

CHAPTER IV

RESULTS AND DISCUSSION

The pulverized seeds of *Pachyrrhizus erosus* (L.) Urban (2 kg) were extracted with hexane, chloroform and then ethanol. The oil portion from hexane extract was analyzed by Gas-Liquid Column chromatography (GLC) to afford the percentages of fatty acid composition. The chloroform and the ethanol extracts were investigated by several chromatographic techniques to give eight compounds classified as an isoflavone [2], an isoflavanone [15], a 3-phenylcoumarin [17] and five rotenoids [4, 8, 11, 12 and 18]. Their completed structures were determined based on their UV, IR, MS and NMR data, and then discussed by comparison with the literature values. The antimicrobial, anti-HSV, COX-2 inhibitory and cytotoxic activities of these compounds were evaluated.

The ethanol extract of dried stem bark of *Millettia leucantha* Kurz var. *leucantha* (2.7 kg) was investigated by means of chromatographic methods to yield eleven compounds classified as six chalcones [102, 279, 280, 281, 282 and 284] and five flavones [68, 103, 115, 285 and 287]. The structure determinations of these compounds were achieved by interpretation of their UV, IR, MS and NMR data, and then confirmed by comparison with the literature values. Additionally, their antimicrobial, anti-HSV, COX-2 inhibitory and cytotoxic activities were also discussed.

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1. Determination of Oil Compositions from *Pachyrrhizus erosus* seeds

The oil obtained from the seeds of *P. erosus* is clearly yellow. After analysis by GC, the percentages of normal fatty acids were determined and reported in the following table. GC chromatogram (**Figure 12**) were demonstrated in Appendix part.

Peak number	Component name	%Area
1	Capric acid (C10:0) ^A [268]	0.035
2	Myristic acid (C14:0) [269]	0.249
3	Palmitic acid (C16:0) [270]	28.302
4	Haxadecenoic acid (C16:1) [271]	0.193
5	Stearic acid (C18:0) [272]	5.776
6	Oleic acid (C18:1) [273]	29.258
7	Linoleic acid (C18:2) [274]	31.380
8	Linolenic acid (C18:3) [275]	0.790
9	Eicosenoic acid (C20:1) [276]	0.960
10	Docosenoic acid (C22:1) [277]	1.746
11	Docosahexaenoic acid (C22:6) [278]	1.311

A: Abbreviation (C 10:0); C = Carbon atom, 10 = Number of carbon atoms, 0 = Number of double bonds

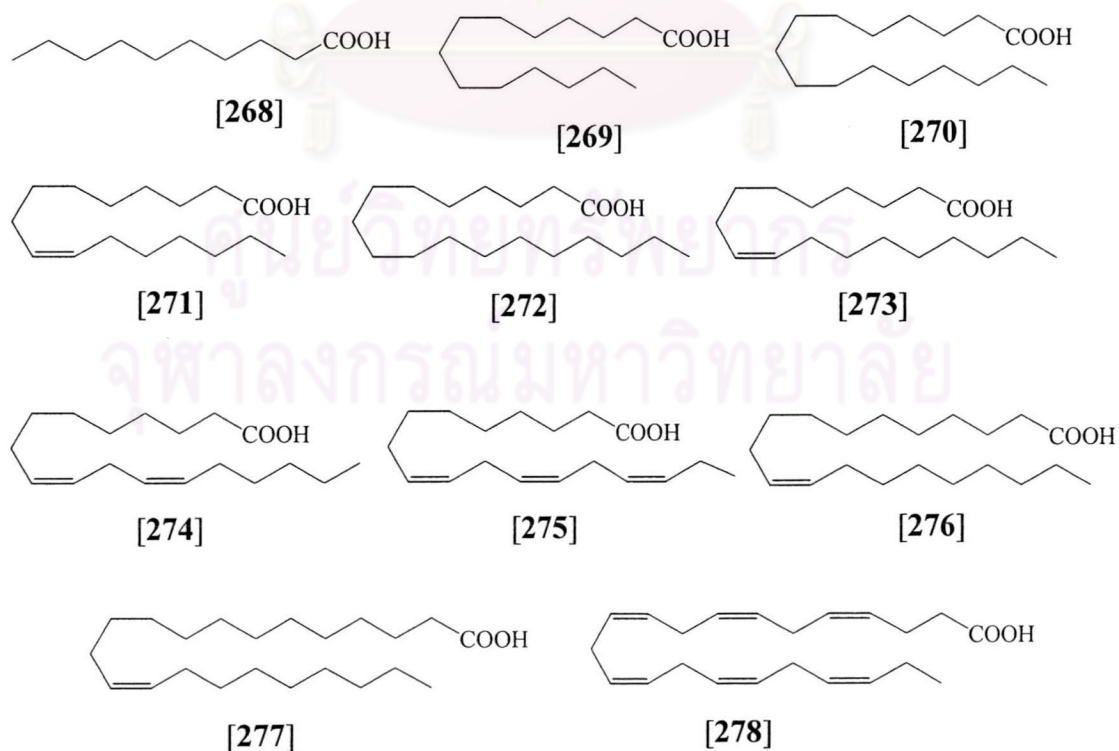


Figure 11 Structures of fatty acids isolated from *P. erosus*

2. Structure Determination of Compounds Isolated from *Pachyrrhizus erosus*

2.1 Structure Determination of Compound 4

Compound **4** was obtained as white crystals. The EI mass spectrum (**Figure 13**) showed the molecular ion peak at m/z 336, consistent with the molecular formula $C_{19}H_{12}O_6$. The UV spectrum (**Figure 14**) showed absorption maxima at 341, 302, 275, 236 and 209 nm. The IR spectrum (**Figure 15**) displayed absorption bands at 1681 (conjugated C=O stretching), 1624-1469 (aromatic ring) and 1154 (C-O stretching) cm^{-1} . This compound was assigned to rotenoid, (+)-dolineone [**4**], which had the *cis*-B/C fusion like (-)-rotenone [**19**]. This conclusion was confirmed by the $[\alpha]_D^{27}$ (+192, $c=0.35$ in $CHCl_3$) and 1H -NMR spectrum (**Figure 16**), which indicated that the H-1 is located at δ 6.74 ppm and was therefore not being strongly negatively shielded by the carbonyl group. All signals of 1H -NMR spectrum were exhibited at the locations, corresponding to those of literature (Puyvelde, 1987). The ^{13}C -NMR spectrum (**Figure 17**) showed the signals at 159.8 and 158.6 ppm due to the carbons 7a and 9, respectively. These signals had been conversely assigned to those in the literature (Puyvelde, 1987). The present work completely assigned the 1H - and ^{13}C -NMR data of this compound by HMQC (**Figure 18**), HMBC (**Figure 19**) experiments and compared all signals with those of literature (**Table 10**).

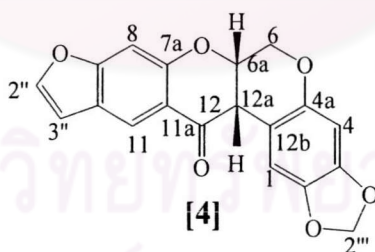


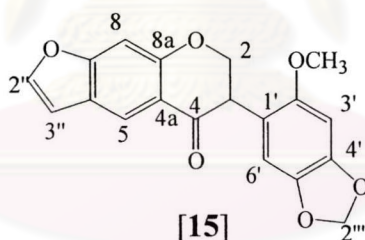
Table 10 The ^1H - and ^{13}C -NMR data of Compound 4 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	(+)-Dolineone	Compound 4		(+)-Dolineone	Compound 4
1	6.72 (1H, s, overlap)	6.74 (1H, s, overlap)	1	106.9	106.9
			2	143.2	142.3
			3	147.9	147.9
4	6.44 (1H, s)	6.45 (1H, s)	4	98.9	98.6
6	4.19 (1H, <i>d</i> , 12.0)	4.19 (1H, <i>d</i> , 12.0)	6	66.4	66.3
	4.63 (1H, <i>dd</i> , 12.0, 3.2)	4.64 (1H, <i>dd</i> , 12.0, 3.5)			
8	7.05 (1H, <i>s</i>)	7.06 (1H, <i>s</i>)	8	99.8	99.8
			9	158.6	159.8
			10	123.1	123.0
11	8.21 (1H, <i>s</i>)	8.21 (1H, <i>s</i>)	11	121.0	120.9
			12	190.6	190.5
			4a	148.5	148.4
6a	4.96 (1H, <i>ddd</i> , 3.9, 3.2, 1.0)	4.96 (1H, <i>ddd</i> , 4.0, 3.0, 1.0)	6a	72.1	72.0
			7a	159.8	158.6
			11a	116.1	116.0
12a	3.89 (1H, <i>d</i> , 3.9)	3.89 (1H, <i>d</i> , 4.0)	12a	45.3	45.2
			12b	105.3	105.3
2''	7.54 (1H, <i>d</i> , 2.3)	7.54 (1H, <i>d</i> , 2.5)	2''	146.2	146.2
3''	6.73 (1H, <i>dd</i> , 2.3, 1)	6.73 (1H, <i>dd</i> , 2.5, 1)	3''	106.9	106.8
2'''	5.80 (1H, <i>d</i> , 1.3)	5.81 (1H, <i>d</i> , 1.3)	2'''	101.2	101.1
	5.86 (1H, <i>d</i> , 1.3)	5.87 (1H, <i>d</i> , 1.3)			

-. The bold values are revised assignments.

2.2 Structure Determination of Compound 15

Compound **15** was acquired as pale yellow crystals. The EI mass spectrum (**Figure 20**) revealed the molecular ion peak at m/z 338, corresponding to the molecular formula $C_{19}H_{14}O_6$. The UV spectrum (**Figure 21**) showed absorptions at 336, 299, 273, 235 and 207 nm. The IR spectrum (**Figure 22**) displayed absorption bands at 2893 (CH stretching), 1687 (C=O stretching) and 1625-1475 (aromatic ring) cm^{-1} . This compound was identified as neotenone [**15**] and has already been isolated from this plant (Krishnamurti and Seshadri, 1966), *Neorautanenia pseudopachyrrhiza* (Crombie and Whiting, 1963) and *N. mitis* (Puyvelde, 1987). Additionally, this compound was always isolated in racemate form (Puyvelde, 1987). The 1H -NMR (**Figure 23**) data exhibited close similarity to those in the literature (Puyvelde, 1987). The ^{13}C -NMR spectrum (**Figure 24**) showed the signals of the carbons 6 and 1' at δ 115.5 and 122.6 ppm, respectively. These were revised from previously report (Puyvelde, 1987). This assignment was confirmed by the application of HMQC (**Figure 25**) and HMBC (**Figure 26**) experiments. The 1H - and ^{13}C -NMR data were demonstrated in **Table 11**.



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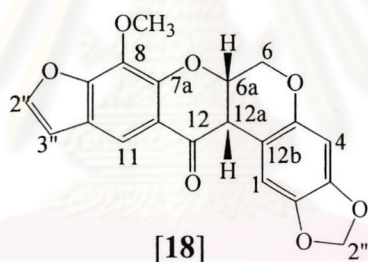
Table 11 The ^1H - and ^{13}C -NMR data of Compound 15 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	Neotenone	Compound 15		Neotenone	Compound 15
2	4.50(1H, <i>dd</i> , 10.8, 5.4)	4.50(1H, <i>dd</i> , 10.6, 5.5)	2	71.3	71.3
	4.58(1H, <i>dd</i> , 10.8, 11.4)	4.56(1H, <i>dd</i> , 10.6, 11.3)			
3	4.31(1H, <i>dd</i> , 11.4, 5.4)	4.31(1H, <i>dd</i> , 11.3, 5.5)	3	48.3	48.3
			4	192.6	192.8
5	8.25(1H, <i>s</i>)	8.25(1H, <i>s</i>)	5	120.8	120.9
			6	115.6	122.6
			7	159.2	159.3
			8	99.6	99.7
8	7.08(1H, <i>s</i>)	7.08(1H, <i>s</i>)	8	99.6	99.7
			4a	118.8	118.8
			8a	159.5	159.9
			1'	122.6	115.5
3'	6.57(1H, <i>s</i>)	6.57(1H, <i>s</i>)	2'	152.8	152.7
			3'	95.4	95.4
			4'	147.8	147.8
			5'	141.4	141.3
6'	6.62(1H, <i>s</i>)	6.61(1H, <i>s</i>)	6'	109.8	109.8
2''	7.57(1H, <i>d</i> , 2.3)	7.57(1H, <i>d</i> , 2.2)	2''	146.0	146.0
3''	6.76(1H, <i>dd</i> , 2.3, 1.0)	6.76(1H, <i>dd</i> , 2.2, 1.0)	3''	107.0	107.0
2'''	5.90(2H, <i>s</i>)	5.91(2H, <i>s</i>)	2'''	101.3	101.3
OCH ₃	3.72(3H, <i>s</i>)	3.72(3H, <i>s</i>)	OCH ₃	56.5	56.5

-: The bold values are revised assignments.

2.3 Structure Determination of Compound 18

Compound **18** was obtained as white crystals. The EI mass spectrum (**Figure 27**) exhibited the molecular ion peak at m/z 366, consistent to the molecular formula $C_{20}H_{14}O_7$. The UV spectrum (**Figure 28**) displayed absorptions at λ_{\max} 344, 283, 243 and 208 nm. The IR spectrum (**Figure 29**) showed absorption bands at 1676 (conjugated C=O stretching) and 1481-1619 (aromatic ring) cm^{-1} . The $[\alpha]_D^{27}$ showed dextrorotatory dispersion at + 116.44 ($c=0.45$, $CHCl_3$), which presents the *cis*-B/C fusion like (-)-rotenone [**19**] and (+)-dolineone [**4**]. This compound was assigned to pachyrrhizone [**18**] already isolated from this plant (Norton and Hansberry, 1945) and *Neorautanenia* species (Crombie and Whiting, 1963). However, the 1H - and ^{13}C -NMR data (**Figure 30** and **31**, respectively) have not been reported. Therefore, the present work completely reported these data of compound **18** for the first time (**Table 12**). This assignment was confirmed by HMQC (**Figure 32**) and HMBC (**Figure 33**) experiments.



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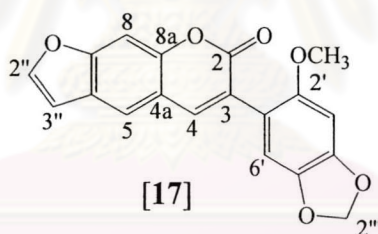
Table 12 The ^1H - and ^{13}C -NMR data of Compound 18 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 18	C	δ_{C} (ppm) of Compound 18
1	6.71 (1H, <i>s</i>)	1	106.9
		2	142.2
		3	147.3
4	6.42 (1H, <i>s</i>)	4	98.9
6	4.2 (1H, <i>d</i> , 11.9)	6	66.2
	4.6 (1H, <i>dd</i> , 11.9, 3.5)	8	133.5
		9	150.9
		10	123.9
11	7.91 (1H, <i>s</i>)	11	114.0
		12	190.7
		4a	148.6
6a	4.97 (1H, <i>m</i>)	6a	72.3
		7a	149.7
		11a	117.1
12a	3.88 (1H, <i>d</i> , 3.84)	12a	45.2
		12b	105.2
2''	7.55 (1H, <i>d</i> , 2.2)	2''	146.1
3''	6.73 (1H, <i>d</i> , 2.2)	3''	107.3
2'''	5.80 and 5.85 (each 1H, <i>d</i> , 1.2)	2'''	101.2
OCH_3	4.13 (3H, <i>s</i>)	OCH_3	61.1

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2.4 Structure Determination of Compound 17

Compound **17** was appeared as green needles. The EI mass spectrum (**Figure 34**) displayed the molecular ion peak at m/z 336, consistent with $C_{19}H_{12}O_6$. The UV absorption bands (**Figure 35**) appeared at λ_{max} 348, 292, 242 and 210 nm. The IR absorption spectrum (**Figure 36**) showed ν_{max} at 1716 (conjugated C=O stretching) and 1429 and 1625 (aromatic) cm^{-1} . This compound demonstrated identical formula with that of compound **4** but different in other major fragment ions in EIMS appeared at m/z 293, 265 and 179. This compound was identified as pachyrrhizin [**17**] by analyses of the 1H - (**Figure 37**) and ^{13}C -NMR (**Figure 38**) data, and by comparison with these data in previously reports (Puyvelde, 1987). Additionally, the carbon signal at δ 116.1 was newly assigned to be carbon 1'', whereas the carbons 7, 2' and 8a should be located at δ 156.1, 152.9 and 151.6, respectively. **Table 13** showed 1H - and ^{13}C -NMR data from the present work compared to these data from literature. The completed assignment of this compound was successfully done by application of 2D-NMR such as HMQC (**Figure 39**) and HMBC (**Figure 40**) experiments.



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Table 13 The ^1H - and ^{13}C -NMR data of Compound 17

H	δ_{H} (ppm), J (Hz)		C	δ_{H} (ppm)	
	Pachyrrhizin (in CDCl_3)	Compound 17 (in CDCl_3)		Pachyrrhizin (in CDCl_3 - $\text{DMSO-}d_6=1:5$)	Compound 17 (in CDCl_3)
			2	173.2	160.7
			3	124.0	123.9
4	7.80 (1H, <i>s</i>)	7.80 (1H, <i>s</i>)	4	142.4	142.4
5	7.69 (1H, <i>s</i>)	7.68 (1H, <i>s</i>)	5	119.6	119.6
			6	124.8	124.8
			7	156.2	156.1
8	7.46 (1H, <i>s</i>)	7.49 (1H, <i>s</i>)	8	99.5	99.4
			4a	116.2	116.1
			8a	156.2	151.6
			1'	-	116.1
			2'	151.7	152.9
3'	6.64(1H, <i>s</i>)	6.63 (1H, <i>s</i>)	3'	95.5	95.4
			4'	148.8	148.7
			5'	141.3	141.2
6'	6.91 (1H, <i>s</i>)	6.89 (1H, <i>s</i>)	6'	110.3	110.3
2''	7.70 (1H, <i>d</i> , 2.2)	7.69 (1H, <i>d</i> , 2.4)	2''	146.7	146.7
3''	6.84(1H, <i>dd</i> ,2.2,1.0)	6.82(1H, <i>dd</i> ,2.4,0.5)	3''	106.4	106.4
2'''	5.98 (2H, <i>s</i>)	5.96 (2H, <i>s</i>)	2'''	101.5	101.5
OCH_3	3.79 (3H, <i>s</i>)	3.77 (3H, <i>s</i>)	OCH_3	56.9	56.8

-: The bold values are revised assignments.

2.5 Structure Determination of Compound 8

Compound **8** was acquired as pale yellow crystals. The EIMS (**Figure 41**) displayed the molecular ion peak at m/z 352, agreeing with the molecular formula $C_{19}H_{12}O_7$. The UV spectrum (**Figure 42**) showed the absorption maxima identical with those of compound **4** at λ_{max} 339, 304, 276, 237 and 208 nm. The IR spectrum (**Figure 43**) revealed absorption bands at 3461 (OH stretching), 2907 (CH stretching), 1682 (C=O stretching) and 1480 and 1625 (aromatic ring) cm^{-1} . The $[\alpha]^{27}_D$ was +140.0, it was therefore the *cis*-B/C fusion like compound **4**. The 1H -NMR spectrum (**Figure 44**) showed all signals corresponding to those in the literature (Puyvelde, 1987). This compound was identified as (+)-12a-hydroxydoloneone [**8**], derivative of compound **4**. The furano protons (H-2'' and H-3'') connected to the A-ring were observed at δ 7.55 (H-2'', *d*, $J=2.3$ Hz) and 6.74 (H-3'', *dd*, $J=2.3, 1$ Hz). Four singlet aromatic protons were established as H-1 (δ 6.51 ppm), H-4 (δ 6.47 ppm), H-8 (δ 7.02 ppm) and H-11 (δ 8.19 ppm). The methylene protons at C-6 showed the signals at δ 4.50 (1H, *dd*, $J=15.8, 4$ Hz) and 4.63 (1H, *dd*, $J=15.8, 2.6$ Hz), whereas the doublet of doublet signals at δ 4.62 (1H, *dd*, $J=4.0, 2.6$ Hz) belonged to H-6a. A pair of doublet signals at δ 5.80 and 5.84 (each 1H, each $J=1.4$ Hz) were assigned to methylenedioxy group connected to the D-ring of rotenoid skeleton. The ^{13}C -NMR spectrum (**Figure 45**) has been reported already (Puyvelde, 1987) but some positions should be revised as C-1 (δ 105.8 ppm), C-9 (δ 160.3 ppm), C-3'' (δ 106.9 ppm) and C-7a (δ 158.3 ppm). This assignment was supported by HMQC (**Figure 46**) and HMBC (**Figure 47**) experiments.

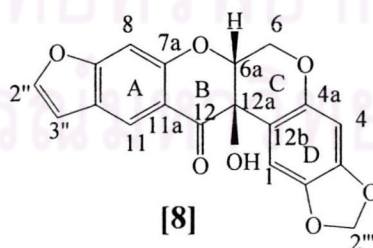


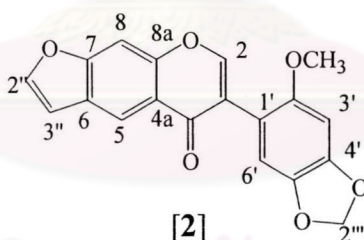
Table 14 The ^1H - and ^{13}C -NMR data of Compound 8 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	(+)-12a-Hydroxydoloneone	Compound 8		(+)-12a-Hydroxydoloneone	Compound 8
1	6.52 (1H, <i>s</i>)	6.51 (1H, <i>s</i>)	1	106.8	105.8
			2	142.3	142.3
			3	149.5	149.5
4	6.48 (1H, <i>s</i>)	6.47 (1H, <i>s</i>)	4	99.9	99.3
6	4.50(1H, <i>dd</i> ,12.9,2.0)	4.50(1H, <i>dd</i> ,15.8,4.0)	6	63.9	64.0
	4.63(1H, <i>dd</i> ,12.9,2.4)	4.63(1H, <i>dd</i> ,15.8,2.6)			
8	7.02(1H, <i>s</i>)	7.02 (1H, <i>s</i>)	8	100.0	100.1
			9	158.3	160.3
			10	123.3	123.4
11	8.19 (1H, <i>s</i>)	8.19 (1H, <i>s</i>)	11	121.0	121.1
			12	192.9	193.0
			4a	149.6	149.6
6a	4.62 (1H, <i>br s</i>)	4.62 (1H, <i>dd</i> ,4.0,2.6)	6a	75.9	75.9
			7a	160.3	158.3
			11a	114.6	114.6
			12a	68.3	68.4
			12b	109.2	109.3
2"	7.55 (1H, <i>d</i> , 2.2)	7.55 (1H, <i>d</i> , 2.3)	2"	146.4	146.5
3"	6.74(1H, <i>dd</i> ,2.2, 1.0)	6.74(1H, <i>dd</i> ,2.3, 1.0)	3"	105.7	106.9
2'''	5.80 and 5.85 (each 1H, each <i>d</i> , 1.2)	5.80 and 5.84 (each 1H, each <i>d</i> , 1.4)	2'''	101.3	101.4
OH	4.46 (1H, <i>s</i>)	4.43 (1H, <i>s</i>)			

-: The bold values are revised assignments.

2.6 Structure Determination of Compound 2

Compound **2** was characterized as white crystals. The EI mass spectrum (**Figure 48**) demonstrated the molecular ion peak at m/z 336, harmonising with the molecular formula $C_{19}H_{12}O_6$. The UV spectrum (**Figure 49**) showed the characteristics of the furanoisoflavonoid chromophore at 303, 237 and 208 nm. The IR absorption spectrum (**Figure 50**) displayed ν_{max} at 1645-1622 and 1474 cm^{-1} (C=O stretching and aromatic ring). This compound was identified as dehydroneotenone [**2**]. Its isolation from *Neorautanenia mitis* and $^1\text{H-NMR}$ data were reported (Puyvelde, 1987). The present work would report the $^{13}\text{C-NMR}$ spectral data (**Figure 52**) at the first time. The $^1\text{H-NMR}$ data (**Figure 51**) showed a singlet signal at δ 7.98 ppm. This was evidence of H-2 of isoflavone. Four singlet aromatic protons of H-5, H-8, H-3' and H-6' showed signals at δ 8.54, 7.57, 6.63 and 6.85 ppm, respectively. Thus, the isoflavone skeleton was substituted by furan ring (7.72 ppm, H-2'' and 6.91 ppm, H-3'') at positions 6 and 7. The 3 positions on phenyl ring were substituted by OCH_3 (δ 3.73 ppm) at 2' and methylenedioxy group (δ 5.96 ppm) at 3' and 4'. The complete assignment was managed by performing HMQC (**Figure 53**) and HMBC (**Figure 54**) experiments.



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Table 15 The ^1H - and ^{13}C -NMR data of Compound 2

H	δ_{H} (ppm), J (Hz)		C	δ_{H} (ppm) of Compound 2 (in CDCl_3)
	Dehydroneotenone (in CDCl_3 : $\text{DMSO}-d_6$ =1:4)	Compound 2 (in CDCl_3)		
2	7.98 (1H, <i>s</i>)	7.98 (1H, <i>s</i>)	2	154.7
			3	121.1
			4	176.6
			5	119.0
5	8.28 (1H, <i>s</i>)	8.54 (1H, <i>s</i>)	6	126.0
			7	157.2
8	7.63 (1H, <i>br s</i>)	7.57 (1H, <i>s</i>)	8	99.8
			4a	121.1
			8a	154.2
			1'	112.8
			2'	153.0
3'	6.64 (1H, <i>s</i>)	6.63 (1H, <i>s</i>)	3'	95.5
			4'	148.4
			5'	141.2
6'	6.70 (1H, <i>s</i>)	6.85 (1H, <i>s</i>)	6'	111.3
2''	7.88 (1H, <i>d</i> , 2.0)	7.72 (1H, <i>d</i> , 2.2)	2''	147.4
3''	6.93 (1H, <i>dd</i> , 2.0, 1.0)	6.91 (1H, <i>d</i> , 2.2)	3''	107.0
2'''	5.86 (2H, <i>s</i>)	5.96 (2H, <i>s</i>)	2'''	101.4
OCH_3	3.78 (3H, <i>s</i>)	3.73 (3H, <i>s</i>)	OCH_3	56.9

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2.7 Structure Determination of Compound 11

Compound **11** was acquired as pale yellow crystals. The EIMS (**Figure 55**) exhibited the molecular ion peak at m/z 382, suggesting the molecular formula $C_{20}H_{14}O_8$. The UV spectrum (**Figure 56**) revealed absorptions at λ_{max} 349, 284, 243 and 206 nm. The IR spectrum (**Figure 57**) provided absorption bands at 3461 (OH stretching), 2937 (CH stretching), 1682 (C=O stretching) and 1482 and 1621 (aromatic ring) cm^{-1} . The 1H - and ^{13}C -NMR spectral data (**Figures 58** and **59**, respectively) showed very similar patterns with those of (+)-pachyrrhizone [**18**], whereas MS data and IR spectrum confirmed the presence of hydroxy group. Furthermore, the connection of B and C-rings was proved to be the *cis*-B/C fusion as those of compound **18** and (-)-rotenone [**19**] by exhibiting of $[\alpha]_D^{27}$ at +91.6 and displaying of the singlet signal of H-1 at δ 6.49 ppm, which was highly deshielded located. Thus, this compound was eventually identified as (+)-12a-hydroxy pachyrrhizone [**11**]. In the 1H -NMR, two protons at δ 5.80 and 5.84 ppm were characterized as methylenedioxy group and the spin-coupled protons at δ 7.55 and 6.73 ppm ($J=2.3$ Hz) confirmed the presence of furan moiety connected to A-ring. Three benzenoid protons identified as H-1, H-4 and H-11 showed all singlet signals at δ 6.49, 6.46 and 7.88 ppm, respectively. Additionally, the methoxy substitution at C-8 displayed singlet signal at δ 4.10 ppm, whereas the signal at δ 4.40 ppm belonged to OH group at C-12, and three coupled protons at δ 4.50, 4.61 and 4.71 ppm analyzed for H-6 and H-6a. This compound was known, however, the ^{13}C -NMR, HMQC (**Figure 60**) and HMBC (**Figure 61**) spectra were presented at the first time in this work.

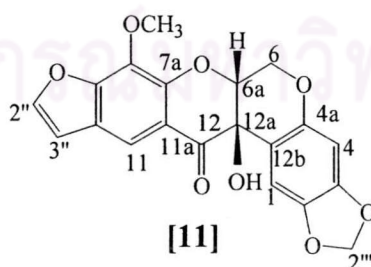


Table 16 The ^1H - and ^{13}C -NMR data of Compound 11 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm) of Compound 11
	(+)-12a- Hydroxypachyrrhizone	Compound 11		
1	6.45 (1H, <i>s</i>)	6.49 (1H, <i>s</i>)	1	105.6
			2	142.2
			3	149.4
4	6.50 (1H, <i>s</i>)	6.46 (1H, <i>s</i>)	4	99.2
6	4.60 (2H, <i>m</i>)	4.50 (1H, <i>dd</i> , 12.1, 1.1)	6	63.8
		4.71 (1H, <i>dd</i> , 12.1, 2.4)		
			8	133.5
			9	151.4
			10	124.3
11	7.85 (1H, <i>s</i>)	7.88 (1H, <i>s</i>)	11	113.9
			12	193.1
			4a	149.7
6a	4.60 (1H, <i>m</i>)	4.61 (1H, <i>dd</i> , 2.4, 0.9)	6a	75.9
			7a	149.3
			11a	115.7
			12a	68.2
			12b	109.0
2''	7.55(1H, <i>d</i> , 3.0)	7.55(1H, <i>d</i> , 2.3)	2''	146.3
3''	6.70(1H, <i>d</i> , 3.0)	6.73 (1H, <i>d</i> , 2.2)	3''	107.2
2'''	5.80 (2H, <i>s</i>)	5.90 and 5.84 (each H, each <i>d</i> , 1.5)	2'''	101.3
OCH_3	4.10 (3H, <i>s</i>)	4.10 (3H, <i>s</i>)	OCH_3	61.1
OH	4.60 (1H, <i>s</i>)	4.40 (1H, <i>s</i>)		

2.8 Structure Determination of Compound 12

Compound **12** was obtained as colourless oil. The EI mass spectrum (**Figure 62**) showed the molecular ion peak at m/z 410, belonging to the molecular formula $C_{23}H_{22}O_7$. The UV absorption spectrum (**Figure 63**) showed λ_{\max} at 293, 244 and 205 nm. The IR spectrum (**Figure 64**) exhibited absorption bands at 3446 (OH stretching), 2962 (CH stretching), 1673 (conjugated C=O stretching) and 1507 and 1614 (aromatic ring) cm^{-1} . This compound provided $[\alpha]_D^{27}$ at -145, which was related to that of (-)-rotenone [**19**]. The $^1\text{H-NMR}$ spectral data (**Figure 65**) showed the singlet signal of H-1 at δ 6.55 ppm. These data confirmed the junction between B and C rings as *cis*-B/C ring junction. Compound **12** was determined as rotenone derivative, (-)-12a-hydroxyrotenone [**12**] previously isolated from this plant and *Neorautanenia* species (Oberholzer, Rall and Roux, 1974; Puyvelde, 1987). The pattern of $^1\text{H-NMR}$ in the present work was closely similar to that in the literature (Puyvelde, 1987) but the $^{13}\text{C-NMR}$ data (**Figure 66**) should be revised some positions, including C-1 and C-12b. The C-1 should be located at δ 109.3 ppm, while the C-12b should be located at δ 108.7 ppm. The successful elucidation was supported by application of 2D-NMR as HMQC (**Figure 67**) and HMBC (**Figure 68**).

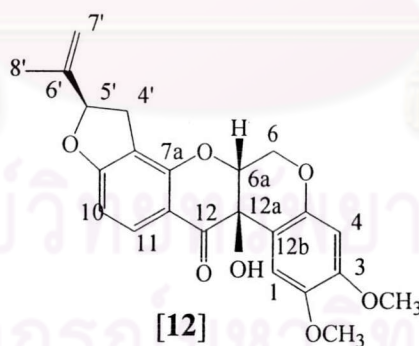


Table 17 The ^1H - and ^{13}C -NMR data of Compound 12 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	(+)-12a-Hydroxyrotenone	Compound 12		(+)-12a-Hydroxyrotenone	Compound 12
1	6.53(1H, <i>s</i>)	6.55 (1H, <i>s</i>)	1	108.8	109.3
			2	142.9	143.9
			3	151.2	151.1
4	6.44 (1H, <i>s</i>)	6.48 (1H, <i>s</i>)	4	101.1	101.0
6	4.50 (2H, <i>m</i>)	4.50 (1H, <i>m</i>)	6	63.9	63.8
		4.62 (overlap)	8	113.2	113.2
			9	168.0	168.0
10	6.80 (1H, <i>d</i> , 8.5)	6.53 (1H, <i>d</i> , 8.5)	10	105.3	105.3
11	7.70 (1H, <i>d</i> , 8.5)	7.82 (1H, <i>d</i> , 8.5)	11	130.2	130.1
			12	191.1	191.1
6a	4.50 (1H, <i>m</i>)	4.58 (overlap)	4a	148.4	148.3
			6a	76.1	76.0
			7a	157.7	157.7
			11a	111.8	111.7
			12a	67.6	67.5
			12b	109.5	108.7
4'	~ 3 (1H, <i>m</i>)	2.94(1H, <i>dd</i> ,16.0,9.0) 3.29(1H, <i>dd</i> ,16.0,9.0)	4'	31.1	31.1
5'	5.20 (1H, <i>m</i>)	5.24 (1H, <i>t</i> , 9.0)	5'	88.0	87.9
			6'	142.9	142.8
7'	4.80-5.10(1H, <i>m</i>)	4.94 (1H, <i>s</i>)	7'	112.6	112.7
		5.07 (1H, <i>s</i>)			
8'	1.73 (3H, <i>br s</i>)	1.76 (3H, <i>s</i>)	8'	17.1	17.1
OCH ₃	3.70 (3H, <i>s</i>)	3.72 (3H, <i>s</i>)	OCH ₃	55.9 and 56.4	55.8 and
	3.78 (3H, <i>s</i>)	3.82 (3H, <i>s</i>)			56.3
OH	4.50 (1H, <i>br s</i>)	4.48 (1H, <i>s</i>)			

∴ The bold values are revised assignments.

3. Structure Determination of Compounds Isolated from *Millettia leucantha*

3.1 Structure Determination of Compound 279

Compound **279** was acquired as yellow needles. The EI mass spectrum (**Figure 69**) displayed the molecular ion peak at m/z 312, agreeing with the molecular formula $C_{18}H_{16}O_5$, which was supported by elemental analysis (*Anal.* Calcd for $C_{18}H_{16}O_5 \cdot 1/6H_2O$: C, 68.60; H, 5.21. Found: C, 68.66; H, 5.00). The UV spectrum (**Figure 70**) showed λ_{max} at 348, 304, 245 and 206 nm. The IR absorption spectrum (**Figure 71**) exhibited ν_{max} at 1651 (C=O stretching) and 1489 and 1601 (aromatic ring) cm^{-1} . This compound was analyzed as 2',4'-dimethoxy-3,4-methylene dioxychalcone. The 1H -NMR spectrum (**Figure 72**) showed a set of *trans*-olefinic protons at δ 7.35 (H- α) and 7.60 (H- β) ppm, each proton exhibited a coupling constant $J=16$ Hz. This spectrum also revealed the presence of aromatic coupling pattern of two ABX systems, a methylenedioxy group at δ 6.01 (2H, s) and two methoxy substituents at δ 3.87 and 3.91 ppm. Additionally, two fragments of EIMS at m/z 165 [$2,4-(MeO)_2C_6H_3-CO^+$, 43%] indicated that two methoxy groups were located on the A ring, while a methylenedioxy group was on the B ring. This compound has already been synthesized for cosmetic purpose (Salem *et al.*, 2000). The present work, however, was the first time to report this compound as a natural product. The precise assignment was proceeded by the ^{13}C -NMR (**Figure 73**) and 2D-NMR as HMQC (**Figure 74**) and HMBC (**Figure 75**).

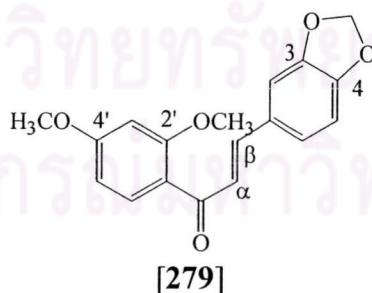


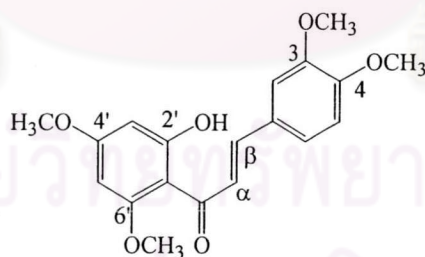
Table 18 The ^1H - and ^{13}C -NMR data of Compound 279 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 279	C	δ_{C} (ppm) of Compound 279
		1	129.9
2	7.12 (1H, <i>d</i> , 1.6)	2	106.6
		3	148.2
		4	149.3
5	6.82 (1H, <i>d</i> , 8.0)	5	108.5
6	7.07 (1H, <i>dd</i> , 8.0,1.6)	6	124.7
		1'	122.3
		2'	160.3
3'	6.50 (1H, <i>d</i> , 2.0)	3'	98.6
		4'	164.0
5'	6.56 (1H, <i>dd</i> , 8.8,2.0)	5'	105.1
6'	7.75 (1H, <i>d</i> , 8.8)	6'	132.8
α	7.35 (1H, <i>d</i> , 16.0)	α	125.3
β	7.60 (1H, <i>d</i> , 16.0)	β	141.0
OCH_2O	6.01 (1H, <i>s</i>)	OCH_2O	101.4
		$\text{C}=\text{O}$	190.3
2'- OCH_3	3.91 (3H, <i>s</i>)	2'- OCH_3	55.7
4'- OCH_3	3.87 (3H, <i>s</i>)	4'- OCH_3	55.5

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3.2 Structure Determination of Compound 280

Compound **280** was obtained as orange needles. The EI mass spectrum (**Figure 76**) displayed $[M]^+$ at m/z 344, suggesting the molecular formula $C_{19}H_{20}O_6$. The UV absorption spectrum (**Figure 77**) provided λ_{max} at 370, 257 and 220 nm. The IR spectrum (**Figure 78**) exhibited absorption bands at 3445 (OH stretching) and 1622 (C=O stretching) cm^{-1} . In 1H -NMR spectrum (**Figure 79**), a set of *trans*-olefinic protons at δ 7.75 (H- α) and 7.80 (H- β) (each *d*, $J=15.5$ Hz) and a chelated hydroxy group at δ 14.40 ppm assigned to OH-2' based on a 2'-hydroxychalcone were observed. This spectrum also exhibited the presence of four methoxy groups at δ 3.83, 3.91, 3.93 and 3.94 ppm. In the aromatic region, a set of two *meta*-coupled protons at δ 5.96 and 6.11 ppm ($J=2$ Hz), and one ABX system at δ 6.90(1H, *d*, $J=8$ Hz), 7.13 (1H, *d*, $J=2$ Hz) and 7.21 (1H, *dd*, $J=8, 2$ Hz) were observed. The EIMS fragments at m/z 181 and 164 indicated that a hydroxy group and two methoxy groups were on the A ring, while two other methoxy groups were on the B ring. Consequently, this compound was determined to be 2'-hydroxy-3,4,4',6'-tetramethoxychalcone [**280**]. It has already been isolated from *Merrilla caloxylon* (Rutaceae) (Fraser and Lewis, 1974) and *Pongamia pinnata* (Leguminosae) (Tanaka *et al.*, 1992). The present work also reported the ^{13}C -NMR spectral data (**Figure 80**) at the first time.



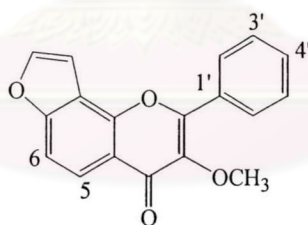
[**280**]

Table 19 The ^1H - and ^{13}C -NMR data of Compound 280 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm) of Compound 280
	2'-hydroxy-3,4,4',6'- tetramethoxychalcone	Compound 280		
2	7.13 (1H, <i>d</i> , 2.0)	7.13 (1H, <i>d</i> , 2.0)	1	128.6
			2	110.4
			3	151.1
			4	149.1
5	6.90 (1H, <i>d</i> , 8.0)	6.90 (1H, <i>d</i> , 8.0)	5	111.2
6	7.22 (1H, <i>dd</i> , 8.0,2.0)	7.21 (1H, <i>dd</i> , 8.0,2.0)	6	122.6
3'	5.97 (1H, <i>d</i> , 2.0)	5.96 (1H, <i>d</i> , 2.0)	1'	106.3
			2'	168.4
			3'	91.2
			4'	166.0
5'	6.12 (1H, <i>d</i> , 2.0)	6.11 (1H, <i>d</i> , 2.0)	5'	93.8
α	7.75 (1H, <i>d</i> , 16.0)	7.75 (1H, <i>d</i> , 16.0)	6'	162.4
			α	125.4
β	7.82 (1H, <i>d</i> , 16.0)	7.80 (1H, <i>d</i> , 16.0)	β	142.6
			C=O	192.4
2'-OH	14.40 (1H, <i>s</i>)	14.40 (1H, <i>s</i>)		
OCH ₃	3.84 (3H, <i>s</i>)	3.83 (3H, <i>s</i>)	OCH ₃	55.5
OCH ₃	3.92 (9H, <i>s</i>)	3.91 (3H, <i>s</i>)	OCH ₃	55.7
OCH ₃		3.93 (3H, <i>s</i>)	OCH ₃	55.8
OCH ₃		3.94 (3H, <i>s</i>)	OCH ₃	56.0

3.3 Structure Determination of Compound 115

Compound **115**, $[M+H]^+$ at m/z 293 in the ESI mass spectrum (**Figure 81**), was isolated as colourless plates from $CHCl_3$. The UV spectrum (**Figure 82**) showed a characteristic of furanoflavonoid chromophore at λ_{max} 304, 260 and 219 nm. The IR spectrum (**Figure 83**) displayed absorption bands at 1633 (C=O stretching) and 1625 and 1458 (aromatic ring) cm^{-1} . The 1H -NMR (**Figure 84**), ^{13}C -NMR (**Figure 85**) spectra and ESIMS confirmed the molecular formula of this compound as $C_{18}H_{12}O_4$. The *ortho*-coupled protons were observed at δ 8.21 (1H, *d*, $J=8.8$ Hz) and 7.56 that located the same position with the other three aromatic protons, including H-3', H-4' and H-5'. Thus, this position showed multiplet signal. Another multiplet signal at δ 8.15 ppm belonged to H-2' and H-6'. The 1H -NMR spectrum also provided the signal of angular furan ring at δ 7.77 (H-2'', *d*, $J=2.4$ Hz) and 7.19 (H-3'', *dd*, $J=2.4, 1.2$ Hz). By comparison with very similar compound, lanceolatin B [**103**], this compound was identified as karanjin (3-methoxy derivative of lanceolatin B) [**115**]. This compound was well known from many plant species. In earlier work, no the 1H -NMR and ^{13}C -NMR was reported. Thus, the present work was the first report for these data.



[115]

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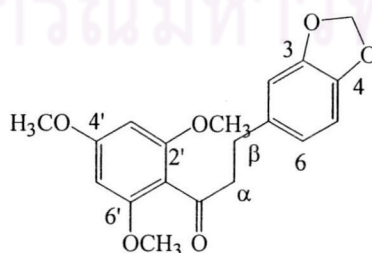
Table 20 The ^1H - and ^{13}C -NMR data of Compound 115 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 115	C	δ_{C} (ppm) of Compound 115
		2	154.9
		3	141.8
		4	175.3
5	8.21 (1H, <i>d</i> , 8.8)	5	121.8
6	7.56 (1H, <i>m</i>)	6	110.0
		7	158.2
		8	117.0
		9	150.0
		10	119.7
		1'	131.1
2'	8.15 (1H, <i>m</i>)	2'	128.4
3'	7.56 (1H, <i>m</i>)	3'	128.7
4'	7.56 (1H, <i>m</i>)	4'	130.7
5'	7.56 (1H, <i>m</i>)	5'	128.7
6'	8.15 (1H, <i>m</i>)	6'	128.4
2''	7.77 (1H, <i>d</i> , 2.4)	2''	145.6
3''	7.19 (1H, <i>dd</i> , 2.4, 1.2)	3''	104.2
OCH_3	3.93 (3H, <i>s</i>)	OCH_3	60.3

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3.4 Structure Determination of Compound 281

Compound **281** was obtained as a pale yellow oil and observed a molecular formula as $C_{19}H_{20}O_6$. The EIMS (**Figure 86**) exhibited a molecular ion peak at m/z 344 and major fragment at m/z 195 [$2,4,6-(MeO)_3C_6H_2CO^+$, 82 %] arose from the ketonic A ring fragment substituted by three methoxy groups, and other major peak at m/z 148 [$3,4-OCH_2O-C_6H_3-CH_2=CH^+$, 100%] belonged to the B ring attached by methylenedioxy group. HRFABMS showed m/z 345.1333 (M+H); (cald. For $C_{19}H_{21}O_6$: 345.1347). The UV spectrum (**Figure 87**) showed maxima absorption bands at 285, 233 and 207 nm. The IR absorption spectrum (**Figure 88**) displayed ν_{max} at 2940 (CH stretching), 1698 (C=O stretching) and 1606 and 1455 (aromatic ring) cm^{-1} . In the 1H -NMR spectrum (**Figure 89**), the presence of two sets of methylene protons at δ 3.02 (2H, *m*, H- α) and 2.91 ppm (2H, *m*, H- β), the unclear ABX coupling pattern at δ 6.71 (2H, *m*, H-2 and H-5) and 6.65 (H-6, *dd*, $J=7.5, 2.0$ Hz), and symmetry meta-protons located at δ 6.09 ppm (2H, *s*, H-3' and H-5') were observed. The ^{13}C -NMR spectral data (**Figure 90**) showed three positions of symmetry carbons, including two methoxy carbons at δ 55.8, two methine carbons of the A-ring at δ 90.6 and two quaternary carbons of the A-ring at δ 158.2 ppm. Furthermore, the data from DEPT-135 (**Figure 91**) provided the appearance of three groups of methylene carbons assigned to one methylenedioxy group at δ 100.7 ppm, and α, β -ethylene carbon, typical of dihydrochalcone, at δ 46.5 and 29.7 ppm, respectively. These data indicated that compound **281** was 2',4',6'-trimethoxy-3,4-methylenedioxydihydrochalcone. This work was the first report for this compound. The precise elucidation was achieved by 2D-NMR techniques as HMQC (**Figure 92**) and HMBC (**Figure 93**).



[281]

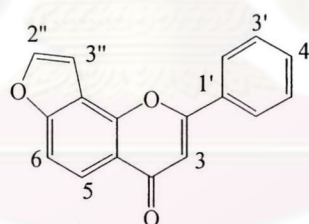
Table 21 The ^1H - and ^{13}C -NMR data of Compound 281 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 281	C	δ_{C} (ppm) of Compound 281
		1	135.4
2	6.71 (1H, <i>d</i> , 2.0)	2	108.9
		3	147.4
		4	145.5
5	6.71 (1H, <i>d</i> , 7.5)	5	108.0
6	6.65 (1H, <i>dd</i> , 7.5, 2.0)	6	121.1
		1'	113.3
		2'	158.2
3'	6.09 (1H, <i>s</i>)	3'	90.5
		4'	162.3
5'	6.09 (1H, <i>s</i>)	5'	90.5
		6'	158.2
α	3.02 (2H, <i>m</i>)	α	46.5
β	2.91 (2H, <i>m</i>)	β	29.7
OCH_2O	5.90 (2H, <i>s</i>)	OCH_2O	100.7
		$\text{C}=\text{O}$	203.4
2'- OCH_3	3.76 (3H, <i>s</i>)	2'- OCH_3	55.8
4'- OCH_3	3.82 (3H, <i>s</i>)	4'- OCH_3	55.4
6'- OCH_3	3.76 (3H, <i>s</i>)	6'- OCH_3	55.8

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3.5 Structure Determination of Compound 103

Compound **103** was colourless needles. The EI mass spectrum (**Figure 94**) appeared the molecular ion peak at m/z 262, consistent with $C_{17}H_{10}O_3$ as the molecular formula. The UV spectrum (**Figure 95**) showed λ_{max} at 297, 263 and 219 nm. The IR spectrum (**Figure 96**) displayed conjugated C=O stretching band at 1646 cm^{-1} . The $^1\text{H-NMR}$ spectrum (**Figure 97**) demonstrated that compound **103** was a furanoflavone [δ 6.90 (H-3, *s*); 7.78 (H-2'', *d*, $J=2.4\text{ Hz}$); 7.22 (H-3'', *dd*, $J=2.4, 1.2\text{ Hz}$)]. This spectrum further exhibited two multiplet signals at δ 7.58 (4H, *m*) assigned to H-6, H-3', H-4' and H-5', and at δ 7.97 (2H, *m*) belonged to H-2' and H-6'. Additionally, an aromatic singlet proton at δ 8.17 ppm was observed. This compound eventually identified as lanceolatin B [**103**], which had already been isolated from many plants, for instance *Pongamia glabra* (Talapatra, Mallik and Talapatra, 1980), *P. pinnata* (Tanaka *et al.*, 1992) and *Millettia sanagana* (Mbafor, *et al.*, 1995). Comparison of the $^1\text{H-}$ and $^{13}\text{C-NMR}$ (**Figure 98**) spectra between compound **103** from this work and lanceolatin B from the literature (Tanaka *et al.*, 1992; Mbafor, *et al.*, 1995) has been given in **Table 22**.



[103]

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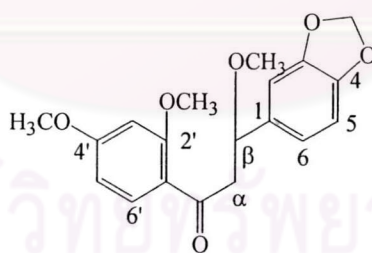
Table 22 The ^1H - and ^{13}C -NMR data of Compound 103 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	Lanceolatin B	Compound 103		Lanceolatin B	Compound 103
3	6.90 (1H, <i>s</i>)	6.90 (1H, <i>s</i>)	2	162.7	162.6
			3	108.1	108.0
			4	178.2	178.2
5	8.18 (1H, <i>d</i> , 9.0)	8.17(1H, <i>d</i> , 8.8)	5	121.8	121.8
6	7.58 (1H, <i>m</i>)	7.56 (1H, <i>m</i>)	6	110.2	110.2
2'	7.97 (1H, <i>m</i>)	7.97 (1H, <i>m</i>)	7	158.4	158.3
			8	117.2	117.1
			9	150.9	150.8
			10	119.4	119.4
			1'	131.8	131.8
			2'	126.2	126.2
			3'	129.1	129.1
			4'	131.5	131.5
			5'	129.1	129.1
			6'	126.2	126.2
2''	7.79 (1H, <i>d</i> , 2.0)	7.78(1H, <i>d</i> , 2.4)	2''	145.8	145.8
3''	7.26 (1H, <i>d</i> , 2.0)	7.22 (1H, <i>dd</i> , 2.4, 1.2)	3''	104.2	104.2

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3.6 Structure Determination of Compound 102

Compound **102** was acquired as a colourless oil. The EI mass spectrum (**Figure 99**) showed the molecular ion peak at m/z 344, analyzed for $C_{19}H_{20}O_6$. The UV spectrum (**Figure 100**) displayed absorptions at 295, 269 and 220 nm. The IR spectrum (**Figure 101**) exhibited absorption bands at 2935 (CH stretching), 1666 (C=O stretching) and 1600 and 1488 (aromatic ring) cm^{-1} . The base peak of EIMS at m/z 165 was attributed to both $[2,4-(OMe)_2-C_6H_3-CO]^+$ and $[3,4-OCH_2O-C_6H_3-CH-OMe]^+$. The 1H -NMR spectrum (**Figure 102**) presented an ABX system centred at δ 3.19, 3.48 and 4.74 ppm and a singlet (3H) at δ 3.19 ppm, typical of a methoxy substituent on the aliphatic β -carbon of dihydrochalcone. This spectrum also exhibited two aromatic ABX systems, one was located at δ 6.43 (H-3', *d*, $J=2.4$ Hz), 6.51 (H-5', *dd*, $J=8.0, 2.4$ Hz) and 7.77 (H-6', *d*, $J=8.8$ Hz), and another was located at δ 6.88 (H-2, *d*, $J=1.6$ Hz), 6.77 (H-5, *d*, $J=8.0$ Hz) and 6.82 (H-6, *dd*, $J=8.0, 1.6$ Hz). Additionally, this compound also contained two methoxy groups on the A-ring and methylenedioxy group on the B-ring. Thus, this compound was assigned to dihydromillettone methyl ether [**102**] by comparison of the above data with those of the literature (Mahmoud and Waterman, 1985). However, this work was the first time to report the ^{13}C -NMR spectral data (**Figure 103**).



[**102**]

Table 23 The ^1H - and ^{13}C -NMR data of Compound **102** in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm) of Compound 102
	Dihydromillettene methyl ether	Compound 102		
2	6.88 (1H, <i>d</i> , 2.0)	6.88 (1H, <i>d</i> , 1.6)	1	135.9
			2	108.0
			3	147.8
			4	146.9
5	6.76 (1H, <i>d</i> , 8.0)	6.77(1H, <i>d</i> , 8.0)	5	107.0
6	6.82 (1H, <i>dd</i> , 8.0,2.0)	6.82 (1H, <i>dd</i> , 8.0,1.6)	6	120.4
2'	7.78 (1H, <i>d</i>, 8.0)	6.43 (1H, <i>d</i>, 2.4)	1'	121.2
			2'	160.6
3'	6.51 (1H, <i>dd</i>, 8.0,2.0)	6.43 (1H, <i>d</i>, 2.4)	3'	98.3
5'	6.43 (1H, <i>d</i>, 2.0)	6.51 (1H, <i>dd</i>, 8.8,2.4)	4'	164.4
			5'	105.1
6'		7.77 (1H, <i>d</i>, 8.8)	6'	132.8
α_{eq}	3.22 (1H, <i>dd</i> , 16.9, 4.9)	3.19(1H, <i>dd</i> ,16.8,4.8)	α	52.0
α_{ax}	3.47 (1H, <i>dd</i> , 16.9, 8.0)	3.48(1H, <i>dd</i> ,16.8,8.0)		
β_{ax}	4.73 (1H, <i>dd</i> , 8.0, 4.9)	4.74(1H, <i>dd</i> ,8.0, 4.8)	β	79.5
OCH_2O		5.95 (2H, <i>d</i> , 1.2)	OCH_2O	100.9
			$\text{C}=\text{O}$	197.2
$\beta\text{-OCH}_3$	3.18 (3H, <i>s</i>)	3.19 (3H, <i>s</i>)	OCH_3	55.4
2'- OCH_3		3.86 (3H, <i>s</i>)	OCH_3	55.4
4'- OCH_3	3.84 (3H, <i>s</i>)	3.84 (3H, <i>s</i>)	OCH_3	56.5
6'- OCH_3	3.85 (3H, <i>s</i>)			

-: The bold values should be noticed.

3.7 Structure Determination of Compound 282

Compound **282** was obtained as pale yellow needles. The EI mass spectrum (**Figure 104**) showed the molecular ion peak at m/z 372, agreeing with $C_{20}H_{20}O_7$, which was supported by elemental analysis (*Anal.* Calcd for $C_{20}H_{20}O_7 \cdot 1/4H_2O$: C, 63.74; H, 5.48. Found: C, 64.04; H, 5.48). Substitution of the ketonic A ring by a methylenedioxy group deduced by the appearance of peaks at m/z 149 [3,4-OCH₂O-C₆H₃-CO⁺, 27 %] and 121 [3,4-OCH₂O-C₆H₃⁺, 20 %]. Additionally, a base peak (m/z 341) was reasonably assigned to be a benzopyrilium cation (Kiuchi, Chen and Tsuda, 1990) produced by a loss of a methoxy group as shown in **Chart 1**. The UV spectrum (**Figure 105**) displayed λ_{max} at 320, 277 and 211 nm. In the IR spectrum (**Figure 106**), absorption bands at 2940 (CH stretching), 1659 (C=O stretching), and 1587 and 1487 (aromatic ring) cm^{-1} were appeared. The ¹H-NMR spectral data (**Figure 107**) exhibited an olefinic proton as singlet at δ 6.33 assigned as H- α , a singlet (3H) at δ 3.83 belonged to β -methoxy group confirmed by HMBC signal of methoxy proton into C- β , and other three methoxy groups substituted on the B-ring at δ 3.73 (6H, *s*) and 3.80 (3H, *s*). In DEPT-135 experiment (**Figure 109**), the presence of methylene carbon signal at δ 101.4 also supported that compound **282** contains only one methylenedioxy group (¹H-NMR signal of methylenedioxy protons as singlet at δ 5.98 ppm), whereas the signal at δ 90.8 belonged to both C-3 and C-5 methine carbons under symmetrical condition (¹H-NMR signal of these carbons as singlet at δ 6.10 ppm). This compound was suggested to be a new β -methoxychalcone, 2,4,6, β -tetramethoxy-3',4'-methylenedioxychalcone [**282**]. The assignment was supported by the ¹³C-NMR (**Figure 108**) resonances and 2D-NMR as HMQC (**Figure 110**) and HMBC (**Figure 111**) experiments. Furthermore, the configuration of the olefinic function was determined by NOE experiment (the selected NOE enhancements were shown in structure **283**). An *E*-isomer is known to be the preferred isomer in naturally occurring β -methoxychalcones (Kiuchi, Chen and Tsuda, 1990). Thus, the structure **282** was completely elucidated by this observations and the data from HMBC experiment.

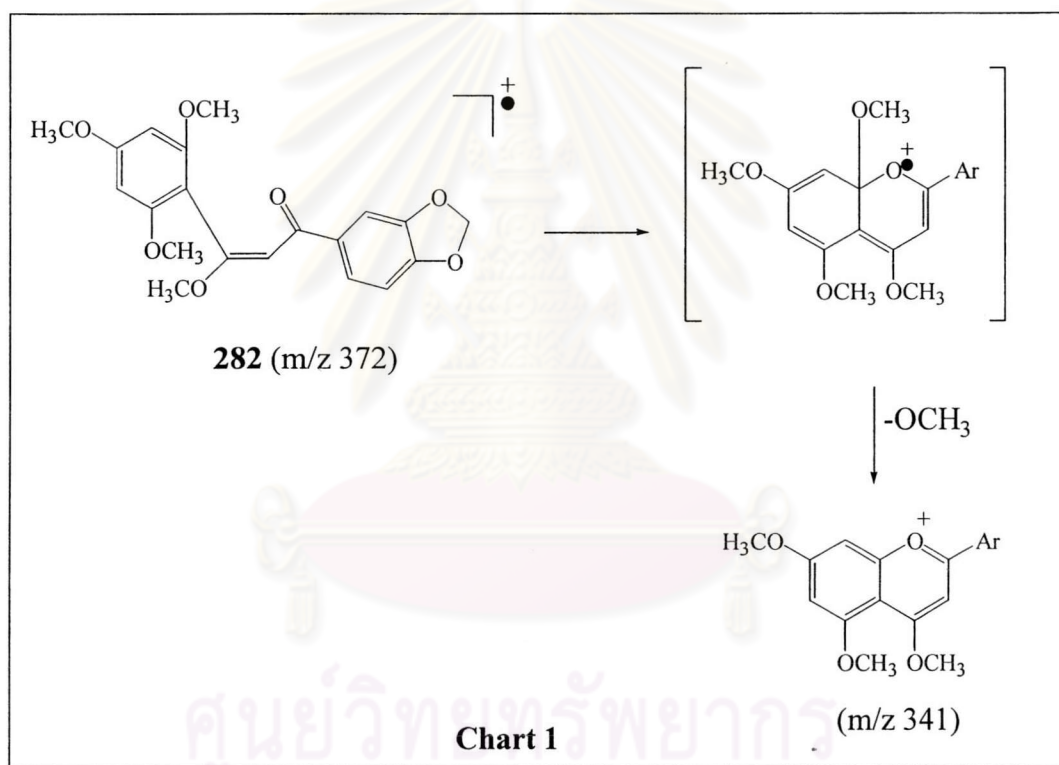
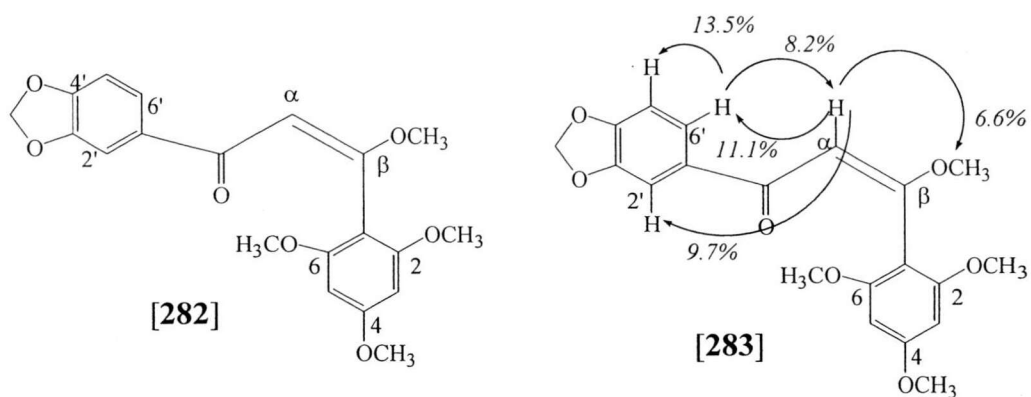


Chart 1 A Possible Formation of Benzopyrilium cation from β -Methoxychalcone 282 in EIMS

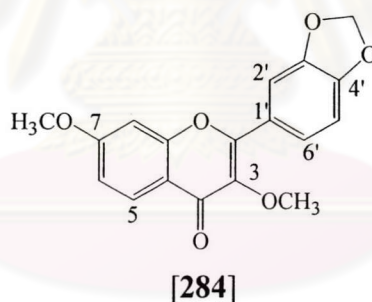
Table 24 The ^1H - and ^{13}C -NMR data of Compound 282 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 282	C	δ_{H} (ppm) of Compound 282
		1	107.0
		2	158.5
3	6.10 (1H, <i>s</i>)	3	90.8
		4	162.1
5	6.10 (1H, <i>s</i>)	5	90.8
		6	158.5
		1'	134.7
2'	7.32 (1H, <i>d</i> , 2.0)	2'	108.2
		3'	147.5
		4'	150.4
5'	6.76 (1H, <i>d</i> , 8.4)	5'	107.4
6'	7.46 (1H, <i>dd</i> , 8.4, 2.0)	6'	123.4
α	6.33 (1H, <i>s</i>)	α	101.2
		β	165.7
OCH_2O	5.98 (2H, <i>s</i>)	OCH_2O	101.4
		$\text{C}=\text{O}$	188.4
$\beta\text{-OCH}_3$	3.83 (3H, <i>s</i>)	$\beta\text{-OCH}_3$	55.9
2- OCH_3	3.73 (3H, <i>s</i>)	2- OCH_3	55.9
4- OCH_3	3.80 (3H, <i>s</i>)	4- OCH_3	55.2
6- OCH_3	3.73 (3H, <i>s</i>)	6- OCH_3	55.9

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3.8 Structure Determination of Compound 284

Compound **284** was obtained as white solids. The EI mass spectrum (**Figure 112**) showed the molecular ion peak at m/z 326, corresponding to the molecular formula $C_{18}H_{14}O_6$. The UV spectrum (**Figure 113**) showed absorption maxima at 339, 314, 242 and 208 nm. The IR absorption spectrum (**Figure 114**) displayed ν_{\max} at 1619 and 1445 cm^{-1} belonged to the aromatic region. The 1H - and ^{13}C -NMR spectra (**Figure 115** and **116**, respectively) revealed the presence of methylenedioxy group at δ_H 6.06 (2H, *s*) and δ_C 101.6 ppm, two series of three coupled aromatic protons showing ABX patterns and two methoxy substituents assigned as 3-OCH₃ at δ 3.88 and 7-OCH₃ at δ 3.91. Furthermore, the EIMS gave ions at m/z 146 [$3,4-OCH_2O-C_6H_3-C\equiv CH$]⁺, typical of a methylenedioxy substituted B-ring. These data were identical with those in the literature (Das *et al.*, 1994), thus requiring the flavone to be assigned as desmethoxykanugin [284]. This compound was earlier isolated from *Pongamia glabra* (Subrahmanyam, Rao and Rao, 1977) and *Gelonium multiflorum* (Das *et al.*, 1994).



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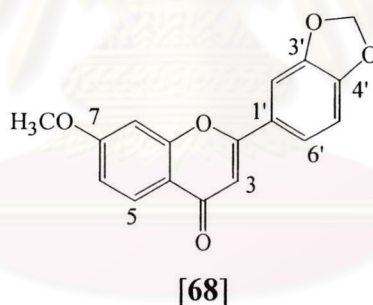
Table 25 The ^1H - and ^{13}C -NMR data of Compound 284 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	Desmethoxykanugin	Compound 284		Desmethoxykanugin	Compound 284
			2	154.7	154.6
			3	140.8	140.7
			4	174.4	174.4
5	8.14(1H, <i>d</i> , 8.8)	8.14(1H, <i>d</i> , 9.0)	5	127.1	127.0
6	6.96(1H, <i>dd</i> , 8.8, 2.4)	6.95(1H, <i>dd</i> , 9.0, 2.0)	6	114.3	114.3
			7	156.8	156.8
8	6.89 (1H, <i>d</i> , 2.4)	6.89(1H, <i>d</i> , 2.0)	8	99.9	99.8
			9	164.0	164.0
			10	118.0	118.0
			1'	124.8	124.7
2'	7.61 (1H, <i>d</i> , 1.8)	7.61(1H, <i>d</i> , 2.0)	2'	108.6	108.5
			3'	147.9	147.8
			4'	149.5	149.4
5'	6.94(1H, <i>d</i> , 8.2)	6.94(1H, <i>d</i> , 8.0)	5'	108.4	108.3
6'	7.69(1H, <i>dd</i> , 8.2, 1.8)	7.69(1H, <i>dd</i> , 8.0, 2.0)	6'	123.4	123.3
OCH ₂ O	6.06 (2H, <i>s</i>)	6.06 (2H, <i>s</i>)	OCH ₂ O	101.6	101.6
3-OCH ₃	3.88 (3H, <i>s</i>)	3.88 (3H, <i>s</i>)	3-OCH ₃	60.0	60.0
7-OCH ₃	3.91 (3H, <i>s</i>)	3.91 (3H, <i>s</i>)	7-OCH ₃	55.8	55.8

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3.9 Structure Determination of Compound 68

Compound **68** was acquired as colourless needles. The EI mass spectrum (**Figure 117**) displayed the molecular ion peak at m/z 296, analysed for $C_{17}H_{12}O_5$. The UV spectrum (**Figure 118**) exhibited absorption maxima at 334, 311, 237 and 220 nm. The IR spectrum (**Figure 119**) showed ν_{max} at 1638, 1502 and 1432 cm^{-1} . The $^1\text{H-NMR}$ spectrum (**Figure 120**) showed an aromatic singlet signal at δ 6.64 ppm for H-3, a B-ring spin system for 3',4'-substitution at δ 6.08 (2H, *s*), one methoxy substituent on the A-ring at δ 3.93 (3H, *s*), and also showed two typical of ABX systems. In EIMS, the fragment ions at m/z 146 and 134, which were the characteristic of methylenedioxy substituted B-ring, were observed. Thus, the combination of $^1\text{H-NMR}$, EIMS data and comparison of ^1H - and $^{13}\text{C-NMR}$ (**Figure 121**) data with those in the literature, fully supported that this flavone was 3',4'-methylenedioxy-7-methoxyflavone [**68**] that previously reported from *Millettia hemsleyana* (Mahmoud and Waterman, 1985). However, its $^{13}\text{C-NMR}$ spectral data of this compound was reported here for the first.



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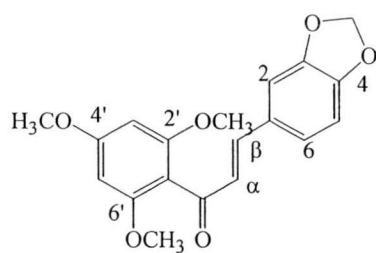
Table 26 The ^1H - and ^{13}C -NMR data of Compound 68 in CDCl_3

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm) of Compound 68 (in CDCl_3)
	3',4'-methylenedioxy-7- methoxyflavone (in $\text{C}_5\text{D}_5\text{N}$)	Compound 68 (in CDCl_3)		
3	7.08 (1H, <i>s</i>)	6.64 (1H, <i>s</i>)	2	162.7
			3	108.7
5	8.35 (1H, <i>d</i> , 9.0)	8.12 (1H, <i>d</i> , 8.8)	4	177.8
			5	127.0
6	7.05 (1H, <i>dd</i> , 9.0, 2.0)	6.99 (1H, <i>dd</i> , 8.8, 2.4)	6	114.3
			7	157.9
8	7.15 (1H, <i>d</i> , 2.0)	6.95 (1H, <i>d</i> , 2.4)	8	100.4
			9	164.1
2'	7.60 (1H, <i>d</i> , 2.0)	7.35 (1H, <i>d</i> , 2.0)	10	117.8
			1'	125.9
5'	6.99 (1H, <i>d</i> , 8.0)	6.93 (1H, <i>d</i> , 8.4)	2'	106.6
			3'	148.4
6'	7.54 (1H, <i>dd</i> , 8.0, 2.0)	7.48 (1H, <i>dd</i> , 8.4, 2.0)	4'	150.4
			5'	106.2
OCH ₂ O	6.08 (2H, <i>s</i>)	6.08 (2H, <i>s</i>)	6'	121.2
7-OCH ₃	3.80 (3H, <i>s</i>)	3.90 (3H, <i>s</i>)	OCH ₂ O	101.9
			7-OCH ₃	55.8

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3.10 Structure Determination of Compound 285

Compound **285** was obtained as pale yellow needles. The EI mass spectrum (**Figure 122**) exhibited the molecular ion peak at m/z 342, and the analytical calculation gave C, 66.66; H, 5.30 (Found C, 66.49; H, 5.24). These data were consistent with $C_{19}H_{18}O_6$. The UV spectral data (**Figure 123**) displayed λ_{\max} at 344, 296, 254 and 208 nm. The IR spectrum (**Figure 124**) showed ν_{\max} at 1646 (C=O stretching) and 1603 and 1489 (aromatic ring) cm^{-1} . The $^1\text{H-NMR}$ spectrum (**Figure 125**) revealed the presence of a set of *trans*-olefinic protons at δ 6.78 and 7.28 (each *d*, $J=16.0$ Hz), a series of three coupled aromatic protons presenting ABX system at δ 7.05 (H-2, *d*, $J=2.0$ Hz), 6.79 (H-5, *d*, $J=8.0$ Hz) and 6.98 (H-6, *dd*, $J=8.0, 2.0$ Hz) in addition to 2',4',6'-trimethoxy substitution on the A-ring and 3,4-methylenedioxy substitution on the B-ring. The DEPT-135 (**Figure 127**) encouraged the $^1\text{H-NMR}$ and other spectral data as mentioned above by presenting one methylene carbon at δ 101.5 belonged to methylenedioxy carbon and the signal at δ 90.7 ppm assigned to both C-3' and C-5' methine carbons, whilst their $^1\text{H-NMR}$ signals were located at the same position of δ 6.16 (2H, *s*). Additionally, the ^1H -decoupling experiment (**Figure 128**) was proceeded to confirm the overlapping signals at δ 6.78-6.79 ppm (H- α and H-5) by irradiation of the signals at δ 6.78-6.79 (H- α and H-5) and 7.28 (H- β). These data showed the presence of a chalcone skeleton and a similar substitution pattern of those of dihydrochalcone **281**. This compound also exhibited chemical correlation with compound **281** confirmed by the successful process of 1,4-reduction with $\text{Et}_3\text{SiH}/\text{CF}_3\text{CO}_2\text{H}$, in which perhydrochalcone **286** was obtained as an over-reduction by-product (**Chart 2**). This compound was consequently identified as 2',4',6'-trimethoxy-3,4-methylenedioxychalcone [**285**], which was isolated from a natural source at the first time but has been patented for synthetic product without $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectral data (Klein, 1993). The assignment of this compound was completely done by $^{13}\text{C-NMR}$ (**Figure 126**), HMQC (**Figure 129**) and HMBC (**Figure 130**) experiments.



[285]

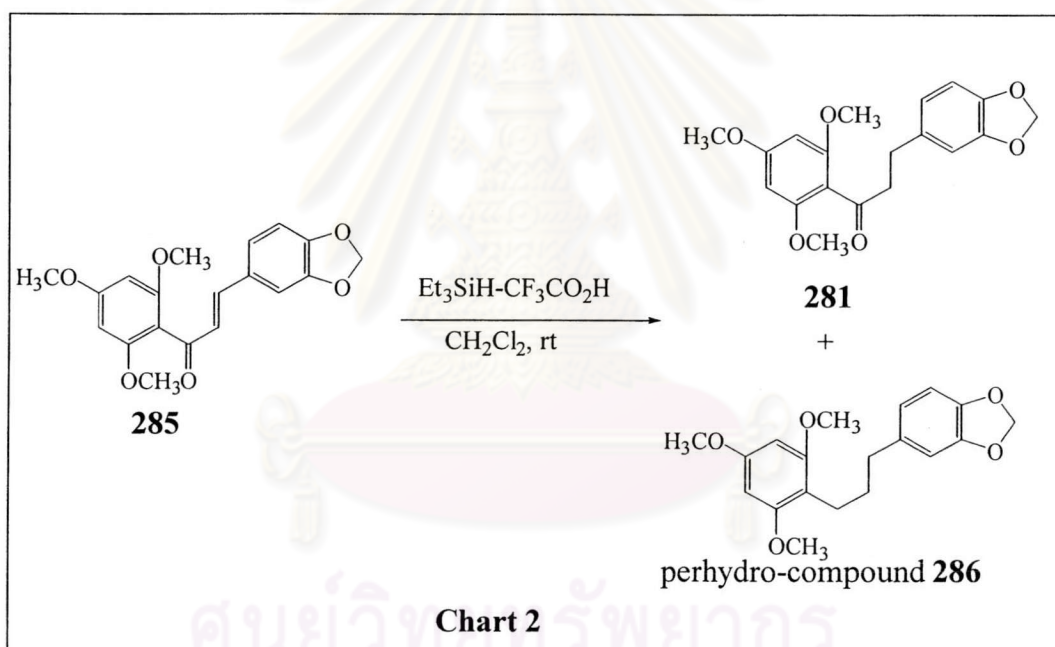


Chart 2 Chemical Correlation of **285** to **281** by Et_3SiH Reduction

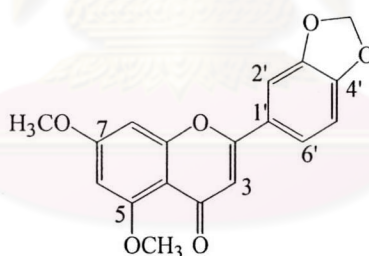
Table 27 The ^1H - and ^{13}C -NMR data of Compound 285 in CDCl_3

H	δ_{H} (ppm), J (Hz) of Compound 285	C	δ_{C} (ppm) of Compound 285
		1	129.4
2	7.05 (1H, <i>d</i> , 2.0)	2	106.7
		3	148.2
		4	149.5
5	6.79 (1H, <i>d</i> , 8.0)	5	108.4
6	6.98 (1H, <i>dd</i> , 8.0, 2.0)	6	124.7
		1'	111.9
		2'	158.7
3'	6.16 (1H, <i>s</i>)	3'	90.7
		4'	162.3
5'	6.16 (1H, <i>s</i>)	5'	90.7
		6'	158.7
α	6.78 (1H, <i>d</i> , 16.0)	α	127.3
β	7.28 (1H, <i>d</i> , 16.0)	β	144.0
OCH_2O	5.99 (2H, <i>s</i>)	OCH_2O	101.5
		$\text{C}=\text{O}$	194.1
2'- OCH_3	3.77 (3H, <i>s</i>)	2'- OCH_3	55.9
4'- OCH_3	3.86 (3H, <i>s</i>)	4'- OCH_3	55.4
6'- OCH_3	3.77 (3H, <i>s</i>)	6'- OCH_3	55.9

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3.11 Structure Determination of Compound 287

Compound **287** was obtained as colourless needles. The EI mass spectrum (**Figure 131**) exhibited the molecular ion peak at m/z 326, agreeing with the molecular formula $C_{18}H_{14}O_6$. This spectrum also showed the fragment ions at m/z 146 (42 %) and 134 (10 %), typical of a methylenedioxy substituted B-ring like compound **68** and **284**. The UV absorption spectrum (**Figure 132**) showed λ_{max} at 334, 265, 240 and 220 nm. The IR spectrum (**Figure 133**) showed ν_{max} at 1653 (C=O stretching) and 1616 and 1456 (aromatic ring) cm^{-1} . On the 1H -NMR spectrum (**Figure 134**), a B-ring spin pattern for 3',4'-methylenedioxy substitution at δ 6.08 (2H, *s*), and one ABX system at δ 7.55 (H-2', *d*, $J=1.8$ Hz), 6.99 (H-5', *d*, $J=7.9$ Hz) and 7.52 (H-6', *dd*, $J=7.9, 1.8$ Hz) were appeared. This spectrum also exhibited the signals for *meta*-coupled A-ring protons at δ 6.55 (H-6, *d*, $J=2.4$ Hz) and 6.79 (H-8, *d*, $J=2.4$ Hz), and two methoxy groups at δ 3.86 (6H, *s*). By comparison with the NMR data in the literature (Tomazela *et al.*, 2000) and its similar compounds (**68** and **284**), and from supporting of the ^{13}C -NMR data (**Figure 135**), this compound was assigned to 3',4'-methylenedioxy-5,7-dimethoxyflavone [**287**].



[287]

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Table 28 The ^1H - and ^{13}C -NMR data of Compound 287 in $\text{C}_5\text{D}_5\text{N}$

H	δ_{H} (ppm), J (Hz)		C	δ_{C} (ppm)	
	3',4'- methylenedioxy - 5,7-dimethoxy flavone	Compound 287		3',4'- methylenedioxy - 5,7-dimethoxy flavone	Compound 287
3	6.92 (1H, <i>s</i>)	6.95 (1H, <i>s</i>)	2	160.2	160.2
			3	108.7	108.7
			4	176.6	176.7
			5	161.3	161.3
			6	96.8	96.8
6	6.55 (1H, <i>d</i> , 2.3)	6.55 (1H, <i>d</i> , 2.4)	7	164.4	164.4
			8	93.7	93.6
8	6.78 (1H, <i>d</i> , 2.3)	6.79 (1H, <i>d</i> , 2.4)	9	160.1	160.1
			10	109.7	109.7
2'	7.54 (1H, <i>d</i> , 1.8)	7.56 (1H, <i>d</i> , 1.8)	1'	126.0	126.0
			2'	106.6	106.5
			3'	148.9	148.9
			4'	150.6	150.6
			5'	108.9	108.9
6'	7.51 (1H, <i>dd</i> , 8.1, 1.8)	7.52 (1H, <i>dd</i> , 8.0, 1.8)	6'	121.3	121.3
OCH ₂ O	6.07 (2H, <i>s</i>)	6.08 (2H, <i>s</i>)	OCH ₂ O	101.6	101.6
OCH ₃	3.85 (3H, <i>s</i>)	3.86 (3H, <i>s</i>)	OCH ₃	55.9	55.9
OCH ₃	3.85 (3H, <i>s</i>)	3.86 (3H, <i>s</i>)	OCH ₃	56.2	56.2

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4. Biological Activities of Compounds from *Pachyrrhizus erosus*

All isolated compounds from *Pachyrrhizus erosus* in the present work, including compounds **2**, **4**, **8**, **11**, **12**, **15**, **17** and **18** were subjected to cytotoxicity test against NCI-H460 cell line, while compounds **4**, **8**, **11**, **15**, **17** and **18** were also subjected to antimicrobial activity test using agar diffusion method. In evaluation of COX-2 selectively inhibitory activity using Kirtikara's method (Kirtikara *et al.*, 2001), compounds **17** and **18** were tested at 10 µg/ml. All compounds from the above activity tests were inactive (data not shown). However, anti-HSV activity test using inactivated plaque reduction assay (Abou-Karam and Shier, 1990) for crude CHCl₃ extract of *P. erosus* seeds and pure compounds **4**, **8**, **11**, **15**, **17** and **18** showed that compound (+)-12a-hydroxydolineone [**8**] could inhibit HSV-1 activity at IC₅₀ of 25.5 µg/ml, and compound (+)-12a-hydroxypachyrrhizone [**11**] could inhibit both HSV-1 and HSV-2 activities at IC₅₀ of 18.0 and 18.5 µg/ml, respectively. Moreover, the investigation of anti-HSV activity were also observed cytotoxic doses to normal cell (Vero cell) of **8** at concentration >50 µg/ml and **11** at concentration 50 µg/ml. This result (**Table 29**) demonstrated the importance of the hydroxy substitution at C-12a to the activity.

5. Biological Activities of Compounds from *Millettia leucantha*

5.1 Antimicrobial Activity Test

Compounds **102**, **103**, **279**, **280**, **281**, **282**, **284**, **285** and **287** were subjected to antimicrobial activity test using agar diffusion method. No compound quite showed this inhibition activity (data not shown).

5.2 Anti-HSV Activity Test

Nine compounds, comprising **102**, **103**, **279**, **280**, **281**, **282**, **284**, **285** and **287** were subjected to anti-HSV activity test using inactivated plaque reduction assay (Abou-Karam and Shier, 1990). Compounds **102** and **281** showed moderate activity when compared to the positive control, acyclovir (IC₅₀ at 0.06 µg/ml for HSV-1 and 0.5 µg/ml for HSV-2). Compound **102** displayed inhibition activity at IC₅₀ 17.0 µg/ml for HSV-1 and 36.3 µg/ml for HSV-2, whereas compound **281** inhibited HSV-1 activity at IC₅₀ 15.5 µg/ml and inhibited HSV-2 activity at IC₅₀ 17.0 µg/ml. The cytotoxic dose (CC₅₀) to normal cell of **102** was observed at 45.5 µg/ml, whilst this of **281** was displayed at 38.5 µg/ml. Both compounds **102** and **281** were

dihydrochalcones, suggesting that two saturated carbon bridges might be responsible for this activity. This result was deduced in **Table 30**.

5.3 COX-2 Inhibitory Activity Test

Seven compounds as compounds **103**, **279**, **280**, **282**, **284**, **285** and **287** were tested for selective COX-2 inhibitory activity by means of Kirtikara's method (Kirtikara *et al.*, 2001). The only compound, desmethoxykanugin [**284**] exhibited significantly selective COX-2 inhibitory activity at IC_{50} 0.96 μ M. Aspirin, indomethacin and NS-398 were employed as positive controls. This result was demonstrated in **Table 31**.

5.4 Cytotoxic Activity Test

All isolated compounds from *Millettia leucantha* except compound **115** were subjected to cytotoxicity test against human lung cancer NCI-H460 cell line. 2',4'-Dimethoxy-3,4-methylenedioxychalcone [**279**] and 2',4',6'-trimethoxy-3,4-methylene dioxychalcone [**285**] exhibited activity at IC_{50} 7.36 and 3.69 μ g/ml, respectively. Both compounds were chalcones without any substituents on α - and β -carbons, suggesting that the *trans*-olefinic protons might be important for the activity. This result was summarized in **Table 32**.

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Table 29 Inhibitory Effect of Isolates from *Pachyrrhizus erosus* Against HSV-1 and HSV-2

Tested sample	Conc. (µg/ml)	% Inhibition ^{a)}		IC ₅₀ (µg/ml) ^{b)}		CC ₅₀ (µg/ml) ^{c)}	Selectivity Index ^{d)}	
		HSV-1	HSV-2	HSV-1	HSV-2		HSV-1	HSV-2
Crude CHCl ₃ extract	5	84.7	46.6	-	-	-	-	-
(+)-Dolineone [4]	10	0	0	-	-	-	-	-
	50	15.0	24.4	-	-	-	-	-
(+)-12a-Hydroxy dolineone [8]	10	31.0	29.0	-	-	-	-	-
	50	82.7 ^{f)}	42.5	25.5	-	>50	-	ND
(+)-12a-Hydroxy pachyrrhizone [11]	10	27.5	13.5	-	-	-	-	-
	20	56.6 ^{f)}	56.3 ^{f)}	18.0	18.5	35	1.9	1.9
Neotenone [15]	10	0	0	-	-	-	-	-
	50	0	0	-	-	-	-	-
Pachyrrhizin [17]	10	0	0	-	-	-	-	-
	50	26.1	23.7	-	-	-	-	-
Pachyrrhizone [18]	10	0	0	-	-	-	-	-
	50	0	15.5	-	-	-	-	-
Acyclovir ^{e)}	-	-	-	0.06	0.50	-	-	-

a)-: Inactivation, Plaque reduction assay

b)-: IC₅₀ (50% Inhibitory concentration, Mean of 3 independent experiments)

c)-: CC₅₀ (50% Cytotoxic concentration, Examined by trypan blue exclusion method, Mean of 3 independent experiments)

d)-: Selectivity Index = CC₅₀/IC₅₀

e)-: Acyclovir as positive control

f)-: Compounds exhibiting more than 50% inhibition at ≤ 50 µg/ml were further determined for IC₅₀.

Table 30 Inhibitory Effect of Compounds from *Millettia leucantha* Against HSV-1 and HSV-2

Compound	Conc. ($\mu\text{g/ml}$)	% Inhibition ^{a)}		IC ₅₀ ($\mu\text{g/ml}$) ^{b)}		CC ₅₀ ($\mu\text{g/ml}$) ^{c)}	Selectivity Index ^{d)}	
		HSV-1	HSV-2	HSV-1	HSV-2		HSV-1	HSV-2
Dihydromillettone methyl ether [102]	10	38	16					
	20	53	33					
	30	65 ^{f)}	41					
	40	72 ^{f)}	56 ^{f)}	17.0	36.3	45.5	2.7	1.3
Lanceolatin B [103]	10	0	0					
	50	0	0					
2',4'-Dimethoxy-3,4-methylenedioxy chalcone [279]	10	0	0					
	50	0	0					
2'-Hydroxy-3,4,4',6'-tetramethoxy chalcone [280]	10	0	0					
	50	0	0					
2',4',6'-Trimethoxy-3,4-methylenedioxy dihydrochalcone [281]	10	37	33					
	20	58 ^{f)}	55 ^{f)}					
	30	80 ^{f)}	79 ^{f)}	15.5	17.0	38.5	2.5	2.3
2,4,6, β -Tetramethoxy-3',4'-methylenedioxy chalcone [282]	10	0	0					
	50	0	0					
Desmethoxykanugin [284]	10	0	0					
	50	0	0					
2',4',6'-Trimethoxy-3,4-methylenedioxy chalcone [285]	10	0	0					
	50	0	0					
3',4'-Methylenedioxy-5,7-dimethoxy flavone [287]	10	0	0					
	50	0	0					
Acyclovir ^{e)}	-	-	-	0.06	0.50			

a)-: Inactivation, Plaque reduction assay; b)-: IC₅₀ (50% Inhibitory concentration, Mean of 3 independent experiments); c)-: CC₅₀ (50% Cytotoxic concentration, Examined by trypan blue exclusion method, Mean of 3 independent experiments); d)-: Selectivity Index = CC₅₀/IC₅₀;

e)-: Acyclovir as positive control; f)-: Compounds exhibiting more than 50% inhibition at $\leq 50 \mu\text{g/ml}$ were further determined for IC₅₀.

Table 31 Selective COX-2 Inhibitory Activity of Compounds from *Millettia leucantha*

compound	%inhibition at 10 µg/ml ^{b)}		IC ₅₀ (µM)	
	COX-1	COX-2	COX-1	COX-2
Lanceolatin B [103]	-	NI ^{c)}	-	-
2',4'-Dimethoxy-3,4- methylenedioxy chalcone [279]	-	NI ^{c)}	-	-
2'-Hydroxy-3,4,4',6'- tetramethoxy chalcone [280]	-	NI ^{c)}	-	-
2,4,6,β-Tetramethoxy- 3',4'methylenedioxy chalcone [282]	-	NI ^{c)}	-	-
Desmethoxykanugin [284]	~50	80	-	0.96±0.003 ^{d)}
2',4',6'-Trimethoxy-3,4-methylenedioxy chalcone [285]	-	NI ^{c)}	-	-
3',4'-Methylenedioxy-5,7-dimethoxyflavone [287]	-	NI ^{c)}	-	-
Aspirin	93.1	35.6	11.41±3.71	19.80±11.20
Indomethacin	-	-	0.005±0.003	0.006±0.002
NS-398	-	-	NI ^{c)}	0.01±0.01

a):- NS-398 = N-(2-[cyclohexyloxy]-4-nitrophenyl)methanesulfonamide

b):- compounds with ≥ 80% inhibition were further analyzed for IC₅₀ value.

c):- NI = no inhibition

d):- mean ± SE (n)

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Table 32 Cytotoxic Activity of Compounds from *Millettia leucantha* Against NCI-H460

Compound	IC ₅₀ (µg/ml)
3',4'-Methylenedioxy-7-methoxyflavone [68] ^{a)}	>10
Dihydromilletinone methyl ether [102] ^{a)}	>10
Lanceolatin B [103] ^{a)}	>10
2',4'-Dimethoxy-3,4-methylenedioxychalcone [279] ^{b)}	7.36
2'-Hydroxy-3,4,4',6'- tetramethoxychalcone [280] ^{a)}	>10
2',4',6'-Trimethoxy-3,4-methylenedioxydihydrochalcone [281] ^{a)}	>10
2,4,6,β-Tetramethoxy-3',4'-methylenedioxychalcone [282] ^{b)}	>10
Desmethoxykanugin [284] ^{a)}	>10
2',4',6'-Trimethoxy-3,4-methylenedioxychalcone [285] ^{b)}	3.69
3',4'-Methylenedioxy-5,7-dimethoxyflavone [287] ^{a)}	>10

a):- Dissolved in DMSO

b):- Dissolved in EtOH, but insoluble material remains

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