $C_7H_6O_4$. The UV absorption bands (Figure 153) were found at λ_{max} 220, 258 and 295 nm. This compound was identified as protocatechuic acid (Torssell, 1997). Complete ^1H and ^{13}C assignments of this compound were obtained by HMQC (Figure 156) and HMBC (Figures 157-158) experiments, as summarized in Table 39.



(180)

Table 39 The ^1H and ^{13}C NMR data of compound 180 in acetone-d_6

Position	**^1H δ (ppm), *J* (Hz)**	**^{13}C δ (ppm)**
1		167.4
2		122.9
3	7.46 (1H, *dd*, 8.3, 1.5)	123.3
4	6.88 (1H, *d*, 8.3)	115.4
5		150.4
6		145.3
7	7.52 (1H, *d*, 1.5)	117.2

2. DPPH Free Radical Scavenging activity

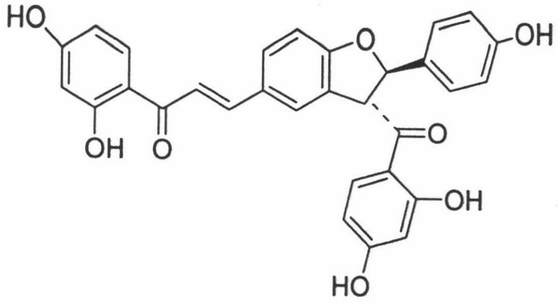
Nine compounds, i. e. **1, 21, 27, 60, 171, 173, 175, 177** and **179**, from *O. integerrima* were subjected to DPPH radical scavenging activity test. Compounds **171, 177** and **179** exhibited moderate activity when compared with the positive control quercetin (**83**) (Table 20).

2.1 Biflavonoids

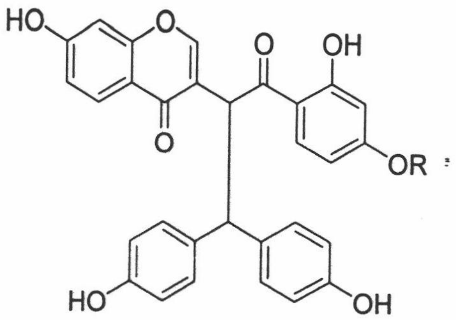
Compound **171** showed moderate activity while compound **1** exhibited weak activity. Compounds **173**, **27** and **21** showed very weak activity. Compound **171** had a very rigid planar structure, suggesting that a planar structure might be important for the activity.

2.2 Isoflavonoids

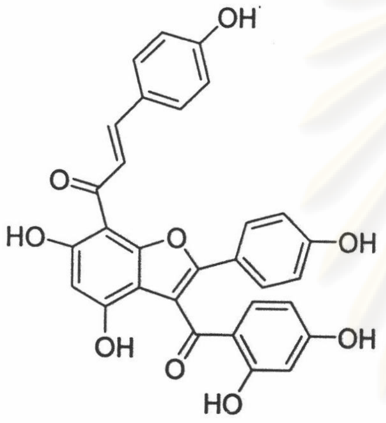
Compound **179** showed moderate activity whereas compound **177** was slightly less active. The isoflavonoids with 6,7 methylenedioxy group such as compounds **60** and **175** displayed weak activity.



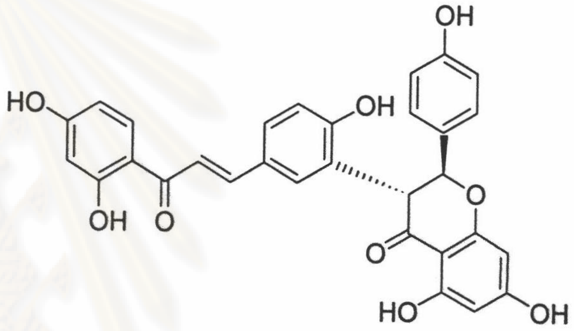
(1)



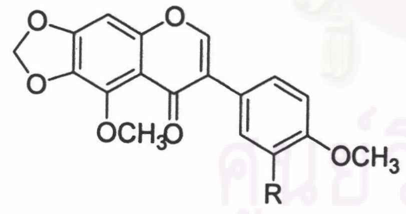
	R
(21)	**CH_3**
(27)	**H**



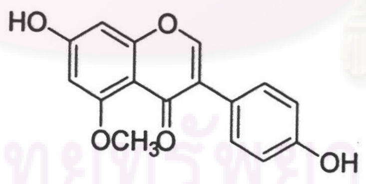
(171)



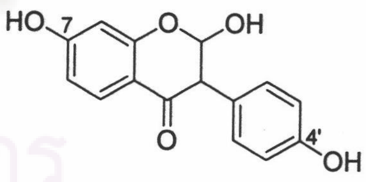
(173)



	R
(60)	**OCH_3**
(175)	**H_3**



(177)



(179)

Figure 4 Structures of flavonoids with DPPH radical scavenging activity from *O. integerrima*