CHAPTER IV

RESULTS AND DISCUSSION

From the hexane, $CHCl_3$ and MeOH extracts of the leaves of *Chisocheton penduliflorus* Planch. ex Hiern (family Meliaceae) three compounds (CP-1, CP-2 and CP-3) were isolated. Eleven compounds (CP-3, CP-4, CP-5, CP-6, CP-7, CP-8, CP-9, CP-10, CP-11, CP-12 and CP-13) were isolated from similar extracts of the wood and six compounds (CP-3, CP-8, CP-9, CP-12, CP-14 and CP-15) were isolated from the stem bark extracts.

The dried, ground leaves, fruits and seeds of another meliaceous plant, cf. *Aglaia erythrosperma* C.M. Pannell, also yielded three major extracts: hexane, $CHCl_3$ and MeOH extracts. Four compounds (AE-1, AE-2, AE-3 and AE-4) were isolated from the leaf extracts. The extracts from the fruits were extensively chromatographed to yield six compounds (AE-1, AE-2, AE-5, AE-6, AE-7 and AE-8). Similar method yielded five compounds (AE-1, AE-2, AE-6, AE-9 and AE-10) from the seed extracts.

The structures of all isolated compounds were identified and elucidated by interpretation of their UV, IR, MS and NMR spectral data, and confirmed by comparison with the literatures. Compounds AE-1, AE-2, AE-3, AE-6, AE-8 and AE-9 were found to be identical to compounds CP-3, CP-8, CP-9, C-6, CP-10 and CP-7, respectively.

1. Structure Determination of Compounds Isolated from *Chisocheton penduliflorus*

1.1 Identification of Compound CP-1

Compound CP-1 was obtained as white amorphous powder, soluble in $CHCl_3$. Its IR spectrum (**Figure 9**) showed absorption band at 1700 cm^{-1} (carbonyl groups). The molecular formula of $C_{24}H_{38}O_2$ was determined from its $[M+Na]^+$ peak at *m/z* 381, displayed in the ESI mass spectrum (**Figure 10**).

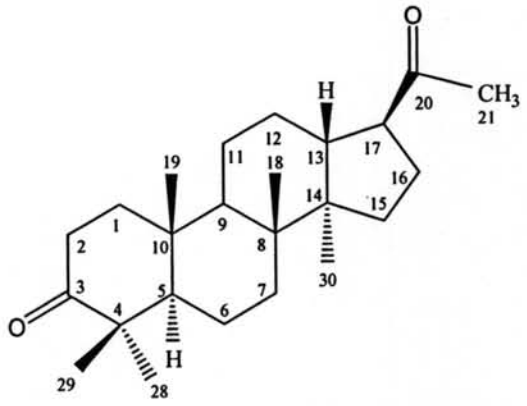
The ^1H NMR spectrum (**Figures 11a-11c** and **Table 16**) showed five tertiary methyl singlets at δ 0.85 (H_3-30), 0.92 (H_3-18), 0.99 (H_3-19), 1.01 (H_3- 29) and 1.05 (H_3-28), a most downfield methyl singlet at δ 2.10 (H_3-21) and a couple of methylene proton ddd at δ 2.39 (1H, J = 15.6, 7.8, 4.6 Hz, H-2a) and δ 2.46 (1H, J = 15.6, 9.8,

7.6 Hz, H-2b). The resonance at δ 2.56 (1H, *dt*, *J* = 11.0, 6.0 Hz) is characteristic of H-17 of the dammarane type triterpenes (Aalbersberg and Singh, 1991).

The ^{13}C NMR (**Figure 12** and **Table 16**) and DEPT 135 (**Figures 13a** and **13b**) spectra of compound CP-1 displayed five methyl signals at δ 15.3 (C-19), 15.7 (C-30), 16.0 (C-18), 21.0 (C-29) and 26.7 (C-28), whereas eight methylene carbons resonated at δ 19.6 (C-6), 21.7 (C-11), 25.6 (C-12), 25.9 (C-16), 31.5 (C-15), 34.1 (C-2), 34.8 (C-7) and 39.9 (C-1). Four methine carbons exhibited their signals at δ 45.1 C-13), 50.0 (C-9), 54.1 (C-17) and 55.3 (C-5), and four quaternary carbon signals at δ 36.9 (C-10), 40.4 (C-8), 47.4 (C-4) and 49.9 (C-14). The most downfield signal at δ 217.9 was assignable to the carbonyl moiety at C-3. Two other signals represent the side chain with a keto carbonyl carbon at C-20. These signals consist of one methyl carbon which resonated at δ 30.0 (C-21) and one carbonyl carbon at δ 212.2.

The long-range HMBC correlations (**Figure 16c**) between both H-1b (δ 1.90) and H-2b (δ 2.46) with the signal of C-3 keto carbonyl at δ 217.9 confirmed the position of that carbonyl group at C-3. HMBC correlations could also be observed between the proton signal (δ 2.10) of methyl group at the end of the side chain (H_3-21) to C-20 signal at δ 212.2 and C-17 signal at δ 54.1, indicating another keto carbonyl to be at position 20.

Based on the spectral data analysis, compound CP-1 was identified as the hexanordammarane triterpenoid 22,23,24,25,26,27-hexanordammaran-3,20-dione (hollongdione). This compound has been reported as a constituent of *Dipterocarpus pilosus* (family Dipterocarpaceae) (Gupta and Dev, 1971) and has been synthesized from 3*β*-hydroxy-22,23,24,25,26,27-hexanordammaran-20-one (Tanaka *et al.*, 1987).



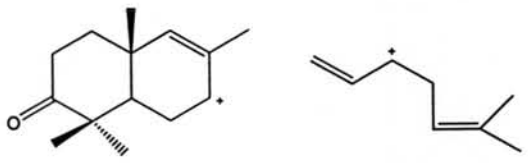
Hollongdione (Compound CP-1)

Table 16. NMR spectral data of hollongdione (compound CP-1) ($CDCl_3$, 500 MHz)

Position	^1H	^{13}C	HMBC
1	1.43 (1H, *m*)	39.9	C-2, C-10
	1.90 (1H, *m*)		C-2, C-3, C-10
2	2.39 (1H, *ddd*, *J* = 15.6, 7.8, 4.6 Hz)	34.1	C-10
	2.46 (1H, *ddd*, *J* = 15.6, 9.8, 7.6 Hz)		C-3
3	-	217.9	
4	-	47.4	
5	1.33 (1H, *m*)	55.3	C-4
6	1.42 (2H, *m*)	19.6	C-8, C-10
7	1.29 (1H, *m*)	34.8	C-5, C-8
	1.56 (1H, *m*)		C-18
8	-	40.4	
9	1.36 (1H, *m*)	50.0	C-7, C-8, C-10, C-11
10	-	36.9	
11	1.49 (1H, *m*)	21.7	C-12
	1.54 (1H, *m*)		C-8, C-9
12	1.20 (2H, *m*)	25.6	C-13, C-17
13	1.92 (1H, *m*)	45.1	C-30, C-20
14	-	49.9	
15	1.69 (2H, *m*)	31.5	C-14, C-17
16	1.65 (1H, *m*)	25.9	C-15
	1.71 (1H, *m*)		C-15, C-20
17	2.56 (1H, *dt*, *J* = 11.0, 6.0 Hz)	54.1	C-13, C-16, C-20
18	0.92 (3H, *s*)	16.0	C-8, C-9, C-14
19	0.99 (3H, *s*)	15.3	C-1, C-9
20	-	212.2	
21	2.10 (3H, *s*)	30.0	C-17, C-20
28	1.05 (3H, *s*)	26.7	C-4, C-5, C-29
29	1.01 (3H, *s*)	21.0	C-3, C-4, C-5, C-28
30	0.85 (3H, *s*)	15.7	C-8, C-13

1.2 Identification of Compound CP-2

Compound CP-2 was obtained as white amorphous powder, soluble in $CHCl_3$. Its IR spectrum (**Figure 18**) exhibited the absorption peaks of carbonyl group at 1706 cm^{-1}, double bond at 1456 cm^{-1} and geminal dimethyl at 1375 cm^{-1}. Its mass spectrum (**Figure 19**) exhibited a molecular ion peak at *m/z* 424, consistent with a molecular formula of $C_{30}H_{48}O$, and also showed other major peaks at *m/z* 205 (the fragment ion representing rings A and B after ring C cleavage with concerted hydrogen transfer of dammarane skeleton) and 109 (the fragment ion of the side chain of the dammarane skeleton) as shown below (van der Doelen *et al.*, 1998).



m/z 205 *m/z* 109

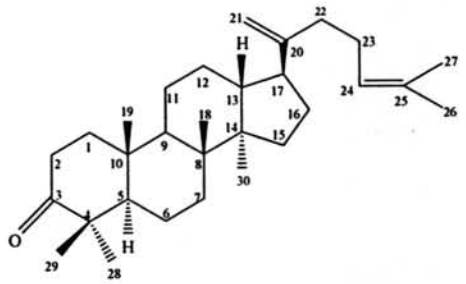
The ^1H NMR spectrum of compound CP-2 (**Figures 20a-20c** and **Table 17**) displayed five tertiary methyl signals at δ 0.86 (H_3-30), 0.92 (H_3-19), 0.99 (H_3-18), 1.02 (H_3-29) and 1.06 (H_3-28), and olefinic geminal dimethyl signals at δ 1.59 (*d*, *J* = 1.2 Hz, H_3-27) and 1.59 (*d*, *J* = 1.2 Hz, H_3-26). Furthermore, the presence of two exomethylene proton signals at δ 4.69 and 4.72 (*br s*, H_2-21) and an olefinic methine signal at δ 5.11 (*tdd*, *J* = 7.0, 1.5, 1.2 Hz, H-24) suggested that the compound should have an exomethylene group and another double bond in the side chain.

As for the main tetracyclic ring of the dammarane skeleton, the ^{13}C NMR (**Figure 21** and **Table 17**), DEPT 135 (**Figures 22a-22b**) and HMQC spectra (**Figures 24a-24f**) of CP-2 displayed five methyl carbon signals at δ 15.3 (C-30), 15.8 (C-18), 16.0 (C-19), 21.0 (C-29) and 26.7 (C-28), whereas eight methylene carbons resonated at δ 19.7 (C-6), 21.9 (C-11), 25.0 (C-12), 28.9 (C-16), 31.3 (C-15), 34.1 (C-2), 34.7 (C-7) and 40.0 (C-1). Four methine carbons exhibited their signals at δ 50.3 (C-9), 55.4 (C-5), 45.4 (C-13) and 47.7 (C-17), and four quaternary carbons resonatted at δ 36.9 (C-10), 40.4 (C-8), 47.4 (C-4) and 49.4 (C-14). The most downfield signal at δ 218.1 was assignable to the keto carbonyl moiety at C-3. Eight other signals represent the side chain with an exomethylene group at C-20, and olefinic geminal dimethyl groups at C-25. These signals consist of olefinic geminal dimethyl signals at δ 17.7 (C-27) and 25.7 (C-26), two methylene signals at δ 27.1

(C-23) and 34.1 (C-22), an exomethylene carbon at δ 107.6 (C-21), one olefinic methine carbon at δ 124.4 (C-24) and two olefinic quaternary carbons at δ 131.4 (C-25) and 152.6 (C-20).

The HMBC correlations (**Figure 25a-25j**) from H-17 signal at δ 2.18 to C-20 (δ 152.6), C-21 (δ 107.6) and C-22 (δ 34.1), from H-22 signal at δ 1.97 to C-21 and C-23 (δ 27.1), and from both H-21a and H-21b to C-22 and C-17 (δ 47.7) confirmed that the exomethylene group was at positions 20 and 21 of the side chain. Cross peaks were observed between H-24 (δ 5.11) with both C-27 (δ 17.7) and C-26 (δ 25.7), and, alternately, between, both, H-27 (δ 1.60) and H-26 (δ 1.67) and C-24 (δ 124.4), confirming the position of olefinic geminal dimethyl groups at the end of the side chain. Long-range HMBC correlation could also be observed between H-1b signal at δ 1.94 and C-3 (δ 218.1), confirming the keto carbonyl group at position 3.

On the basis of the above results, compound CP-2 was characterized as dammara-20,24-diene-3-one (dammaradienone), previously isolated from the oleoresin of *Dipterocarpus pilosus* (family Dipterocarpaceae) (Gupta and Dev, 1971) and from the resin of *Pistacia terebinthus* (family Anacardiaceae) (Assimopoulou and Papageorgiou, 2005).



Dammaradienone (Compound CP-2)

Table 17. NMR spectral data of dammaradienone (compound CP-2) ($CDCl_3$, 500 MHz)

Position	^1H	^{13}C	HMBC
1	1.42 (1H, *m*)	40.0	
	1.94 (1H, *m*)		C-2, C-3, C-5, C-10, C-19
2	2.42 (1H, *m*)	34.1	C-1, C-3, C-10
	2.48 (1H, *m*)		C-1, C-10
3	-	218.1	
4	-	47.4	
5	1.37 (1H, *m*)	55.4	
6	1.35 (1H, *m*)	19.7	
	1.50 (1H, *m*)		
7	1.29 (1H, *m*)	34.7	
	1.62 (1H, *m*)		

Table 17. NMR spectral data of dammarandienone (compound CP-2) ($CDCl_3$, 500 MHz) (continued)

Position	^1H	^{13}C	HMBC
8	-	40.4	
9	1.40 (1H, *m*)	50.3	
10	-	36.9	
11	1.40 (2H, *m*)	21.9	
12	1.55 (1H, *m*)	25.0	
	1.84 (1H, *m*)		
13	1.67 (1H, *m*)	45.4	
14	-	49.4	
15	1.10 (1H, *m*)	31.3	
	1.57 (1H, *m*)		
16	1.89 (2H, *m*)	28.9	C-15
17	2.18 (1H, *m*)	47.7	C-12, C-13, C-16, C-20, C-21, C-22
18	0.99(3H, *s*)	15.8	C-7, C-8, C-14
19	0.92 (3H, *s*)	16.0	C-1, C-5, C-9, C-10
20	-	152.6	
21	4.69 (1H, *br s*)	107.6	C-17, C-22
	4.72 (1H, *br s*)		C-17, C-22
22	1.97 (2H, *m*)	34.1	C-21, C-23
23	2.10 (2H, *q*, $J = 7.3$)	27.1	C-20, C-22, C-24, C-25
24	5.11 (1H, *tdd*, J = 7.0,1.5,1.3 Hz)	124.4	C-26, C-27
25	-	131.4	
26	1.59 (3H, *d*, $J = 1.2$ Hz)	25.7	C-24, C-27
27	1.59 (3H, *d*, $J = 1.2$ Hz)	17.7	C-24, C-26
28	1.06 (3H, *s*)	26.7	C-3, C-4, C-5, C-29
29	1.02 (3H, *s*)	21.0	C-3, C-4, C-5, C-29
30	0.86 (3H, *s*)	15.3	C-13, C-14, C-15

1.3 Identification of Compound CP-3

Compound CP-3 (AE-1) was obtained as white amorphous powder, soluble in $CHCl_3$. The IR spectrum (**Figure 27**) indicated the presence of hydroxyl group (s) (3457 cm^{-1}) and ether group (1380 cm^{-1}). The EI mass spectrum (**Figure 28**) showed a $[M-H_2O]^+$ peak at *m/z* 442, indicating a molecular formula of $C_{30}H_{52}O_3$.

The ^1H NMR spectrum (**Figures 29a-29c** and **Table 18**) showed singlet signals for eight tertiary methyl groups at δ 0.82 (H_3-29), 0.84 (H_3-19), 0.87 (H_3-30), 0.92 (H_3-28), 0.95 (H_3-18), 1.09 (H_3-27), 1.12 (H_3-21) and 1.17 (H_3-26) and a carbinol methine proton at δ 3.37 (*t*, *J* = 2.8 Hz, H-3). Furthermore, the presence of one oxymethine proton at δ 3.62 (*dd*, *J* = 10.4, 5.5 Hz, H-24) suggested that this compound should have a tetrahydrofuran ring in the side chain (Mohamad *et al.*, 1999).

The ^{13}C NMR spectrum (**Figure 30** and **Table 18),** analysed by the aid of DEPT 135 experiment (**Figures 31**), indicated the presence of five methyl carbons at δ 15.5 (C-18), 16.0 (C-19), 16.5 (C-30), 22.1 (C-29) and 28.3 (C-28), whereas eight methylene carbons resonated at δ 18.2 (C-6), 21.6 (C-11), 25.4 (C-2), 25.8 (C-16), 27.0 (C-12), 31.4 (C-15), 33.6 (C-1) and 35.2 (C-7). Four methine carbons exhibited their signals at δ 42.8 (C-13), 49.5 (C-5), 49.8 (C-17) and 50.6 (C-9), one oxymethine carbon at δ 76.3 (C-3), and four quaternary carbons at δ 37.3 (C-10), 37.6 (C-4), 40.6 (C-8) and 50.1 (C-14). Eight other signals represent the side chain with a tetrahydrofuran ring between C-20 and C-24, and a hydroxyl-substituted C-25. These signals consist of three methyl signals at δ 24.0 (C-27), 27.1 (C-21) and 27.8 (C-26), two methylene signals at δ 26.4 (C-23) and 34.8 (C-22), one oxymethine carbon at δ 86.3 (C-24), and two oxygenated quaternary carbons at δ 70.3 (C-25) and 86.6 (C-20). These data were similar to those of cabraleone, previously isolated from *Cabralea polytricha* of the family Meliaceae (Cascon and Brown, 1972), except its C-3 keto carbonyl was replaced by a hydroxyl group.

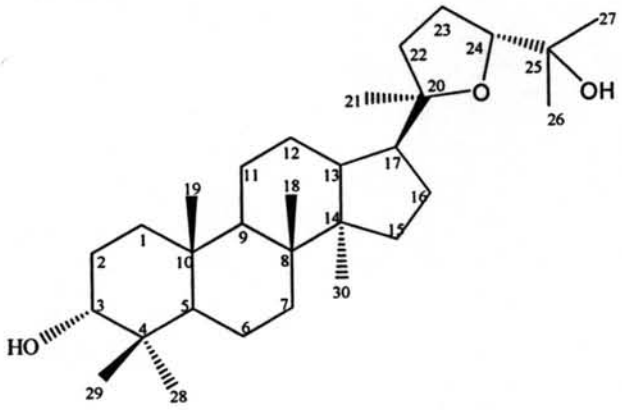
Long-range HMBC correlations (**Figures 34g**) could be observed between the proton signals (δ 1.17 and 1.09) of both H_3-26 and H_3-27 methyl groups at the end of the side chain to C-24 signal at δ 86.3 and the hydroxy-substituted C-25 at δ 70.3. Both positions 20 and 24 were assigned *S* configuration. Position 20 was assigned *S* configuration according to the majority of dammarane triterpenes, while *S* configuration of position 24 was assigned following comparison of the proton and

carbon resonances, together with the coupling patterns of the H-24 oxymethine proton (δ_H 3.62, J = 10.4, 5.5 Hz; δ_C 86.3) (Roux *et al.*, 1998).

The relative configuration at C-3 was established according to NOESY correlations (**Figure 35**) observed between H-18 and H-3 signals, as well as between H-3 and H-29 signals.

From the above ^{1}H and ^{13}C NMR data, together with the information from ^{1}H-^{1}H COSY (**Figures 32**), HMQC (**Figures 33a-33b**) and HMBC experiments, compound CP-3 (AE-1) was identified as a dammarane-type triterpene, cabraleadiol (Hisham *et al.*, 1996).

This compound has previously been isolated from several plants of the family Meliaceae e.g. *Amoora yunnanensis* (leaves) (Luo *et al.*, 2000), *A. cucullata* (stem bark) (Haque *et al.*, 1995), *Cabralea eichleriana* (wood) (Rao *et al.*, 1975), *C. polytricha* (fruits) (Cascon and Brown, 1972), *Dysoxylum malabaricum* (stem bark) (Hisham *et al.*, 1996), *Aglaia crassinervia* (bark) (Su *et al.*, 2006), *A. lawii* (stem bark) (Qiu *et al.*, 2001) and *A. tomentosa* (leaves) (Mohamad *et al.*, 1999). It was found in other plant families, i.e. in the leaves of *Cordia spinescens*, family Boraginaceae (Nakamura *et al.*, 1997) and the stem bark of *Commiphora dalzielii*, family Burseraceae (Waterman and Ampofo, 1985).



Cabraleadiol (Compound CP-3)

Table 18. Comparison of ^{1}H and ^{13}C NMR spectral data of cabraleadiol and compound CP-3 ($CDCl_3$, 500 MHz)

Position	Cabraleadiol*		Compound CP-3 (AE-1)		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
1	1.30 (1H, *m*)	33.6	1.29 (1H, *m*)	33.6	
	1.42 (1H, *m*)		1.41 (1H, *m*)		
2	1.54 (1H, *m*)	25.3	1.51 (1H, *m*)	25.4	C-4
	1.95 (1H, *m*)		1.93 (1H, *m*)		
3	3.40 (1H, *t*, *J* = 2 Hz)	76.2	3.37 (1H, *t*, *J* = 2.8 Hz)	76.3	C-1, C-4, C-5, C-29
4	-	37.6	-	37.6	
5	1.25 (1H, *m*)	49.5	1.25 (1H, *m*)	49.5	C-4, C-10, C-19, C-29
6	1.42 (2H, *m*)	18.2	1.39 (2H, *m*)	18.2	C-4, C-7, C-8
7	1.26 (1H, *m*)	35.1	1.24 (1H, *m*)	35.2	C-6
	1.57 (1H, *m*)		1.57 (1H, *m*)		C-8, C-9, C-18
8	-	40.6	-	40.6	
9	1.45 (1H, *m*)	50.6	1.45 (1H, *m*)	50.6	C-10, C-11
10	-	37.2	-	37.3	
11	1.19 (1H, *m*)	21.6	1.19 (1H, *m*)	21.6	
	1.54 (1H, *m*)		1.53 (1H, *m*)		C-8, C-10, C-13
12	1.23 (1H, *m*)	27.0	1.23 (1H, *m*)	27.0	
	1.80 (1H, *m*)		1.77 (1H, *m*)		
13	1.66 (1H, *m*)	42.7	1.65 (1H, *m*)	42.8	C-14, C-30
14	-	50.1	-	50.1	
15	1.09 (1H, *m*)	31.4	1.05 (1H, *m*)	31.4	
	1.45 (1H, *m*)		1.45 (1H, *m*)		C-30
16	1.30 (1H, *m*)	25.8	1.27 (1H, *m*)	25.8	
	1.75 (1H, *m*)		1.73 (1H, *m*)		
17	1.87 (1H, *m*)	49.8	1.83 (1H, *m*)	49.8	C-13, C-22
18	0.97 (3H, *s*)	15.5	0.95 (3H, *s*)	15.5	C-7, C-8, C-14
19	0.86 (3H, *s*)	16.0	0.84 (3H, *s*)	16.0	C-1, C-5, C-9, C10
20	-	86.5	-	86.6	
21	1.15 (3H, *s*)	27.1	1.12 (3H, *s*)	27.1	C-17, C-20, C-22
22	1.68 (1H, *m*)	34.7	1.65 (1H, *m*)	34.8	C-24, C-17
	1.87 (1H, *m*)		1.86 (1H, *m*)		C-24, C-17
23	1.75 (1H, *m*)	26.3	1.75 (1H, *m*)	26.4	C-20, C-22, C-24
	1.87 (1H, *m*)		1.84 (1H, *m*)		C-22, C-24, C-25
24	3.65 (1H,*dd*, *J* = 6, 10 Hz)	86.2	3.62 (1H, *dd*, *J*=10.4, 5.5 Hz)	86.3	C-25, C-26
25	-	70.2	-	70.3	
26	1.19 (3H, *s*)	27.8	1.17 (3H, *s*)	27.8	C-24, C-25, C-27

Table 18. Comparison of ^{1}H and ^{13}C NMR spectral data of cabraleadiol and compound CP-3 ($CDCl_3$, 500 MHz) (continued)

Position	Cabraleadiol		Compound CP-3 (AE-1)		
	^{1}H*	^{13}C**	^{1}H	^{13}C	HMBC
27	1.11 (3H, *s*)	24.0	1.09 (3H, *s*)	24.0	C-24, C-25, C-26
28	0.94 (3H, *s*)	28.3	0.92 (3H, *s*)	28.3	C-3, C-4, C-5, C-29
29	0.84 (3H, *s*)	22.1	0.82 (3H, *s*)	22.1	C-3, C-4, C-5, C-28
30	0.89 (3H, *s*)	16.5	0.87 (3H, *s*)	16.5	C-8, C-13, C-14, C-15

*Hisham *et al.*, 1996

** Hisham *et al.*, 1996 (in $CDCl_3$, 125 MHz)

1.4 Identification of Compound CP-4

Compound CP-4 was obtained as colorless needle crystals, soluble in $CHCl_3$. Its IR absorption maximum at 3517 cm^{-1} (**Figure 36**) suggested the presence of hydroxy group(s). Its molecular formula, $C_{15}H_{26}O_2$, determined by ESI-TOF mass spectrometry (**Figure 37**).

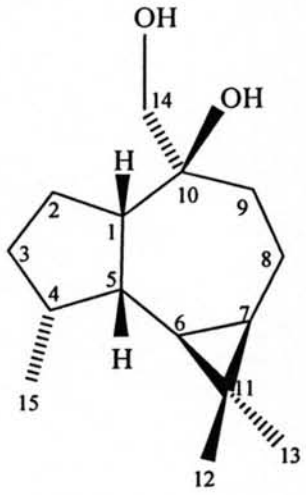
The ^{13}C NMR spectrum of CP-4 (**Figure 39** and **Table 19**) exhibited 15 carbon peaks, indicating its sesquiterpenoid nature. These peaks were classified, according to DEPT 135 experiment (**Figure 40a-40b**), into those of three methyl (δ 16.1, 16.2 and 29.0), five methylene (δ 18.3, 24.5, 29.0, 32.0 and 70.7), five methine (δ 22.4, 28.6, 38.2, 40.0 and 53.5) and two quaternary carbons (δ 19.1 and 76.5).

The ^1H NMR spectrum (**Figure 38a-38d**) of compound CP-4 displayed two most upfield signals of typical methine protons of the aromadendrane framework at δ 0.12 (1H, *t*, *J* = 9.5 Hz, H-6) and 0.66 (1H, *ddd*, *J* = 11.2, 9.5, 5.9 Hz, H-7) (Vizzotto *et al.*, 2003). Two sharp singlets at δ 1.01 and 1.04, each integrating for three protons, could be assigned to H_3-12 and H_3-13, respectively. A couple of methylene protons bearing hydroxy group resonated separately at δ 3.29 (1H, *d*, *J* = 10.9 Hz, H-14a) and 3.42 (1H, *d*, *J* = 10.9 Hz, H-14b). Another methyl group resonated as a doublet at δ 0.94 (3H, *d*, *J* = 6.6 Hz, H_3-15). As shown in the ^1H-^1H COSY spectrum (**Figure 41**), this methyl doublet was coupled to a methine proton (H-4) appearing at δ 1.98. Connectivity could also be observed from the methine triplet of H-6 at δ 0.12 to both the methine H-5 peak at δ 1.86 and H-7 signal at δ 0.64.

Long-range HMBC correlations (**Figure 43a-43f**) from both H-14a and H-14b signals at δ 3.29 and 3.42, respectively, to C-10 (δ 76.5), C-9 (δ 32.0) and C-1 (δ 53.5) established the connectivity between the oxymethylene group at C-14 and C-10 and helped in placing another hydroxyl group at C-10. Cross correlations were observed between CH_3-12 and CH_3-13, and between both methyl protons to C-6, C-7 and C-11 at δ 22.4, 28.6 and 19.1, respectively. The larger than 10 ppm difference in the carbon chemical shifts between these two methyls results from the position of CH_3-12 in a shielding region of the β-face of the aromadendrane seven-membered ring, making it more shielded than CH_3-13. The HMBC spectrum also showed the cross peaks between H_3-15 and C-3 (δ 29.0), C-4 (δ 38.2) and C-5 (δ 40.0), confirming that a methyl group is attached to C-4.

To determine the relative configuration, NOESY experiment **(Figure 44)** was performed. The 2D spectrum exhibited the correlations between H-1 and H-12, H-5 and H-12, H-13 and H-15, and H-6 and H-13, suggesting the relative configuration of those positions. Based on these spectral data analysis and comparison with reported values **(Table 19)**, compound CP-4 was identified as an aromadendrane sesquiterpenoid (+)-10β,14-dihydroxy-*allo*-aromadendrane or 14-hydroxyviridiflorol (Vizzotto *et al.*, 2003).

14-Hydroxyviridiflorol has been reported as a constituent of *Pulicaria paludosa* (family Compositae) (San Feliciano *et al.*, 1989) and *Duguetia glabriuscula* (family Annonaceae) (De Siqueira *et al.*, 2003). The compound has been shown as possessing antibacterial (Vizzotto *et al.*, 2003) and antineoplastic activity against human larynx carcinoma (Hep_2) cells, with IC_{50} value of 11.6 µg/ml (Matos *et al.*, 2006).



14-Hydroxyviridiflorol (Compound CP-4)

Table 19. Comparison of ^{1}H and ^{13}C NMR spectral data (in $CDCl_3$, 500 MHz) of 14-hydroxyviridiflorol and compound CP-4

Position	14-Hydroxyviridiflorol		Compound CP-4		
	^{1}H*	^{13}C**	^{1}H	^{13}C	HMBC
1	1.75-1.90 (1H, *m*)	53.9	1.86 (1H, *m*)	53.5	C-2, C-4, C-5, C-6, C-9, C-10, C-14
2	1.45-1.60 (2H, *m*)	25.0	1.59 (2H, *m*)	24.5	C-1, C-3, C-5, C-10
3	1.20-1.34 (1H, *m*)	29.4	1.28 (1H, *m*)	29.0	C-1, C-4, C-15
	1.72-1.82 (1H, *m*)		1.81 (1H, *m*)		C-2, C-4, C-5
4	1.88-2.00 (1H, *m*)	38.6	1.98 (1H, *m*)	38.2	C-2, C-5, C-6,C-15
5	1.77-1.86 (1H, *m*)	40.4	1.86 (1H, *m*)	40.0	C-1, C-2, C-3, C-4, C-6, C-10, C-11
6	0.09 (1H, *dd*, *J* = 9.6, 8.3 Hz)	22.8	0.12 (1H, *t*, *J* = 9.5 Hz)	22.4	C-4, C-7, C-8, C-11, C-12, C-13
7	0.63 (1H, *ddd*, *J* = 11.0, 9.6, 5.5 Hz)	29.5	0.64 (1H, *ddd*, *J* = 11.2, 9.5, 5.9 Hz)	28.6	C-5, C-6, C-11, C-13
8	1.30-1.47 (1H, *m*)	18.7	1.41 (1H, *m*)	18.3	C-7, C-9, C-10, C-11
	1.58-1.70 (1H, *m*)		1.68 (1H, *m*)		C-6, C-7, C-9, C-10
9	1.35-1.45 (1H, *m*)	32.5	1.51 (1H, *m*)	32.0	C-1, C-7, C-8, C-10, C-14
	1.57-1.68 (1H, *m*)		1.68 (1H, *m*)		
10	-	76.8	-	76.5	
11	-	19.5	-	19.1	
12	0.98 (3H, *s*)	16.6	1.01 (3H, *s*)	16.2	C-6, C-7 , C-11, C-13
13	1.01 (3H, *s*)	29.1	1.04 (3H, *s*)	29.0	C-6, C-7 , C-11, C-12
14	3.25 (1H, *d*, *J* = 10.8 Hz)	71.1	3.29 (1H, *d*, *J* = 10.9 Hz)	70.7	C-1, C-9, C-10
	3.38 (1H, *d*, *J* = 10.8 Hz)		3.42 (1H, *d*, *J* = 10.9 Hz)		
15	0.91 (3H, *d*, *J* = 6.7 Hz)	16.5	0.94 (d, *J* = 6.6 Hz)	16.1	C-3, C-4, C-5

*Vizzotto *et al.*, 2003 (in $CDCl_3$, 300 MHz)

** Vizzotto *et al.*, 2003 (in $CDCl_3$, 75 MHz)

1.5 Identification of Compound CP-5

Compound CP-5 was obtained as colorless needle crystals. Its IR absorption band (**Figure 45**) at 3544 cm^{-1} was indicative of the presence of hydroxyl group(s) in the structure. The high resolution ESI-TOF mass spectrum (**Figure 46**) exhibited $[M+Na]^+$ ion peak at *m/z* 261.1830, indicating the molecular formula of $C_{15}H_{26}O_2$.

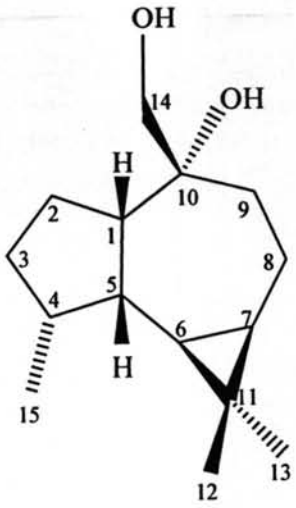
Analysis of the NMR spectra of compound CP-5 pointed out that it occurred as the major component in an inseparable 3:2 mixture with compound CP-4 (14-hydroxyviridiflorol), and might therefore be another aromadendrane sesquiterpenoid with very similar chemical structure.

The ^1H NMR spectrum of compound CP-5 (**Figure 47a** and **47b**) exhibited the signals of two methine protons of the cyclopropane ring at δ 0.34 (1H, *t*, *J* = 9.5 Hz, H-6) and 0.69 (1H, *ddd*, *J* = 15.8, 9.5, 6.6 Hz, H-7), respectively. Both signals, especially that of H-6, are more deshielded than their counterparts in compound CP-4. Two methyl singlets at δ 0.98 and 1.05 could be ascribed to CH_3-12 and CH_3-13, respectively, while CH_3-15 appeared as a doublet (*J* = 6.8 Hz) at δ 0.95. The oxymethylene protons at position 14 separately resonated as a couple of the most downfield doublets at δ 3.37 (1H, *d*, *J* = 11.0 Hz, H-14a) and 3.46 (1H, *d*, *J* = 11.0 Hz, H-14b).

The correlations between H-6 and H-5 at δ 1.74 (1H, *m*), as well as between H-5 and H-1 at δ 2.08 (1H, *m*), which, in turn, coupled to H_2-2 at δ 1.90 (2H, *m*), and between CH_3-15 and H-4 at δ 2.01 (1H, *m*) were observed from the ^1H-^1H COSY spectrum (**Figure 50**). All of these protons and carbons were assigned according to their correlations in the HMQC spectrum (**Figure 51**). HMBC correlations (**Figure 52**) between both oxymethylene H_2-14 to C-1 (δ 50.0) and C-9 (δ 33.5) helped confirming the position of this side chain at the hydroxyl-substituted C-10 (δ 76.3).

The relative configuration at C-1, C-5 and C-10 was established according to NOESY correlations (**Figure 53a and 53b**) observed between H-1 and H-14 signals, as well as between H-1 and H-5 signals. The only difference from compound CP-4 being in the relative configuration at C-10 of compound CP-5, which is *α*-OH and *β*-CH_2OH instead of *vice versa* as in 14-hydroxyviridiflorol (CP-4). The deshielded H-6 is thus resulted from the closer proximity of this proton to the 10-OH group. Therefore, compound CP-5 was elucidated as the new aromadendrane-type

sesquiterpenoid which is a stereoisomer of 14-hydroxyviridiflorol, and was named 14-hydroxyepiviridiflorol.



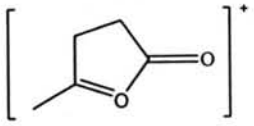
14-Hydroxyepiviridiflorol (Compound CP-5)

Table 20. ^{1}H and ^{13}C NMR spectral data of compound CP-5 (14-hydroxyepi-viridiflorol) (in $CDCl_3$, 500 MHz)

Position	Compound CP-5		
	^{1}H*	^{13}C	HMBC
1	2.08 (1H, *m*)	50.0	C-2, C-5, C-6, C-9, C-10, C-14
2	1.90 (2H, *m*)	23.8	C-3, C-4, C-5, C-10
3	1.30 (1H, *m*)	30.2	C-15, C-4
	1.80 (1H, *m*)		C-1, C-2, C-4, C-5
4	2.01 (1H, *m*)	38.3	C-2, C-6
5	1.74 (1H, *m*)	40.7	C-1, C-2, C-3, C-6, C-7, C-10
6	0.34 (*t*, *J* = 9.5 Hz)	23.3	C-4, C-8, C-13
7	0.69 (*ddd*, *J* = 15.8, 9.5, 6.6 Hz)	25.1	C-13
8	1.67 (1H, *m*)	19.2	C-6, C-10 , C-11
	1.77 (1H, *m*)		
9	1.71 (1H, *m*)	33.5	C-8, C-10
	1.92 (1H, *m*)		C-1, C-8, C-10
10	-	76.3	
11	-	19.1	
12	0.98 (3H, *s*)	16.0	C-6, C-7, C-11, C-13
13	1.05 (3H, *s*)	28.6	C-6, C-7, C-11, C-12
14	3.37 (*d*, *J* =11.0 Hz)	69.9	C-1, C-9
	3.46 (*d*, *J* = 11.0 Hz)		C-1, C-9, C-10
15	0.95 (*d*, *J* = 6.8 Hz)	15.6	C-3, C-4, C-5

1.6 Identification of Cmpound CP-6

Compound CP-6 was obtained as white amorphous powder, soluble in $CHCl_3$. The IR spectrum (**Figure 54**) showed bands at 1710 cm^{-1} (carboxyl group) and 1743 cm^{-1} (γ-lactone). Its EI mass spectrum (**Figure 55**) exhibited the molecular ion peak at *m/z* 430, in agreement with $C_{27}H_{42}O_4$ as the molecular formula. The base peak at *m/z* 99 indicated that compound CP-6 was a trisnor-triterpene γ-lactone (losing three carbons at the end of the side chain) as shown below (Cascon and Brown, 1972).



m/z 99

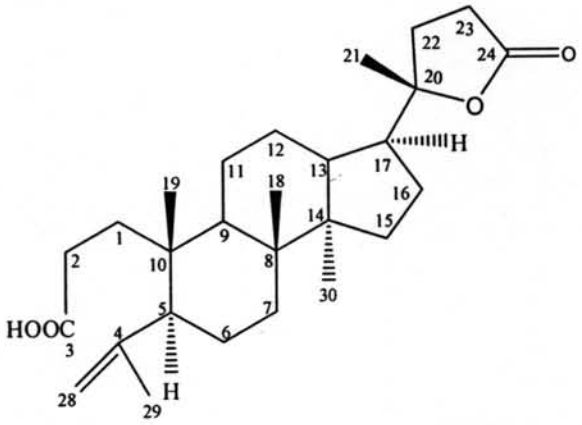
The ^1H NMR spectrum (**Figures 56a** and **56b**) displayed five tertiary methyl signals at δ 0.86 (H_3-19), 0.91 (H_3-30), 1.02 (H_3-18), 1.38 (H_3-21) and 1.74 (H_3-29). Two broad singlets at δ 4.67 and 4.86, each integrating for one proton, were assignable to olefinic methylene protons of the isopropenyl group attached to C-5.

The ^{13}C NMR (**Figures 57a** and **57b**), DEPT 135 (**Figure 58**) and HMQC spectra (**Figures 60a** and **60b**) of compound CP-6 helped in differentiating the signals of a 3,4-secodammarane fused rings with an isopropenyl group at C-5. Four methyl carbon signals appeared at δ 15.3 (C-18), 16.1 (C-30), 20.1 (C-19) and 23.2 (C-29), whereas nine methylene carbons resonated at δ 21.9 (C-11), 24.5 (C-1), 25.0 (C-12), 26.7 (C-16), 28.2 (C-2), 31.2 (C-6), 31.4 (C-15), 33.7 (C-7) and 113.5 (C-28). Four methine carbons exhibited their signals at δ 41.0 (C-5), 43.2 (C-13), 49.3 (C-9) and 50.7 (C-17), and four quaternary signals at δ 39.0 (C-10), 40.0 (C-8), 50.5 (C-14) and 147.3 (C-4). The most downfield signal at δ 179.9 was assignable to the carboxy carbonyl moiety at C-3. Five other signals represent the side chain with a γ-lactone ring between C-20 and C-24. These signals consist of a methyl signal at δ 25.1 (C-21), two methylene signals at δ 29.2 (C-22) and 34.1 (C-23), and two oxygenated quaternary carbons at δ 90.1 (C-20) and 176.9 (C-24). These data were similar to those of the 3,4-secodammarane eichlerianic acid, previously isolated from *Aglaia foveolata* (Roux *et al.*, 1998), except for the γ-lactone ring between C-20 and C-24 at the side chain of this triterpenoid in place of a tetrahydrofuran ring and a hydroxyl-substituted C-25 in eichlerianic acid.

In the HMBC spectrum (**Figures 61a-61c**), correlations between both exomethylene protons at position 28 with the signal of C-29 at δ 23.2 and between

H_3-29 methyl proton with the C-5 signal at δ 41.0 could be observed and, therefore, revealed clevage of the A ring and the attachment of the isopropenyl group at C-5 (Luo *et al.*, 2000). HMBC correlations could also be seen between both methylene protons signals at δ 2.58 and 2.69 (H-22a and H-22b, respectively) of the γ-lactone side chain to C-24 (δ 176.9) and the quaternary C-20 (δ 90.1). The configuration at C-20 was also assigned the *S* configuration similar to most dammarane triterpenes.

Therefore, compound CP-6 was identified as 20*S*,24-epoxy-25,26,27-trisnor-24-oxo-3,4-seco-4(28)-dammaren-3-oic acid (eichlerialactone) from its spectral data and comparison to previous work (Singh and Aalbersberg, 1992). This triterpene has been found in several plants in the family Meliaceae, such as from *Dysoxylum richii* leaves (Singh and Aalbersberg, 1992), *D. cauliflorum* fruits (Huang *et al.*, 1999), *Cabralea canjerana* branches (De Campos Braga *et al.*, 2006), *C. eichleriana* wood (Rao *et al.*, 1975) and *Amoora yunnanensis* bark (Luo *et al.*, 2000).



Eichlerialactone (Compound CP-6)

Table 21. Comparison of NMR spectral data of eichlerialactone and compound CP-6 ($CDCl_3$, 500 MHz)

Position	Eichlerialactone*	Compound CP-6		
	^{13}C	^{1}H	^{13}C	HMBC
1	24.6	1.82 (1H, *m*)	24.5	
		1.97 (1H, *m*)		
2	28.2	2.19 (1H, *m*)	28.2	C-3
		2.40 (1H, *m*)		
3	177.2	-	179.9	
4	147.3	-	147.3	
5	41.0	1.51 (1H, *m*)	41.0	
6	31.2	1.93 (1H, *m*)	31.2	
		2.12 (1H, *m*)		

Table 21. Comparison of NMR spectral data of eichlerialactone and compound CP-6 ($CDCl_3$, 500 MHz) (continued)

Position	Eichlerialactone*	Compound CP-6		
	^{13}C	^{1}H	^{13}C	HMBC
7	33.8	1.23 (1H, *m*)	33.7	C-14
		1.54 (1H, *m*)		C-18
8	40.1	-	40.0	
9	49.4	2.04 (1H, *m*)	49.3	
10	31.9	-	39.0	
11	22.0	1.28 (1H, *m*)	21.9	
		1.43 (1H, *m*)		C-13
12	25.2	1.26 (1H, *m*)	25.0	
		1.84 (1H, *m*)		C-9, C-14
13	43.3	1.63 (1H, *m*)	43.2	C-8
14	50.5	-	50.5	
15	31.4	1.16 (1H, *m*)	31.4	C-13, C-14, C-17
		1.47 (1H, *m*)		
16	26.8	1.30 (1H, *m*)	26.7	
		1.77 (1H, *m*)		
17	50.8	1.98 (1H, *m*)	50.7	C-20
18	15.3	1.02 (3H, *s*)	15.3	C-7, C-8, C-9, C-14
19	20.1	0.86 (3H, *s*)	20.1	C-5, C-9, C-10
20	90.0	-	90.1	
21	25.0	1.38 (3H, *s*)	25.1	C-17, C-20
22	29.2	2.58 (1H, *m*)	29.2	C-20, C-24
		2.69 (1H, *m*)		
23	34.2	1.62 (2H, *m*)	34.1	C-20
24	176.8	-	176.9	
28	113.6	4.67 (1H, *br s*)	113.5	C-29
		4.86 (1H, *br s*)		
29	23.2	1.74 (3H, *s*)	23.2	C-4, C-5, C-28
30	16.2	0.91 (3H, *s*)	16.1	C-8, C-13, C-14, C-15

* Singh and Aalbersberg, 1992 (in $CDCl_3$, 300 MHz)

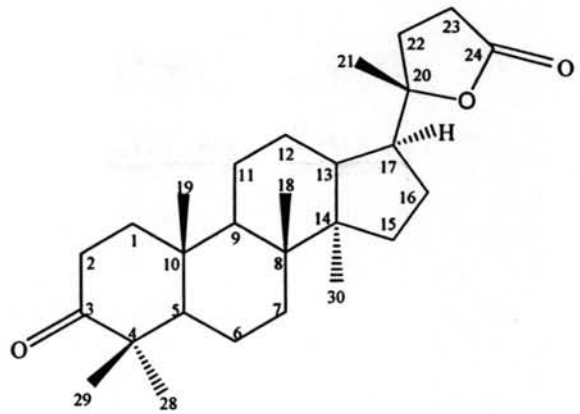
1.7 Identification of Compound CP-7

Compound CP-7 was obtained as white amorphous powder, soluble in $CHCl_3$. The IR spectrum (**Figure 63**) displayed absorption bands for keto carbonyl group (1704 cm^{-1}) and γ-lactone (1739 cm^{-1}). The EI mass spectrometry (**Figure 64**) exhibited the molecular ion at *m/z* 414, corresponding to the molecular formula $C_{27}H_{42}O_3$. The base peak at m/z 99 corresponded to the γ-lactone fragment of a trisnor-triterpene (Cascon and Brown, 1972).

The ^1H NMR spectrum (**Figure 65** and **Table 22**) of compound CP-7 exhibited six methyl singlets at δ 0.91 (H_3-30), 0.96 (H_3-18), 1.01 (H_3-19), 1.05 (H_3-29), 1.10 (H_3-28) and 1.39 (H_3-21). The ^{13} C NMR (**Figure 66** and **Table 22**) and DEPT 135 spectra (**Figures 67a-67c**) displayed six methyl carbon signals at δ 15.2 (C-19), 16.0 (C-18), 16.1 (C-30), 21.0 (C-29), 25.5 (C-21) and 26.7 (C-28), ten methylene carbon signals at δ 19.6 (C-6), 21.9 (C-11), 25.0 (C-22), 26.8 (C-16), 29.2 (C-23), 31.0 (C-12), 31.2 (C-15), 34.1 (C-2), 34.5 (C-7) and 39.8 (C-1), four methine carbon signals at δ 43.3 (C-13), 49.2 (C-17), 49.9 (C-9) and 55.3 (C-5), four quaternary carbon signals at δ 36.8 (C-10), 40.2 (C-8), 47.4 (C-4) and 50.1 (C-14). One quaternary carbon bearing oxygen resonated at δ 90.0 (C-20), whereas a carbonyl ester appeared at δ 176.7 (C-24). The most downfield quaternary signal at δ 217.9 was assignable to the keto carbonyl moiety at C-3. These data suggested the presence of a dammaran-3-one skeleton (Luo *et al.*, 2000).

Based on the spectral analysis and comparison to previous data (Ahmad and Alvi, 1987), compound CP-7 was identified as the dammarane triterpenoid, cabralealactone.

This compound has previously been isolated from several plants of the family Meliaceae e.g. from *Cabralea polytricha* fruits (Cascon and Brown, 1972), *C. eichleriana* wood (Rao *et al.*, 1975), *Aglaia leucophylla* stem bark (Benosman *et al.*, 1994), *A. tomentosa* leaves (Mohamad *et al.*, 1999), *Amoora cucullata* stem bark (Haque *et al.*, 1996) and *Dysoxylum cauliflorum* fruits (Huang *et al.*, 1999). In addition, it was found in the aerial plants of *Cleome brachycarpa* of the family Capparidaceae (Ahmad and Alvi, 1987).



Cabralealactone (Compound CP-7)

Table 22. Comparison of NMR spectral data of cabralealactone and compound CP-7 ($CDCl_3$, 500 MHz)

Position	Cabralealactone*	Compound CP-7	
	^{13}C	^{1}H	^{13}C
1	39.9	1.43 (1H, *m*)	39.8
		1.64 (1H, *m*)	
2	34.1	2.35 (1H, *m*)	34.1
		2.57 (1H, *m*)	
3	217.3	-	217.9
4	47.4	-	47.4
5	55.5	1.36 (1H, *m*)	55.3
6	19.7	1.48 (1H, *m*)	19.6
		1.60 (1H, *m*)	
7	34.7	1.34 (1H, *m*)	34.5
		1.56 (1H, *m*)	
8	40.5	-	40.2
9	50.0	1.45 (1H, *m*)	49.9
10	36.9	-	36.8
11	22.0	1.15 (1H, *m*)	21.9
		1.43 (1H, *m*)	
12	26.9	1.27 (1H, *m*)	31.0
		1.93 (1H, *m*)	
13	43.4	1.60 (1H, *m*)	43.3
14	50.3	-	50.1
15	31.3	1.12 (1H, *m*)	31.2
		1.48 (1H, *m*)	
16	26.8	1.19 (1H, *m*)	26.8
		1.78 (1H, *m*)	
17	49.4	2.03 (1H, *m*)	49.2
18	16.0	0.96 (3H, *s*)	16.0
19	15.3	1.01 (3H, *s*)	15.2
20	90.0	-	90.0

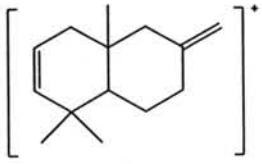
Table 22. Comparison of NMR spectral data of cabralealactone and compound CP-7 ($CDCl_3$, 500 MHz) (continued)

Position	Cabralealactone*	Compound CP-7	
	^{13}C	^{1}H	^{13}C
21	25.6	1.39 (3H, *s*)	25.5
22	25.1	1.86 (1H, *m*)	25.0
		2.14 (1H, *m*)	
23	29.2	2.47 (1H, *m*)	29.2
		2.66 (1H, *m*)	
24	176.3	-	176.7
28	26.8	1.10 (3H, *s*)	26.7
29	21.0	1.05 (3H, *s*)	21.0
30	16.3	0.91 (3H, *s*)	16.1

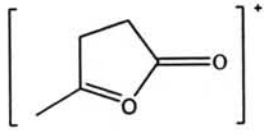
*Ahmad and Alvi, 1987 (in $CDCl_3$, 300 MHz)

1.8 Identification of Compound CP-8

Compound CP-8 was obtained as white amorphous powder. The IR absorption bands (**Figure 68**) at 3463 and 1747 cm^{-1} were indicative of hydroxyl group and γ-lactone, respectively. The EI mass spectrum (**Figure 69**) showed molecular ion peak at m/z 416, corresponding to a molecular formula of $C_{27}H_{44}O_3$, which is 2 mass units more than that of compound CP-7, indicating a secondary hydroxyl in place of carbonyl group at position 3. The base peak at *m/z* 189 represents ring A and B fragment without hydroxyl moiety, whereas the fragment peak at m/z 99 corresponded to the γ-lactone fragment of the trisnor-triterpene (Cascon and Brown, 1972).



m/z 189



m/z 99

The ^1H NMR spectrum (**Figures 70a-70c** and **Table 23**) of compound CP-8 is closely similar to that of compound CP-7, except for an additional downfield oxymethine proton signal at δ 3.38 (*t*, *J* = 2.7 Hz, H-3).

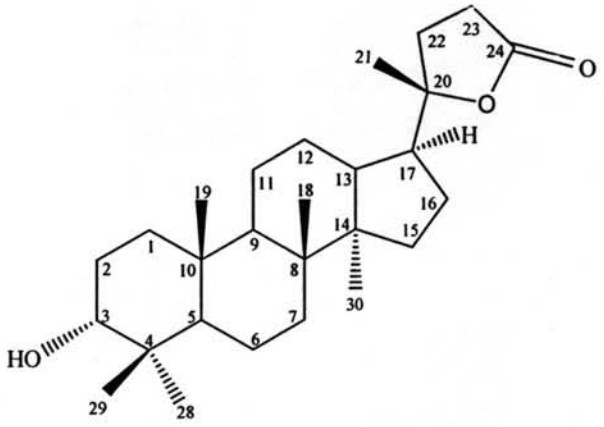
Its ^{13}C NMR spectral data (**Figure 71** and **Table 23**) were also similar to those of compound CP-7, except for an oxymethine carbon signal at δ 76.2 (C-3) in place of one carbonyl carbon signal in compound CP-7. All carbon signals were assigned according to the HMQC (**Figures 74a- 74f**) and HMBC (**Figures 75a-75f**) spectra.

The relative configuration of the chiral C-3 (δ 76.2) was assigned based on NOESY experiment. The NOESY spectrum (**Figure 76**) displayed the correlation between H-3 and H_3-18 and H_3-19, indicating their orientation as on the same side.

Based on these spectral data and comparison with earlier reports (Cascon and Brown, 1972; Su *et al.*, 2006), compound CP-8 was identified as a dammarane-type triterpenoid called cabraleahydroxylactone.

Cabraleahydroxylactone, has been reported as a constituent of several meliaceous plants. For example, it ws isolated from *Cabralea polytricha* fruits (Cascon and Brown, 1972), *C. eichleriana* wood (Rao *et al.*, 1975), *Amoora yunnanensis* bark (Luo *et al.*, 2000) and *Aglaia crassinervia* bark (Su *et al.*, 2006). It

was found in the aerial parts of *Cleome amblyocarpa*, (family Capparidaceae) (Harraz *et al.*, 1995).



Cabraleahydroxylactone (Compound CP-8)

Table 23. Comparison of NMR spectral data of cabraleahydroxylactone and compound CP-8 ($CDCl_3$, 500 MHz)

Position	Cabraleahydroxylactone*	Compound CP-8		HMBC
	^{13}C	^{1}H	^{13}C	
1	33.6	1.29 (1H, *m*)	33.6	
		1.38 (1H, *m*)		
2	25.4	1.55 (1H, *m*)	25.4	
		1.92 (1H, *m*)		
3	76.1	3.38 (1H, *t*, *J* = 2.7 Hz)	76.2	C-1
4	37.6	-	37.6	
5	49.3	1.27 (1H, *m*)	49.3	C-29
6	18.2	1.41 (2H, *m*)	18.2	
7	35.1	1.24 (1H, *m*)	35.1	
		1.57 (1H, *m*)		
8	50.3	-	50.3	
9	50.3	1.44 (1H, *m*)	50.4	
10	37.2	-	37.2	
11	26.8	1.20 (1H, *m*)	26.8	
		1.73 (1H, *m*)		C-8, C-9
12	21.2	1.21 (1H, *m*)	21.3	
		1.56 (1H, *m*)		
13	43.1	1.60 (1H, *m*)	43.1	
14	40.5	-	40.5	
15	31.1	1.09 (1H, *m*)	31.1	
		1.48 (1H, *m*)		C-16
16	25.0	1.29 (1H, *m*)	25.0	
		1.80 (1H, *m*)		C-17
17	49.4	1.98 (1H, *m*)	49.5	C-13, C-22

Table 23. Comparison of NMR spectral data of cabraleahydroxylactone and compound CP-8 ($CDCl_3$, 500 MHz) (continued)

Position	Cabraleahydroxylactone*	Compound CP-8		HMBC
	^{13}C	^{1}H	^{13}C	
18	15.5	0.94 (1H, *m*)	15.5	C-7, C-9, C-14
19	16.0	0.83 (3H, *s*)	16.0	C-1, C-5, C-9, C-10
20	90.2	-	90.2	
21	25.3	1.34 (3H, *s*)	25.4	C-17, C-20, C-22
22	31.2	1.93 (1H, *m*)	31.2	C-20, C-24
		2.10 (1H, *m*)		C-17, C-24
23	29.2	2.53 (1H, *m*)	29.2	C-20, C-22, C-24
		2.62 (1H, *m*)		C-22, C-24
24	176.8	-	176.8	
28	28.3	0.92 (3H, *s*)	28.3	C-3, C-4, C-5, C-29
29	22.1	0.82 (3H, *s*)	22.1	C-3, C-4, C-5, C-28
30	16.3	0.88 (3H, *s*)	16.3	C-8, C-13, C-14, C-15

* Su *et al.*, 2006 (in $CDCl_3$, 300 MHz)

1.9 Identification of Compound CP-9

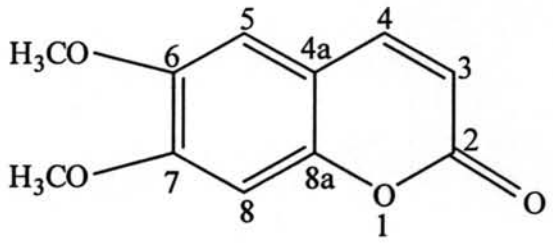
Compound CP-9, which fluoresced under UV light, was obtained as yellow needle crystals. Its molecular formula was determined as $C_{11}H_{10}O_4$ by EI mass spectrum (**Figure 79**), with its $[M+H]^+$ peak at *m/z* 207. The IR spectrum (**Figure 78**) exhibited absorption bands at 1712 cm^{-1} (conjugated carbonyl group) and 1567 and 1463 cm^{-1} (aromatic ring). The ^1H NMR spectrum of compound CP-9 (**Figure 80** and **Table 24**) showed a typical pair of doublets at δ 6.21 and 7.54 (1H each, *d*, J = 9.5 Hz) for H-3 and H-4 of coumarin, respectively. The relatively high field position of H-4 suggested the lack of an oxygen substituent at C-5 (Steck and Mazurek, 1972). The presence of 6,7-disubstituted aromatic ring was suggested by two aromatic proton singlets at δ 6.78 (H-5) and 6.85 (H-8). The presence of a singlet at δ 3.93 integrated for six protons represents two methoxy substituents.

The ^{13}C NMR (**Figure 81**), DEPT 135 (**Figure 82**) and HMQC spectra (**Figures 84**) of compound CP-9 displayed 10 carbons peaks classified into those of two methoxyl groups at δ 56.5, four methine carbons at δ 103.3 (C-8), 107.6 (C-5), 113.5 (C-3) and 143.3 (C-4) and five quaternary carbons at δ 111.6 (C-4a), 144.1 (C-6), 149.8 (C-7), 150.4 (C-8a) and 161.4 (C-2). The HMBC spectrum (**Figures 85** and **Table 24**) showed the correlation between methoxyl protons at δ 3.93 to C-6 singlet at δ 144.1, suggesting the presence of one methoxy group at this position. HMBC correlations were also observed from δ 6.78 (H-5) to carbon signal at δ 143.3 (C-4), 149.8 (C-7) and 150.4 (C-8a), and between H-8 (δ 6.85) and C-4a (δ 111.6), C-6 (δ 144.1), C-7 (δ 149.8) and C-8a (δ 150.4).

From all of the above spectroscopic data and comparison with reported values (Razdan *et al.*, 1987; Ma *et al.*, 2006), compound CP-9 was identified as a 6,7-dimethoxycoumarin named scoparone. Scoparone has previously been isolated from several plants of the family Rutaceae e.g. from *Skimmia laureola* aerial parts (Razdan *et al.*, 1987), *Eriostemon myoporoides* (Sarker *et al.*, 1994), *Ruta angustifolia* (Castillo *et al.*, 1984), *Euodia borbonica* leaves (Valenciennes, Smadja and Conan, 1999), *Afragle paniculata* stem (Adesogan, 1973), *Metrodorea flavida* (Baetas *et al.*, 1999) and *Dictamus angustifolius* root bark (Wu *et al.*, 1999). Plants belonging to other families which also contain scoparone are *Artemisia scoparia* (leaves and flower heads) (Compositae) (Stefanovic *et al.*, 1973), *Artemisia dracunculoides* (Herz, Bhat and Santhanam, 1970), *Echinosophora koreensis* (roots and stems) (Leguminosae) (Iinuma *et al.*, 1993), *Bupleurum fruticosum* (roots) (Apiaceae) (Pistelli *et al.*, 1996),

Aralia bipinnata (woody parts) (Araliaceae) (Hsiao and Chiang, 1995), *Cedrelopsis grevei* (stem bark) (Ptaeroxylaceae) (Mulholland *et al.*, 2003), *Olea capensis* (bark) (Oleaceae) (Tsukamoto *et al.*, 1984) and *Vahlia capensis* (aerial parts) (Vahliaceae) (Majinda *et al.*, 1995).

Scoparone has been shown to possess various biological activities. For example, it has been used as antipyretic, anti-inflammation, diuretic and choleric for the treatment of hepatitis and bilious disorder (Chang and But, 1987). It was reported to reduce the proliferative responses of human peripheral mononuclear cells, to relax smooth muscle, to reduce total cholesterol and triglycerides and to retard the characteristic pathomorphological changes in hypercholesterolaemic diabetic rabbits (Hoult and Paya, 1996). It displayed strong anti-asthma action by directly reducing the intracellular calcium ion concentration in isolated guinea-pig tracheal smooth muscle (Zhao *et al.*, 2000; Liu *et al.*, 2002; Fang *et al.*, 2003). Morover, scoparone reduced pro-imflammatory mediators such as nitric oxide (NO), cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) and IL-6 productions or expressions in Raw 264.7 murine macrophages (Jang *et al.*, 2005).



Scoparone (Compound CP-9)

Table 24. Comparison of NMR spectral data of scoparone and compound CP-9 ($CDCl_3$, 500 MHz)

Position	Scoparone		Compound CP-9		
	^1H*	^{13}C**	^1H	^{13}C	HMBC
2	-	161.7	-	161.4	
3	6.28 (1H, *d*, *J* = 9.6 Hz)	111.7	6.21 (1H, *d*, *J* = 9.5 Hz)	113.5	C-2, C-4a
4	7.65 (1H, *d*, *J* = 9.6 Hz)	143.6	7.54 (1H, *d*, *J* = 9.5 Hz)	143.3	C-2, C-5
4a	-	113.7	-	111.6	
5	6.88 (1H, *s*)	108.2	6.78 (1H, *s*)	107.6	C-4, C-7, C-8a
6	-	150.2	-	144.1	
7	-	153.0	-	149.8	
8	6.83 (1H, *s*)	100.2	6.85 (1H, *s*)	103.3	C-4a, C-6, C-7, C-8a
8a	-	146.6	-	150.4	
6-OCH_3	3.96 (3H, *s*)	56.6	3.93 (3H, *s*)	56.5	C-6
7-OCH_3	3.94 (3H, *s*)	56.6	3.93 (3H, *s*)	56.5	

*Ma *et al.*, 2006 (in $CDCl_3$, 400 MHz)

** Ma *et al.*, 2006 (in $CDCl_3$, 100 MHz)

1.10 Identification of Compound CP-10

Compound CP-10, which was obtained as yellow needle crystals, also fluoresced under UV light, suggesting its coumarin nature. The ESI mass spectrum (**Figure 88**) showed $[M+H]^+$ at *m/z* 193, corresponding to $C_{10}H_8O_4$, which is one methyl unit less than the previous compound. The IR spectrum (**Figure 87**) showed bands at 3297 (OH stretching), 1707 (conjugated C=O stretching) and 1607 and 1453 (aromatic ring) cm^{-1}. The ^1H NMR spectrum of compound CP-10 (**Figure 89**) showed two coupled doublets at δ 6.21 (1H, *d*, *J* = 9.5 Hz) and 7.91 (1H, *d*, *J* = 9.5 Hz) assignable to H-3 and H-4, respectively. The presence of 6,7-dioxygenated aromatic ring was suggested by two singlet signals at δ 6.78 and 7.21 (each 1H, *s*), representing H-8 and H-5, respectively. The ^{13}C NMR spectrum (**Figure 90**) and DEPT 135 spectra (**Figure 91**) exhibited, in total, ten carbon signals which could be classified as those of one methoxy carbon at δ 56.5, four methine carbons at δ 103.3 (C-8), 110.1 (C-5), 112.2 (C-3) and 144.9 (C-4), four quaternary carbons at δ 111.0 (C-4a), 145.7 (C-6), 150.0 (C-8a) and 151.6 (C-7), and the most downfield quaternary signal at δ 161.1 assignable to carbonyl moiety at C-2.

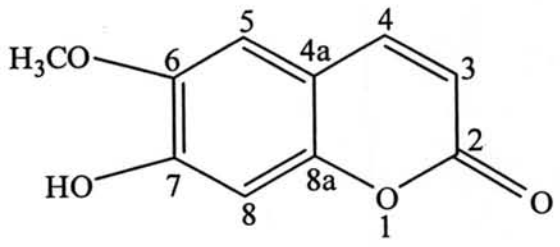
In the HMBC spectrum (**Figures 94**), correlations of the proton signal at δ 3.82 (6-OCH_3) to C-6 signal (δ 145.7), suggesting the presence of methoxy group at C-6. Two singlets at δ 6.78 and 7.21 were assigned to H-8 and H-5, respectively, and confirmed by the HMBC correlations from δ 6.78 (H-8) to C-4a (δ 111.0), C-6 (145.7), C-8a (150.0) and C-7 (151.6), and also between H-5 (δ 7.21) and C-4 (δ 144.9), C-8a (δ 150.0) and C-7 (δ 151.6).

Based on the spectral analysis and comparison with reported values (Razdan *et al.*, 1987; Sibanda *et al.*, 1989), compound CP-10 was identified as another coumarin derivative: scopoletin.

This coumarin has previously been isolated from several plants of the family Rutaceae e.g. from *Skimmia laureola* aerial parts (Razdan *et al.*, 1987), *Eriostemon myoporoides* (Sarker *et al.*, 1994), *Clausena anisata* roots (Ojewole, 2002), *Zanthoxylum schinifolium* bark (Chang *et al.*, 1997), *Pamburus missionnis* fruits (Kumar *et al.*, 1994), *Murraya gleinei* leaves (Wickramaratne *et al.*, 1984), *Aegle marmelos* roots (Shoeb *et al.*, 1973) and *Dictamus angustifolius* root bark (Wu *et al.*, 1999), together with other families such as from *Artemisia dracunculoides* (Herz, Bhat and Santhanam, 1970), *Echinosophora koreensis* roots and stems (Leguminosae) (Iinuma *et al.*, 1993), *Bupleurum fruticosum* roots (Apiaceae) (Pistelli *et al.*, 1996),

Olea africana bark (Oleaceae) (Tsukamoto *et al.*, 1984), *Xeromphis obovata* root bark (Sibanda *et al.*, 1989), *Nyssa sylvatica* wood (Nyssaceae) (Li *et al.*, 2000), *Guarea rhophalocarpa* leaves (Camacho *et al.*, 2001) and *Aglaia crassinervia* bark (Meliaceae) (Su *et al.*, 2006), *Dipterocarpus hasseltii* bark (Dipterocarpaceae) (Muhtadi *et al.*, 2006) and *Vahlia capensis* aerial parts (Vahliaceae) (Majinda *et al.*, 1995).

Investigation of scopoletin, isolated from *Solanum lyratum*, has shown that the coumarin has hepatoprotective and antioxidant activities (Kang *et al.*, 1998; Shaw *et al.*, 2003). Antiproliferative action was also demonstrated through the induction of apoptosis on PC 3 cells (human androgen-independent prostate adenocarcinoma cell) (Liu *et al.*, 2001). Futhermore, scopoletin isolated from *Micromelum integerrium* exhibited antitumoral activity on P-388 lymphocytic leukaemia (Cassady *et al.*, 1979). The compound was able to suppress acetylcholine-induced contractures of the toad rectus abdominis muscle (Ojewoleand and Adesina, 1983). Kang *et al.* (1999) showed that scopoletin inhibited the nitric oxide synthesis in a dose-dependent manner in murine macrophage-like RAW 264.7 cell stimulated with interferon-y (IFN-y) plus lipo-polysaccharide.



Scopoletin (Compound CP-10)

Table 25. Comparison of NMR spectral data of scopoletin and compound CP-10 (DMSO-d_6, 500 MHz)

Position	Scopoletin		Compound CP-10		
	^{1}H*	^{13}C**	^{1}H	^{13}C	HMBC
2	-	160.2	-	161.1	
3	6.10 (1H, *d*, *J* = 9.6 Hz)	112.5	6.21 (1H, *d*, *J* = 9.5 Hz)	112.2	C-2, C-4a
4	7.48 (1H, *d*, *J* = 9.6 Hz)	142.3	7.91 (1H, *d*, *J* = 9.5 Hz)	144.9	C-2, C-5, C-8a,
4a	-	110.5	-	111.0	
5	7.16 (1H, *s*)	107.0	7.21 (1H, *s*)	110.1	C-4, C-8a, C-7
6	-	143.2	-	145.7	
7	-	149.2†	-	151.6	
8	6.22 (1H, *s*)	102.5	6.78 (1H, *s*)	103.3	C-4a, C-6, C-8a, C-7
8a	-	149.8†	-	150.0	
6-OCH_3	3.10 (3H, *s*)	55.2	3.82 (3H, *s*)	56.5	C-6
7-OH	10.0 (1H, *br s*)	-	10.33 (1H, *s*)	-	

*Razdan *et al.*, 1987 (in DMSO-d_6, 90 MHz)

**Sibanda *et al.*, 1989 (in DMSO-d_6, 125 MHz)

†may be exchangeable

1.11 Identification of compound CP-11

Compound CP-11 was obtained as white needle crystals and gave a yellow color when visualized with 10% ethanolic sulphuric acid reagent. Its UV spectrum (**Figure 95**) showed three absorption maxima at λ_{max} 222, 260 and 290 nm, typical of benzoic acid derivatives (Kamath, Mehta and Bafna, 1975), while the IR spectrum (**Figure 96**) showed absorption bands at 2952 cm-1 (C-H stretching), 1599 (aromatic C=C), 1526 (aromatic C=C), 1682 (C=O), 1286, 1240, 1030 (C-O), the hydroxyl absorption band at 3488 cm^{-1} and the broad carboxylic absorption band at 3300-2400 cm^{-1}.

The EIMS of CP-11 (**Figure 97**) displayed a prominent molecular ion peak at *m/z* 168, corresponded to the molecular formula of $C_8H_8O_4$. Intense EIMS fragment peaks at *m/z* 153 ($[M-CH_3]^+$), 125 ($[M-COCH_3]^+$), 123 ($[M-COOH]^+$) , 97 ($[M-CH=CH-COOH]^+$) are important in showing compound CP-11 as having a skeletal structure of phenylpropanoid acid.

The ^1H NMR spectrum of compound CP-11 (**Figures 98a and 98b**) displayed an aromatic proton signal at δ 6.84 (1H, *d*, *J* = 7.9 Hz, H-5) ortho-coupling to another signal at δ 7.44 (1H, *dd*, *J* = 7.9, 1.9 Hz, H-6), which, in turn, meta-coupling to the signal at δ 7.43 (1H, *d*, *J* = 1.9, H-2). A three-proton singlet at δ 3.80 indicated the presence of one methoxy group on the aromatic ring.

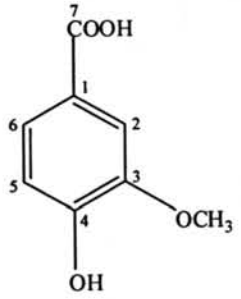
The ^{13}C NMR spectrum (**Figure 99**) of compound CP-11 gave 8 carbon signals. The DEPT-135 spectrum (**Figure 100**), together with the HMQC experiment (**Figure 102**), helped in classifying these signals into those of a carbonyl carbon at δ 167.7, three quaternary carbons at δ 122.1 (C-1), 147.7 (C-3) and 151.6 (C-4), three methine carbons at δ 113.3 (C-2), 115.5 (C-5) and 123.9 (C-6), and one methoxy carbon at δ 56.0 ($O-CH_3$).

The structure of compound CP-11 consists of an aromatic ring substituted with 3 functional groups: hydroxyl, methoxyl and carboxyl group. The positions of these moieties on the benzene ring were confirmed by the HMBC experiment (**Figure 103**). A three-bond correlation between the methoxyl proton at δ 3.80 (OCH_3) to C-3 (δ 147.7) placed this group at position 3. The downfield, hydroxyl-substituted carbon signal of position 4 (δ 151.6) gave cross-peaks with both H-2 (δ 7.43) and H-6 (δ 7.44). These two proton signals also displayed three-bond correlation with the carbonyl carbon (δ 167.7) of the carboxyl group (C-7), therefore establishing the

position of this substituent at C-1. Major HMBC correlations in the structure of compound CP-11 are summarized in **Table 26**.

Based on the spectral data analysis and comparison with reported values (Sakushima *et al.*, 1995), compound CP-11 was identified as a phenolic acid derivative called vanillic acid.

Vanillic acid has been reported as a constituent of *Spathodea campanulata* (Niyonima *et al.*, 1991), *Crescentia cujete* (Binutu, 1997) and *Boreava orientalis* (Sakushima *et al.*, 1995). Bioactivity evaluation of vanillic acid showed antioxidant activity stronger than α-tocopherol, a common natural antioxidant, (Sakushima *et al.*, 1995). Vanillic acid has also been reported to exert antibacterial activity (Fernandez *et al.*, 1996). It possesses antibacterial activity against the growth of *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus cereus* and also exhibited antifungal activity by inhibiting the growth and aflatoxin production by both *Aspergillus flavus* and *Aspergillus parasiticus* (Aziz *et al.*, 1998).



Vanillic acid (Compound CP-11)

Table 26. Comparison of NMR spectral data of vanillic acid and compound CP-11 (DMSO-d_6, 500 MHz)

Position	Vanillic acid*	Compound CP-11		
	^{13}C	^{1}H	^{13}C	HMBC
1	123.1	-	122.1	
2	113.9	7.43 (1H, *d*, J = 1.9 Hz)	113.3	C-3, C-4, C-6, C-7
3	148.6	-	147.7	
4	152.6	-	151.6	
5	115.9	6.84 (1H, *d*, J = 7.9 Hz)	115.5	C-1, C-3, C-4
6	125.3	7.44 (1H, *dd*, J = 7.9, 1.9 Hz)	123.9	C-2, C-4, C-7
7	170.0	-	167.7	
3-OCH_3	56.4	3.80 (3H, *s*)	56.0	C-3

* Sakushima *et al.*, 1995 (in CD_3OD, 100 MHz)

1.12 Identification of Compound CP-12

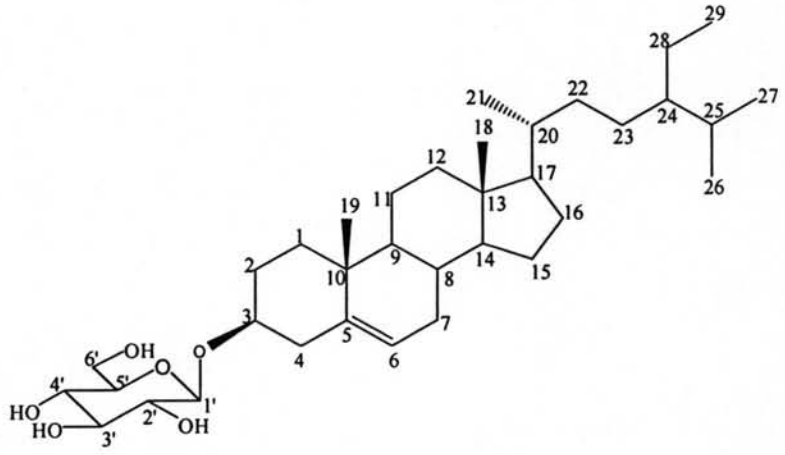
Compound CP-12 was obtained as white amorphous powder. This compound gave purple color upon spraying with anisaldehyde regent. Liebermann-Burchard test of this compound gave green color, suggesting the presence of steroidal skeleton.

In the ^{1}H NMR spectrum (**Figure 104**), the signal at δ 5.33 (2H, *d*, *J* = 5.2 Hz) can be assigned to the vinylic H-6, while a one-proton multiplet at δ 3.42 is attributable to the oxymethine proton at position 3. The signals between δ 0.66-0.99 are those of methyl protons, including the signals at δ 0.65 (3H, *s*, H_3-18), 0.80 (3H, *d*, *J* = 7.0 Hz, H_3-27), 0.80 (3H, *d*, *J* = 7.0 Hz, H_3-26), 0.82 (3H, *t*, *J* = 7.0 Hz, H_3-29), 0.90 (3H, *d*, *J* = 6.4 Hz, H_3-21) and 0.95 (3H, *s*, H_3-19). The signals at δ 1.1-2.3 belong to methylene and methine protons. The multiplet signals at δ 2.80-3.20 were those of the sugar moiety (H-2′, H-3′, H-4′ and H-5′). The signals at δ 3.40 and 3.64 belong to methylene protons at position 6′ and the doublet at δ 4.22 (1H, *J* = 7.8 Hz), was assignable to the β anomeric of the sugar moiety. The sugar component in compound CP-12 was concluded to be *β*-D-glucopyranose.

The ^{13}C NMR spectrum (**Figures 105a-105c** and **Table 27**) showed the signals of 35 carbons atoms, supporting the assignment of this compound as a steroid glucoside. The DEPT experiment (**Figure 106**) were performed to differentiate these 35 signals into those of six methyl carbons at δ 12.2 (C-18), 12.3 (C-29), 19.0 (C-21), 19.1 (C-27), 19.6 (C-19) and 20.2 (C-26), twelve methylene carbons at δ 21.1 (C-11), 23.1 (C-28), 24.3 (C-15), 26.0 (C-23), 28.3 (C-16), 29.9 (C-2), 31.9 (C-7), 33.8 (C-22), 37.3 (C-1), 38.8 (C-12), 42.3 (C-4) and 61.6 (C-6′), fourteen methine carbons at δ 29.2 (C-25), 31.8 (C-8), 36 (C-20), 45.6 (C-24), 50.1 (C-9), 56.0 (C-17), 56.7 (C-14), 70.6 (C-4′), 74.0 (C-2′), 77.2 (C-3), 77.3 (C-5′),77.4 (C-3′), 101.3 (C-1′) and 121.7 (C-6), and three quaternary carbons at δ 36.7 (C-10), 42.3 (C-13) and 141.0 (C-5). The two most downfield signals at δ 141.0 and 121.7 can be assigned to the olefenic C-5 and C-6, respectively. The carbon signal at δ 77.2 represents the oxygenated C-3. The signal at δ 101.3, corresponding to the anomeric carbon (C-1′), confirmed that compound CP-12 should be a monoglycoside.

Comparison of the ^{13}C NMR data of compound CP-12 with those values previously reported for *β*-sitosterol glucoside (Kojima *et al.*, 1990: Mizushina *et al.*, 2006) revealed them to be fully in agreement, as summarized in **Table 27**. Therefore, compound CP-12 was identified as *β*-sitosterol glucoside.

β-Sitosterol glucoside has been isolated from a number of plants from several families, e.g. *Spilanthes acmella* (family Compositae) (Krishnaswamy and Prasanna, 1975), *Aframomum escapum* (Family Zingiberaceae) (Ayimele, Tane and Connolly, 2004) and *Thymelea hirsute* (family Thymelaceae) (Rizk and Rimpler, 1972). It was found to exhibit *in vitro* anthelmintic activity against the nematode *Caenorhabditis elegans* in the bioassay-guided study of *Tribulus terrestris* (family Zygophyllaceae) (Deepak *et al.*, 2002) and also stimulated human peripheral blood lymphocyte proliferation (Bouic *et al.*, 1996). Moreover, this compound was shown to selectively inhibit the activity of mammalian DNA polymerase λ (pol λ) *in vitro* (Mizushina *et al.*, 2006).



β-Sitosterol glucoside (Compound CP-12)

Table 27. Comparison of ^{13}C NMR spectral data of β-Sitosterol glucoside and compound CP-12 (DMSO-d_6, 125 MHz)

Position	β-Sitosterol glucoside*	Compound CP-12
1	37.5	37.3
2	30.3	29.9
3	78.2	77.2
4	39.4	38.8
5	141.0	141.0
6	122.0	121.7
7	32.2	31.9
8	32.1	31.8
9	50.4	50.1
10	37.0	36.7
11	21.3	21.1
12	40.0	42.3
13	42.5	42.3
14	56.9	56.7
15	24.6	24.3
16	28.6	28.3
17	56.3	56.0
18	12.0	12.2
19	19.3	19.6
20	36.4	36.0
21	19.0	19.0
22	34.3	33.8
23	26.4	26.0
24	46.1	45.6
25	29.5	29.2
26	19.5	20.2
27	20.0	19.1
28	23.4	23.1
29	12.2	12.3
1′	102.6	101.3
2′	75.4	74.0
3′	78.7	77.4
4′	71.8	70.6
5′	78.6	77.3
6′	62.9	61.6

* Mizushina *et al.*, 2006 (in pyridine-d_5, 100 MHz)

1.13 Identification of Compound CP-13

Compound CP-13 was obtained as yellow needle crystals, soluble in $CHCl_3$. Its molecular formula was determined by HRESI mass spectrum (**Figure 108**) as $C_{15}H_{26}O_3$, from its $[M+Na]^+$ peak at *m/z* 277.4772. The IR spectrum (**Figure 107**) displayed absorption bands for hydroxy group(s) at 3391 cm^{-1}.

Preliminary examination of the spectral data suggested that compound CP-13 possess similar aromadendrane sesquiterpene structure to compounds CP-4 and CP-5, except for the difference in the number of hydroxyl groups in the structure.

The ^1H NMR spectrum of compound CP-13 (**Figure 109**) displayed two typical, most upfield signals of the methine protons of the aromadendrane framework at δ 0.33 (1H, *t*, *J* = 9.8 Hz, H-6) and 0.85 (1H, *ddd*, *J* = 19.6, 9.1, 6.8 Hz, H-7) (Vizzotto *et al.*, 2003). Two pairs of geminal oxygenated methylene proton resonated at δ 3.29 (1H, *d*, *J* = 10.9 Hz, H-13a), 3.49 (1H, *d*, *J* = 10.9 Hz, H-13b), 3.32 (1H, *d*, *J* = 10.8 Hz, H-14a) and 3.45 (1H, *d*, *J* = 10.8 Hz, H-14b), whereas two methyl signals appeared at δ 1.17 (3H, *s*, H_3-12) and 0.97 (3H, *d*, *J* = 6.8 Hz, H_3-15).

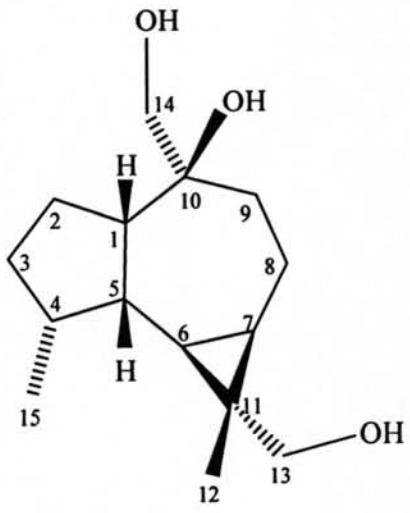
The ^{13}C NMR spectrum of CP-13 (**Figures 110**) exhibited 15 carbon peaks. These peaks were classified, according to DEPT 135 experiment (**Figures 111**), into those of two methyl signals at δ 11.8 (C-12) and 16.3 (C-15), four methylene signals at δ 17.9 (C-8), 24.5 (C-2), 29.1 (C-3) and 31.7 (C-9), two oxygenated methylene carbons at δ 70.7 (C-14) and 73.6 (C-13), five methine carbons at δ 19.4 (C-6), 25.7 (C-7), 38.5 (C-4), 38.9 (C-5) and 53.5 (C-1), one quaternary carbon signal at δ 26.1 (C-11) and one oxygenated quaternary carbon signal at δ 76.1 (C-10).

In the HMBC spectrum (**Figures 114a-114d**), correlations of the H_3-15 methyl proton signal (δ 0.97) with a methylene and two methine carbon signals at δ 29.1 (C-3), 38.5 (C-4) and 38.9 (C-5), respectively, revealed that this methyl group was attached to C-4. The two-bond correlation of the oxygenated H-14a methylene proton (δ 3.32) with C-10 (δ 76.1) established the connectivity between the oxygenated C-14 and C-10. Cross correlations can also be observed between the signal of H-13a (δ 3.29) and carbon signals at δ 19.4 (C-6) and 26.1 (C-11), while the signal of H-13b (δ 3.49) corrates with two methine and quaternary carbon signals at δ 19.4 (C-6), 25.7 (C-7) and 26.1 (C-11), respectively, indicating another oxygenated methylene at position 11.

The NOESY correlations (**Figure 115**) between H_2-13 and H-6, H-1 and H-12, as well as between H-5 and H-12, suggested the relative conformation at positions

13, 1 and 5, respectively. In addition, comparison of the ^{1}H and ^{13}C NMR data of both compound CP-4 (14-hydroxyviridiflorol) and CP-13 suggested the same relative configuration.

In conclusion, compound CP-13 was identified as another aromadendrane - type sesquiterpene named (-)-10*β*,13,14-trihydroxy-*allo*-aromadendrane (Miyazawa *et al.*, 1995). This sesquiterpene was firstly isolated from *Wyethia arizonica* of the family Compositae (Bohlmann *et al.*, 1984). However, no bioactivity of this compound has been reported.



(-)-10*β*,13,14-Trihydroxy-*allo*-aromadendrane (Compound CP-13)

Table 28. Comparison of ^{1}H and ^{13}C NMR spectral data of (-)-10*β*,13,14-trihydroxy-*allo*-aromadendrane and compound CP-13 ($CDCl_3$, 500 MHz)

Position	(-)-10β,13,14-Trihydroxyalloaromadendrane		Compound CP-13		
	^{1}H*	^{13}C**	^{1}H	^{13}C	HMBC
1	1.89 (1H, *m*)	53.5	1.90 (1H, *m*)	53.5	C-6
2	1.52 (1H, *m*)	24.5	1.50 (1H, *m*)	24.5	C-3
	1.68 (1H, *m*)		1.65 (1H, *m*)		C-1
3	1.30 (1H, *m*)	29.1	1.31 (1H, *m*)	29.1	
	1.72 (1H, *m*)		1.85 (1H, *m*)		
4	2.02 (1H, *m*)	38.5	2.01 (1H, *m*)	38.5	
5	1.83 (1H, *m*)	38.9	1.93 (1H, *m*)	38.9	
6	0.32 (1H, *dd*, *J* = 9.5, 10.5 Hz)	19.4	0.33 (1H, *t*, *J* = 9.8 Hz)	19.4	C-13
7	0.83 (1H, *ddd*, *J* = 10.5, 9.5, 6.0 Hz)	25.7	0.85 (1H, *ddd*, *J* = 19.6, 9.1, 6.8 Hz)	25.7	C-8
8	1.49 (1H, *m*)	17.9	1.57 (1H, *m*)	17.9	C-9
	1.68 (1H, *m*)		1.76 (1H, *m*)		C-9, C-10
9	1.54 (1H, *m*)	31.7	1.53 (1H, *m*)	31.7	C-1, C-8
	1.71 (1H, *m*)		1.70 (1H, *m*)		C-1, C-8, C-10
10	-	76.1	-	76.1	
11	-	26.1	-	26.1	
12	1.15 (3H, *s*)	11.7	1.17 (3H, *s*)	11.8	C-6, C-7, C-11, C-13
13	3.26 (1H, *d*, *J* = 11 Hz)	73.6	3.29 (1H, *d*, *J* = 10.9 Hz)	73.6	C-6, C-7, C-11
	3.47 (1H, *d*, *J* = 11 Hz)		3.49 (1H, *d*, *J* = 10.9 Hz)		C-7, C-11
14	3.29 (1H, *d*, *J* = 10.5 Hz)	70.7	3.32 (1H, *d*, *J* = 10.8 Hz)	70.7	C-10
	3.42 (1H, *d*, *J* = 10.5 Hz)		3.45 (1H, *d*, *J* = 10.8 Hz)		
15	0.95 (3H, *d*, *J* = 7 Hz)	16.3	0.97 (3H, *d*, *J* = 6.8 Hz)	16.3	C-3, C-4, C-5

*Miyazawa *et al.*, 1995 (in $CDCl_3$, 400 MHz)

*Miyazawa *et al.*, 1995 (in $CDCl_3$, 400 MHz)

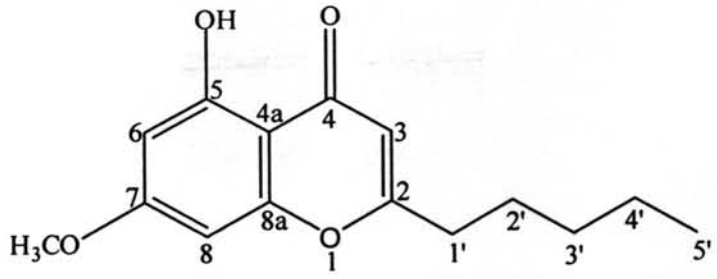
1.14 Identification of compound CP-14

Compound CP-14 was obtained as colorless oil. The ESI-TOF mass spectrum (**Figure 118**) exhibited $[M+H]^+$ ion peak at m/z 263, suggesting the molecular formula $C_{15}H_{18}O_4$. The UV maxima (**Figure 116**) at 246, 272 and 328 nm, are characteristic of a 5,7-dioxygenated chromone (Robeson, Ingham and Harborne, 1980). The presence of an α,β-unsaturated carbonyl group characteristic of chromones was confirmed by the presence of IR absorption bands (**Figure 117**) at 1687 and 1650 cm^{-1} (Jimenez *et al.*, 1989).

The ^1H NMR spectrum (**Figure 119**) showed two doublets at δ 6.33 (1H, *d*, J = 2.2 Hz) and 6.48 (1H, *d*, J = 2.2 Hz) of the meta-coupled aromatic protons at H-8 and H-6, respectively. The resonance at δ 3.88 (3H, *s*), 6.19 (1H, *s*) and 11.14 (1H, *s*) can be readily assigned to methoxyl group at C-7, olefenic proton (H-3) and the hydrogen-bonded phenolic hydroxyl group at C-5, respectively. In addition, the spectrum showed several upfield signals at δ 0.92 (3H, *t*, J = 7.2 Hz, H_3-5′), 1.33 (4H, *m*, H_2-3′, H_2-4′), 1.70 (2H, m, H_2-2′) and 2.50 (2H, *t*, J = 7.6 Hz, H_2-1′) representing a pentyl side chain. The mass fragment at *m/z* 205 $[M-C_4H_8-H]^+$ also confirmed the existence of a pentyl group in the molecule.

The ^{13}C NMR spectrum (**Figure 120** and **Table 29**), DEPT 135 (**Figure 121**) and HMQC experiment (**Figure 123**) displayed the signals of one methyl group at δ 13.9 (C-5′), one methoxy group at δ 55.7 (7-OCH_3), four methylene carbons at δ 22.4 (C-4′), 26.5 (C-2′), 31.2 (C-3′) and 33.3 (C-1′), three methine carbons at δ 100.2 (C-6), 101.1 (C-8) and 103.9 (C-3), five quaternary carbons at δ 100.0 (C-4a), 139.5 (C-2), 158.1 (C-8a), 163.7(C-5), and 166.53 (C-7), and one carbonyl carbon at δ 166.8 (C-4). The HMBC spectrum (**Figures 124** and **Table 29**) revealed the correlation between H-3 (δ 6.19) and C-1′(δ 33.3), indicated that pentyl group should be placed at C-2. The correlation between methoxy proton (7-OCH_3) at δ 3.88 and C-7 (δ 166.53) confirmed the substitution of the methoxyl group at C-7.

From all of the above spectroscopic data in comparison with reported values (Jimenez *et al.*, 1989), compound CP-14 was identified as 5-hydroxy-7-methoxy-2-pentylchromone. This compound was previously isolated from the bark of *Zanthoxylum microcarpum* and *Z. valens* (Jimenez *et al.*, 1989).



5-Hydroxy-7-methoxy-2-pentylchromone (Compound CP-14)

Table 29. Comparison of ^{1}H and ^{13}C NMR spectral data of 5-hydroxy-7-methoxy-2-pentylchromone and compound CP-14 ($CDCl_3$, 500 MHz)

Position	5-hydroxy-7-methoxy-2-pentylchromone*		Compound CP-14		
	^{1}H	^{13}C	^{1}H	^{13}C	HMBC
2	-	139.5	-	139.5	
3	6.17 (1H, *s*)	103.9	6.19 (1H, *s*)	103.9	C-4′, C-1′
4	-	166.9	-	166.8	
4a	-		-	100.0	
5	-	163.8	-	163.7	
6	6.45 (1H, *d*, *J* = 2.2 Hz)	100.2	6.48 (1H, *d*, *J* = 2.2 Hz)	100.2	C-5, C-7, C-8
7	-	166.6	-	166.5	
8	6.31 (1H, *d*, *J* = 2.2 Hz)	101.0	6.33 (1H, *d*, *J* = 2.2 Hz)	101.1	C-4a
8a	-	158.2	-	158.1	
1′	2.48 (2H, *t*, *J* = 7.5 Hz)	33.2	2.50 (2H, *t*, *J* = 7.6 Hz)	33.3	C-2′, C-3′
2′	1.67 (2H, *m*)	31.1§	1.70 (2H, *m*)	26.5	C-3′
3′	1.34 (4H, *m*)	26.3§	1.33 (4H, *m*)	31.2	
4′		22.3		22.4	C-3′
5′	0.91 (3H, *t*, *J* = 7.0 Hz)	13.8	0.92 (3H, *t*, *J* = 7.0 Hz)	13.9	C-3′
7-OCH_3	3.86 (3H, *s*)	55.6	3.88 (3H, *s*)	55.7	C-7
5-OH	11.10 (1H, *s*)	-	11.20 (1H, *s*)	-	C-4a, C-6

* Jimenez *et al.*, 1989 (in $CDCl_3$, 250 MHz)

§ may be interchangeable

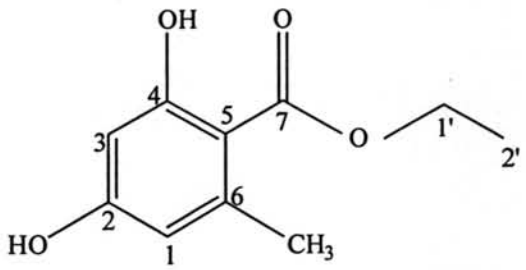
1.15 Identification of Compound CP-15

Compound CP-15 was obtained as yellow amorphous powder. The IR absorption spectrum (**Figure 126**) displayed bands at 3367 (OH stretching), 1643 (conjugated C=O stretching) and 1584 and 1446 cm^{-1} (aromatic ring). Its molecular formula was determined to be $C_{10}H_{12}O_4$ from the $[M-H]^+$ peak at *m/z* 195 in the ESI mass spectrum (**Figure 127**).

The ^1H NMR spectrum (**Figures 128a-128c**) displayed two methyl signals at δ 1.43 (*t*, *J* = 7.1 Hz, H_3-2′) and 2.52 (*s*, 6-CH_3), one oxygenated methylene signal at δ 4.42 (2H, *q*, *J* = 7.1 Hz, H_2-1′), two meta-coupled aromatic protons at δ 6.24 (1H, *d*, *J* = 1.6 Hz, H-1) and 6.29 (1H, *d*, *J* = 1.6 Hz, H-3) and D_2O exchangeable proton signal at δ 11.86 (1H, *s*, 4-OH). The ^{13}C NMR spectrum (**Figure 129**) exhibited, in total, ten carbon signals, which can be classified as those of two methyl carbons at δ 14.3 (C-2′) and 24.4 (6-CH_3), one oxygenated methylene carbon at δ 61.3 (C-1′), six aromatic carbons at δ 101.4 (C-3), 105.9 (C-5), 144.1 (C-6), 111.3 (C-1), 160.1 (C-2) and 165.5 (C-4) and the most downfield signal at δ 171.7 assignable to C-7 ester carbonyl.

Compound CP-15 was identified as, a phenolic compound, ethyl orsellinate by analysis of the above spectral data and confirmed by comparison with previously published data (Lee, Chang and Chen, 2001).

Ethyl orsellinate has been reported as a constituent of the stem and roots of two plants of the family Lauraceae: *Phoebe minutiflora* (Ku, Chen and Lee, 2006) and *Alseodaphne andersonii* (Lee, Chang and Chen, 2001). Bioactivity evaluation of ethyl orsellinate showed weakly nematocidal activity against the second-stage larvae of dog roundworm, *Toxocara canis*, which is a common pathogenic parasite in visceral larva migrans (Ahad *et al.*, 1991).



Ethyl orsellinate (Compound CP-15)

Table 30. Comparison of NMR spectral data of ethyl orsellinate and compound CP-15 ($CDCl_3$, 500 MHz)

Position	Ethyl orsellinate	Compound CP-15	
	^{13}C*	^{1}H	^{13}C
1	111.3	6.24 (1H, *d*, *J* = 1.6 Hz)	111.3
2	160.2	-	160.1
3	101.3	6.29 (1H, *d*, *J* = 1.6 Hz)	101.4
4	165.4	-	165.5
5	108.8	-	105.9
6	144.0	-	144.1
7	171.7	-	171.7
1′	61.3	4.42 (2H, *q*, *J* = 7.1 Hz)	61.3
2′	14.2	1.43 (3H, *t*, *J* = 7.1 Hz)	14.3
6-CH_3	24.3	2.52 (3H, *s*)	24.4
4-OH	-	11.86 (1H, *s*)	-

*Lee, Chang and Chen, 2001 (in $CDCl_3$, 75 MHz)

2. Structure Determination of Compounds Isolated from cf. *Aglaia erythrosperma*

2.1 Identification of Compound AE-4

Compound AE-4 was obtained as white amorphous powder. The EIMS spectrum (**Figure 136**) of compound AE-4 exhibited a molecular ion peak at *m/z* 300, consistent with a molecular formula of $C_{17}H_{16}O_5$. The IR spectrum (**Figure 135**) showed absorption bands at 1751 (conjugated C=O stretching) and 1608 and 1492 (aromatic ring) cm^{-1}.

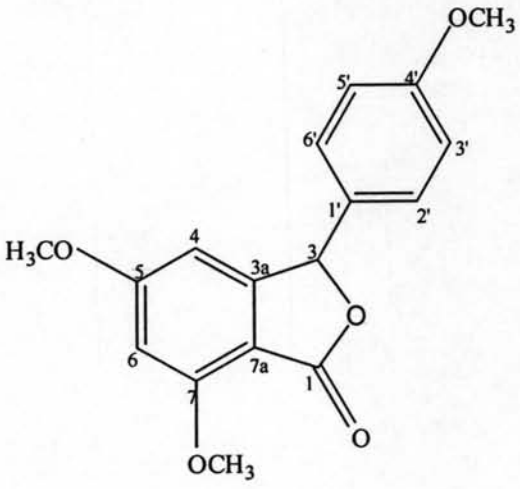
The ^1H NMR spectrum (**Figures 137a-137b**) of this compound exhibited two singlet resonances of 3 methoxyl groups at δ 3.74 (6H, *s*, 5-OCH_3, 4′-OCH_3) and 3.91 (3H, *s*, 7-OCH_3), a downfield singlet at δ 6.17 (1H, *s*, H-3), two *meta*-coupled aromatic proton signals at δ 6.19 (1H, *d*, J = 0.7 Hz, H-4) and 6.37 (1H, *d*, J = 1.6 Hz, H-6) and two doublets of a para-substituted aromatic ring at δ 6.83 (2H, *d*, J = 8.7 Hz, H-3′, H-5′) and 7.12 (2H, *d*, J = 8.7 Hz, H-2′, H-6′).

The ^{13}C NMR (**Figure 138**) and DEPT 135 (**Figure 139**) spectra displayed seventeen carbon signals corresponding to three methoxyls at δ 55.4 (5-OCH_3), 55.9 (4′-OCH_3) and 56.1 (7-OCH_3), six aromatic methine carbons at δ 98.4 (C-4), 99.1 (C-6), 114.4 (C-3′, C-5′) and 128.7 (C-2′, C-6′), one oxygenated methine carbon at δ 81.4 (C-3), three aromatic oxygenated quaternary carbons at δ 159.5 (C-7), 160.4 (C-4′) and 166.9 (C-5), three aromatic quaternary carbons at δ 106.8 (C-7a), 128.8 (C-1′), 154.3 (C-3a), and a carbonyl ester at δ 168.4. These spectral data indicated that the structure of compound AE-4 consists of one tetrasubstituted benzene ring, one disubstituted benzene ring, and three methoxy groups, as parts of a benzofuranone moiety.

The HMBC correlations, (**Figures 142** and **Table 31**), of both methine proton signals of H-2′ and H-6′(δ 7.12) with the methine carbon at δ 81.4 (C-3) and between the signal of H-3 (δ 6.12) and C-1′(δ 128.8), revealed that a phenyl group was attached to C-3. The correlations of each methoxy proton signal to the aromatic C-5, C-7 and C-4′ indicated their substitution at these positions.

According to the above spectral evidence and by comparison of its NMR data with reported data (Salim *et al.*, 2007), compound AE-4 was identified as aglaialactone [5,6-desmethylenedioxy-5-methoxy-aglalactone or 5,7-dimethoxy-3-(4-methoxyphenyl)-1,3-dihydrobenzo[*c*]-furan-1-one].

This compound has recently been isolated from the leaves and twings of *Aglaia ponapensis* (family Meliaceae) (Salim *et al.*, 2007). It exhibited NF-κB inhibitory activity with IC_{50} of 1.9 μM, therefore it could may displayed anti-inflammatory activity.



Aglaialactone (Compound AE-4)

Table 31. Comparison of NMR spectral data of aglaialactone and compound AE-4 ($CDCl_3$, 500 MHz)

Position	Aglaialactone*		Compound AE-4		
	^1H	^{13}C	^1H	^{13}C	HMBC
1	-	168.4	-	168.4	
3	6.17 (1H, *s*)	81.4	6.12 (1H, *s*)	81.4	C-3a, C-1′
3a	-	154.9	-	154.9	
4	6.25 (1H, *d*, *J* = 0.9 Hz)	98.3	6.19 (1H, *d*, *J* = 0.7 Hz)	98.4	C-6, C-7a
5	-	166.8	-	166.9	
6	6.43 (1H, *d*, *J* = 1.4 Hz)	99.0	6.37 (1H, *d*, *J* = 1.6 Hz)	99.1	C-4, C-5, C-7a
7	-	159.4	-	159.5	
7a	-	106.6	-	106.8	
1′	-	128.6	-	128.8	
2′	7.17 (1H, *d*, *J* = 8.5 Hz)	128.7	7.12 (1H, *d*, *J* = 8.7 Hz)	128.7	C-3, C-1′, C-4′
3′	6.88 (1H, *d*, *J* = 8.5 Hz)	114.2	6.83 (1H, *d*, *J* = 8.7 Hz)	114.4	C-1′, C-4′
4′	-	160.2	-	160.4	
5′	6.88 (1H, *d*, *J* = 8.5 Hz)	114.2	6.83 (1H, *d*, *J* = 8.7 Hz)	114.4	C-1′, C-4′
6′	7.17 (1H, *d*, *J* = 8.5 Hz)	128.7	7.12 (1H, *d*, *J* = 8.7 Hz)	128.7	C-3, C-1′, C-4′
7-OCH_3	3.96 (3H, *s*)	56.0	3.91 (3H, *s*)	56.1	C-7
5-OCH_3	3.79 (6H, *s*)	55.3†	3.74 (6H, *s*)	55.4†	C-5
4′-OCH_3		55.9†		55.9†	C-4′

* Salim *et al.*, 2007 (CD_3OD, 400 MHz)

† may be interchangeable

2.2 Identification of Compound AE-5

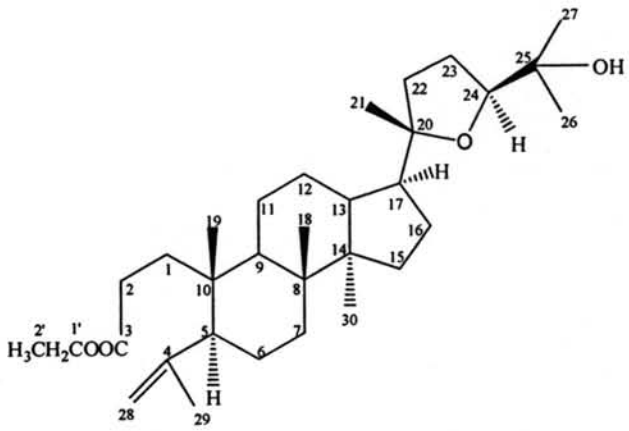
Compound AE-5 was obtained as white amorphous powder. Its IR spectrum (**Figure 143**) exhibited the absorption peak of hydroxyl group at 3489 cm^{-1}, ester carbonyl at 1735 cm^{-1} and ether group at 1384 cm^{-1}. The molecular formula of AE-5 was determined to be $C_{32}H_{54}O_4$ from the $[M+Na]^+$ peak at *m/z* 525 in the ESI-TOF mass spectrum (**Figure 144**), suggesting its nature as a triterpenoid.

The ^1H NMR spectrum of compound AE-5 (**Figures 145a-145c**) displayed seven tertiary methyl signals at δ 0.85 (H_3-19), 0.90 (H_3-30), 1.01 (H_3-18), 1.13 (H_3-21), 1.15 (H_3-26), 1.22 (H_3-27) and 1.75 (H_3-29), and a methyl triplet at δ 1.27 (*t*, J = 7.1 Hz, H_3-2′). Two broad singlets at δ 4.68 and 4.86, each integrating for one proton, were assignable to olefinic methylene protons of the isopropenyl group attached to C-5. An oxymethylene quartet could be observed at δ 4.13 (*q*, J = 7.1 Hz, H_2-1′). Furthermore, the presence of one oxymethine proton signal at δ 3.75 (*t*, J = 7.4 Hz, H-24) suggested that the compound should have a tetrahydrofuran ring in the side chain (Mohamad *et al.*, 1999).

The ^{13}C NMR (**Figure 146** and **Table 32**), DEPT 135 (**Figures 147a-147c**) and HMQC spectra (**Figures 149a** and **149b**) of AE-5 helped in differentiating the signals of a 3,4-secodammarane fused rings with an isopropenyl group at C-5. Four methyl carbon signals appeared at δ 15.3 (C-18), 16.4 (C-30), 20.2 (C-19) and 23.3 (C-29), whereas nine methylene carbons resonated at δ 22.2 (C-11), 24.7 (C-6), 26.2 (C-12), 27.3 (C-16), 28.7 (C-2), 31.5 (C-15), 34.0 (C-7), 34.5 (C-1) and 113.4 (C-28). Four methine carbons exhibited their signals at δ 41.2 (C-9), 43.1 (C-13), 49.6 (C-17) and 50.8 (C-5), and four quaternary signals at δ 39.2 (C-10), 40.1 (C-8), 50.5 (C-14) and 147.6 (C-4). The most downfield signal at δ 174.2 was assignable to the ester carbonyl moiety at C-3. Eight other signals represent the side chain with a tetrahydrofuran ring between C-20 and C-24, and a hydroxyl-substituted C-25. These signals consist of three methyl signals at δ 23.5 (C-21), 24.3 (C-26) and 27.5 (C-27), two methylene signals at δ 25.7 (C-23) and 35.9 (C-22), one oxymethine carbon at δ 83.4 (C-24), and two oxygenated quaternary carbons at δ 71.5 (C-25) and 86.4 (C-20). These data were similar to those of the 3,4-secodammarane eichlerianic acid, previously isolated from *Aglaia foveolata* (Roux *et al.*, 1998). Additional carbon signals of one methyl and one methylene at δ 14.3 and 60.3, respectively, represent the ethyl ester group.

The HMBC correlations (**Figures 150a-150e**) of both olefenic methylene protons at position 28 with the signals of C-29 at δ 23.3 and C-5 at δ 50.8 revealed cleavage of the A ring (Luo *et al.*, 2000) and the attachment of the isopropenyl group at C-5, while the cross peak between the H_2-1′ signal at δ 4.13 and the C-3 carbonyl signal at δ 174.2 confirmed the ethyl ester at this position. HMBC correlations could also be observed between the proton signals (δ 1.15 and 1.12) of both methyl groups at the end of the side chain (H_3-26 and H_3-27, respectively) to C-24 signal at δ 83.4 and the hydroxyl-substituted C-25 at δ 71.5. The NOESY correlation (**Figures 151a** and **151b**) between H-24 and H_3-27, suggested the relative conformation of H-24 to be *anti* (*α*) to H_3-21 (*β*). Moreover, both the proton and carbon resonances, together with the coupling patterns of the oxymethine at C-24 (δ_H 3.75, J = 7.4 Hz; δ_C 83.4), indicated the configuration at this position as *R* (Roux *et al.*, 1998), whereas C-20 was assigned the *S* configuration similar to most dammarane triterpenes isolated from the *Aglaia* genus.

Therefore, compound AE-5 was identified as the 3,4-secodammarane 20*S*,24*R*-epoxy-25-hydroxy-3,4-seco-5*α*-dammar-4(28)-en-3-ethyl ester (ethyl eichlerianoate), previously isolated from the stem bark of *Dysoxylum cauliflorum* (family Meliaceae) (Benosman *et al.*, 2000).



Ethyl eichlerianoate (compound AE-5)

Table 32. NMR spectral data of ethyl eichlerianoate (compound AE-5) ($CDCl_3$, 500 MHz)

Position	^1H	^{13}C	HMBC
1	1.58 (2H, *m*)	34.5	C-3, C-5, C-9, C-10
2	2.17 (1H, *m*)	28.7	C-1, C-3
	2.34 (1H, *m*)		C-1, C-3, C-10
3	-	174.2	
4	-	147.6	
5	2.00 (1H, *dd*, *J* = 12.6, 2.8 Hz)	50.8	C-4, C-10, C-28
6	1.37 (1H, *m*)	24.7	
	1.77 (1H, *m*)		C-10
7	1.23 (1H, *m*)	34.0	
	1.56 (1H, *m*)		
8	-	40.1	
9	1.53 (1H, *m*)	41.2	C-5
10	-	39.2	
11	1.19 (1H, *m*)	22.2	
	1.39 (1H, *m*)		
12	1.20 (1H, *m*)	26.2	
	1.73 (1H, *m*)		
13	1.61 (1H, *m*)	43.1	C-14
14	-	50.5	
15	1.09 (1H, *m*)	31.5	
	1.48 (1H, *m*)		C-8
16	1.79 (1H, *m*)	27.3	
	1.89 (1H, *m*)		C-14
17	1.86 (1H, *m*)	49.6	
18	1.01 (3H, *s*)	15.3	C-7, C-8, C-14
19	0.85 (3H, *s*)	20.2	C-1, C-5, C-9, C-10
20	-	86.4	
21	1.13 (3H, *s*)	23.5	C-17, C-20, C-22
22	1.66 (2H, *m*)	35.9	C-24
23	1.79 (1H, *m*)	25.7	C-20, C-22, C-25
	1.88 (1H, *m*)		C-20, C-22, C-25
24	3.75 (1H, *t*, *J* = 7.4 Hz)	83.4	C-26, C-27
25	-	71.5	
26	1.15 (3H, *s*)	24.3	C-24, C-25
27	1.12 (3H, *s*)	27.5	C-24, C-25
28	4.68 (1H, *s*)	113.4	C-5, C-29
	4.86 (1H, *s*)		C-29
29	1.75 (3H, *s*)	23.3	C-4, C-5, C-28
30	0.90 (3H, *s*)	16.4	C-13, C-14, C-15
1′	4.13 (2H, *q*, *J* = 7.1 Hz)	60.3	C-3, C-2′
2′	1.27 (3H, *t*, *J* = 7.1 Hz)	14.3	C-1′

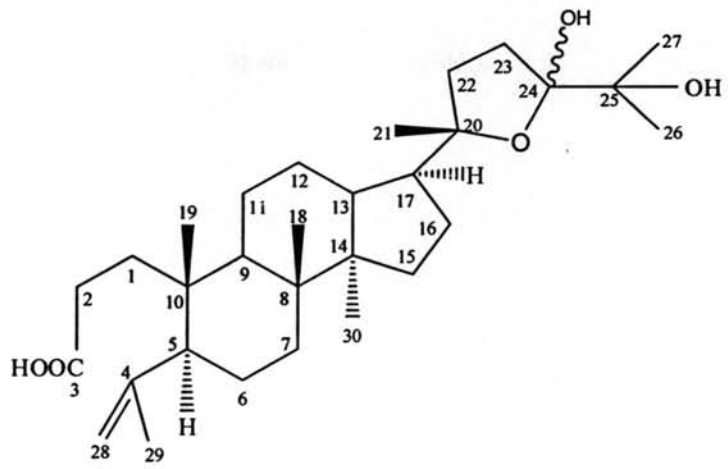
2.3 Identification of Compound AE- 7

Compound AE-7 was obtained as white amorphous powder. Its IR spectrum (**Figure 152**) suggested the presence of carboxylic acid (3412 cm^{-1}), double bond (1636 cm^{-1}), carbonyl (1710 cm^{-1}) and ether (1098 cm^{-1}). The molecular formula of compound AE-7, $C_{30}H_{50}O_5$, was determined by ESI mass spectrometry (**Figure 153**).

Compound AE-7 is another 3,4-secodammarane triterpene, of which its ^1H NMR spectrum (**Figures 154a - 154b** and **Table 33**) displayed seven tertiary methyl signals at δ 0.87 (H_3-19), 0.91 (H_3-30), 1.02 (H_3-18), 1.04 (H_3-21), 1.26 (H_3-26), 1.38 (H_3-27) and 1.75 (H_3-29). Two broad singlets at δ 4.67 and 4.87, each integrating for one proton, were assignable to olefinic methylene protons of the isopropenyl group attached at C-5. ^1H-^1H COSY experiment (**Figure 157**) confirmed these assignments.

The ^{13}C NMR spectral data (**Figure 155** and **Table 33**) of compound AE-7 are also similar to those of compound AE-5, except the typical C-24 oxymethine signal is replaced here by a signal of oxygenated quaternary carbon at δ 108.6, suggesting a five - membered hemiacetal ring instead of a tetrahydrofuran ring. In addition, the signals of ethyl ester are absent, replaced by a carboxyl signal at δ 176.7 (C-3). All carbon signals were assigned according to the HMQC (**Figures 158a-158c**) and HMBC (**Figures 159a-159d**) spectra. Compound AE-7 was in fact a mixture of epimers at C-24 which were inseparable and probably interconvertible. This resulted in the doubling of the ^{13}C NMR signals of the side chain except that of C-24.

Based on these spectral data and comparison with earlier reports (Mohamad *et al.*, 1999; Luo *et al.*, 2000), compound AE-7 was identified as a 3,4-secodammarane triterpenoid called aglinin A. It was previously isolated from the leaves of *Aglaia lawii* (Mohamad *et al.*, 1999) and from the bark of *Amoora yunnanensis* (Luo *et al.*, 2000), both plants belong to the family Meliaceae.



Aglinin A (Compound AE-7)

Table 33. Comparison of NMR spectral data of aglinin A methylester and compound AE-7 ($CDCl_3$, 500 MHz)

Position	Aglinin A methylester*		Compound AE-7		
	^1H	^{13}C	^1H	^{13}C	HMBC
1	1.58 (2H, *m*)	34.7	1.62 (2H, *m*)	34.3	C-2, C-5, C-10, C-19
2	2.31 (1H, *m*)	28.5	2.39 (1H, *m*)	28.2	C-1, C-3
	2.71(1H, *m*)		2.60 (1H, *m*)		
3	-	174.7	-	178.9	
4	-	147.7	-	147.6	
5	1.95 (1H, *m*)	50.9	2.02 (1H, *m*)	51.0	
6	1.37 (1H, *m*)	24.7	1.34 (1H, *m*)	25.0	
	1.77 (1H, *m*)		1.79 (1H, *m*)		
7	1.20 (1H, *m*)	34.0	1.24 (1H, *m*)	34.0	
	1.58 (1H, *m*)		1.55 (1H, *m*)		
8	-	40.2	-	40.1	
9	1.51 (1H, *m*)	41.3	1.52 (1H, *m*)	41.1	
10	-	39.3	-	39.1	
11	1.20 (1H, *m*)	22.2	1.28 (1H, *m*)	22.0	
	1.37 (1H, *m*)	(22.0)	1.43 (1H, *m*)		
12	1.20 (1H, *m*)	27.4	1.20 (1H, *m*)	26.9	
	1.74 (1H, *m*)	(26.9)	1.84 (1H, *m*)		
13	1.66 (1H, *m*)	43.5 (43.0)	1.63 (1H, *m*)	43.3	
14	-	50.9	-	50.4	
15	1.07 (1H, *m*)	31.7	1.09 (1H, *m*)	31.6	
	1.45 (1H, *m*)		1.44 (1H, *m*)		
16	1.38 (1H, *m*)	26.0	1.40 (1H, *m*)	26.0	
	1.74 (1H, *m*)		1.80 (1H, *m*)		
17	1.95 (1H, *m*)	50.6 (49.5)	1.95 (1H, *m*)	50.9	

Table 33. Comparison of NMR spectral data of aglinin A methylester and compound AE-7 ($CDCl_3$, 500 MHz) (continued)

Position	Aglinin A methylester		Compound AE-7		
	^1H*	^{13}C**	^1H	^{13}C	HMBC
18	1.00 (3H, *s*)	15.5	1.02 (3H, *s*)	15.4	C-7, C-8, C-9, C-14
19	0.82 (3H, *s*)	20.3	0.87 (3H, *s*)	20.1	C-1, C-5, C-9, C-10
20	-	88.8 (88.0)	-	88.9	
21	1.09 (3H, *s*)	24.4 (25.2)	1.04 (3H, *s*)	25.2	C-17, C-22
22	1.66 (1H, *m*)	34.7 (37.0)	1.68 (2H, *m*)	33.9	
	2.06 (1H, *m*)				
23	1.81 (1H, *m*)	31.7 (31.2)	2.20 (2H, *m*)	31.3	
	2.06 (1H, *m*)				
24	-	108.6	-	108.6	
25	-	74.2 (74.8)	-	74.4	
26	1.25 (3H, *s*)	24.7 (25.0)	1.26 (3H, *s*)	24.6	C-24, C-25, C-27
27	1.22 (3H, *s*)	24.2	1.38 (3H, *s*)	24.2	C-24, C-25, C-26
28	4.64 (1H, *br s*)	113.5	4.67 (1H, *br s*)	113.6	C-5, C-29
	4.82 (1H, *br s*)		4.87 (1H, *br s*)		C-29
29	1.71 (3H, *s*)	23.4	1.75 (3H, *s*)	23.2	C-4, C-5, C-28
30	0.86 (3H, *s*)	16.5 (16.3)	0.91 (3H, *s*)	16.3	C-8, C-13, C-14, C-15
COOMe	3.64 (3H, *s*)	51.7	-	-	

* Mohamad *et al.*, 1999 ($CDCl_3$, 400 MHz)

** Mohamad *et al.*, 1999 ($CDCl_3$, 125 MHz)

2.4 Identification of Compound AE-10

Compound AE-10 was obtained as white amorphous powder. Its molecular formula was determined by ESI-TOF mass spectrum (**Figure 162**) as $C_{28}H_{26}O_9$, from its $[M]^+$ ion peak at *m/z* 506. The IR spectrum (**Figure 161**) showed bands at 3501 cm^{-1} (OH stretching), 1744 cm^{-1} (carbonyl group) and 1624 and 1437 cm^{-1} (aromatic ring).

The ^1H NMR spectrum (**Figures 163a-163c** and **Table 34**) exhibited one hydroxyl signal at δ 1.91 (1H, *br s*, 1-OH), one methyl ester signal at δ 3.69 (3H, *s*, $COOCH_3$) and two methoxy signals at δ 3.89 (3H, *s*, 6-OCH_3) and 3.92 (3H, *s*, 8-OCH_3). The resonances at δ 5.05 (1H, *d*, *J* = 6.7 Hz), 3.94 (1H, *dd*, *J* = 14.2, 6.7 Hz) and 4.35 (1H, *d*, *J* = 14.2 Hz) can be readily assigned to H-1, H-2 and H-3, respectively. The resonances of two *meta*-coupled aromatic protons can also be observed at δ 6.32 (1H, *d*, *J* = 1.9 Hz, H-5) and 6.17 (1H, *d*, *J* = 1.9 Hz, H-7). Aromatic proton signals at δ 6.75 (1H, *d*, *J* = 1.7 Hz), 6.64 (1H, *d*, *J* = 8.2 Hz) and 6.72 (1H, *dd*, *J* = 8.2, 1.7 Hz) were attributable to H-2′, H-5′ and H-6′, respectively, while the methylenedioxy proton signal at δ 5.89 (2H, *s*, O-CH_2-O), suggested the substitution by a 3′,4′-methylenedioxy group in this aromatic ring. Futhermore, the presence of a groups of five aromatic proton signals at δ 6.95 (2H, *dd*, *J* = 7.7, 2.0 Hz, H-2″, H-6″), 7.14 (1H, *m*, H-3″,H-5″) and 7.13 (1H, *m*, H-4″) indicated that compound AE-10 should be flavagline derivative (Cui *et al.*, 1997).

The ^{13}C NMR (**Figures 164a-164c** and **Table 34**), DEPT 135 (**Figure 165**) and HMQC spectra (**Figures 167**) displayed one methyl ester carbon at δ 52.0, two methoxy signals at δ 55.8 (6-OCH_3 and 8-OCH_3), one methylenedioxy carbon at δ 100.9, two methine carbons at δ 50.5 (C-2), 55.1 (C-3), and one oxygenated methine carbon at δ 79.6 (C-1). Ten aromatic methine carbons exhibited their signals at δ 89.5 (C-5), 92.8 (C-7), 107.2 (C-5′), 108.7 (C-2′), 121.4 (C-6′), 126.7 (C- H-4″) and 127.8 (C-2″, H-3″, H-5″and H-6″). The rest of these carbon signals consists of two oxygenated quaternary carbons at δ 93.8 (C-8b) and 102.0 (C-3a), three aromatic quaternary carbons at δ 107.6 (C-8a), 128.3 (C-1′) and 136.9 (C-1″), and five aromatic oxygenated quaternary carbons at δ 146.7 (C-3′), 147.0 (C-4′), 157.1 (C-8), 160.8 (C-4a) and 164.2 (C-6). The most downfield signal at δ 170.6 is assignable to carbonyl ester at C-2.

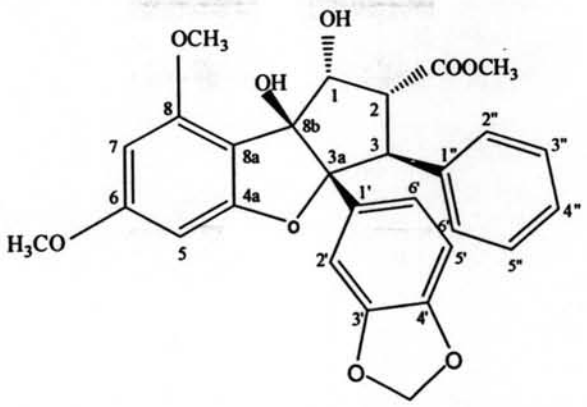
Long-range HMBC correlations (**Figures 168a-168b** and **Table 34**) from hydroxyl proton (1-OH) signal at δ 1.91 to C-1 (δ 79.6), and from H-3 signal at δ 4.35

to C-1′′ (δ 136.9), C-2′′ and C-6′′ (δ 127.8) established the connectivity between hydroxyl group at position 1 and the aromatic ring at position 3. HMBC correlation can also be observed between H-3 and C-1′ (δ 128.3) indicating another aromatic ring at C-3a position. Substitution of two methoxyl groups substitutions at C-6 and C-8, and methylenedioxy group at C-3′ and C-4′ are also confirmed by HMBC experiment.

To determine the relative configuration, the NOESY experiment (**Figure 169**) was performed. The 2D spectrum exhibited the correlation between H-1 and H-2, therefore indicated those protons as on the same side. The coupling constant between the signals of H-1 and H-2 ($J_{1,2}$ = 6.7 Hz) and of H-2 and H-3 ($J_{2,3}$ = 14.2 Hz) in the proton spectrum confirmed that H-1 is on the same side as H-2 and on the opposite side to H-3. The configuration around C-1, C-2, C-3, C-3a, and C-8b in compound AE-10 was also established on the basis of similar chemical shifts and vicinal coupling constant values to those of methyl rocaglate (Cui *et al.*, 1997).

Through analysis of the above spectral data and comparison with literature (Cui *et al.*, 1997), compound AE-10 was identified as a flavagline derivative named 4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate, which has previously been isolated from several *Aglaia* species e.g. *Aglaia elliptica* (stems and fruits) (Cui *et al.*, 1997), *Aglaia dasyclada* (leaves) (Chaidir *et al.*, 2001) and *Aglaia spectabilis* (bark) (Schneider *et al.*, 2000).

This flavagline, isolated from *Aglaia elliptica*, showed very potent cytotoxicity against the U373 (human glioblastoma) and BC1 (human breast cancer) cell lines, with ED_{50} values of 0.0008 and 0.0009 µg/ml, respectively (Cui *et al.*, 1997; Lee *et al.*, 1998). The compound isolated from the bark of *Aglaia spectabilis*, exhibited insecticidal activity toward neonate larvae of the polyphagous pest insect *Spodoptera littoralis* (Schneider *et al.*, 2000).



4'-Demethoxy-3',4'-methylenedioxy-methyl rocaglate (Compound AE-10)

Table 34. Comparison of NMR spectral data of 4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate and compound AE-10 ($CDCl_3$, 500 MHz)

Position	4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate*		Compound AE-10		
	^1H	^{13}C	^1H	^{13}C	HMBC
1	5.02 (1H, *d*, *J* = 6.6 Hz)	79.5	5.05 (1H, *d*, *J* = 6.7 Hz)	79.6	C-3, C-3a
2	3.92 (1H, *dd*, *J* = 6.6, 14.2 Hz)	50.3	3.94 (1H, *dd*, *J* = 6.7, 14.2 Hz)	50.5	C-1, COOCH3
3	4.33 (1H, *d*, *J* = 14.2 Hz)	55.7	4.35 (1H, *d*, *J* = 14.2 Hz)	55.1	C-2, C-3a, C-1′, C-1″, C-2″, C-6″
3a	-	101.8	-	102.0	
4a	-	160.6	-	160.8	
5	6.30 (1H, *d*, *J* = 2.0 Hz)	89.4	6.32 (1H, *d*, *J* = 1.9 Hz)	89.5	C-4a, C-8a
6	-	164.0	-	164.2	
7	6.14 (1H, *d*, *J* = 2.0 Hz)	92.7	6.17 (1H, *d*, *J* = 1.9 Hz)	92.8	C-6, C-8a
8	-	156.9	-	157.1	
8a	-	107.5	-	107.6	
8b	-	93.7	-	93.8	
1′	-	128.1	-	128.3	
2′	6.72 (1H, *d*, *J* = 1.8 Hz)	108.6	6.75 (1H, *d*, *J* = 1.7 Hz)	108.7	C-3′, C-6′
3′	-	146.5	-	146.7	
4′	-	146.8	-	147.0	
5′	6.61 (1H, *d*, *J* = 8.8 Hz)	107.4	6.64 (1H, *d*, *J* = 8.2 Hz)	107.2	C-1′, C-4′
6′	6.70 (1H, *dd*, *J* = 1.8, 8.8 Hz)	121.3	6.72 (1H, *dd*, *J* = 1.7, 8.2 Hz)	121.4	C-2′

Table 34. Comparison of NMR spectral data of 4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate and compound AE-10 ($CDCl_3$, 500 MHz) (continued)

Position	4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate		Compound AE-10		
	^1H*	^{13}C**	^1H	^{13}C	HMBC
1''	-	136.7	-	136.9	
2''	6.94 (1H, *m*)	127.7	6.95 (1H, *dd*, *J* = 2.0, 7.7 Hz)	127.8	C-3
3''	7.11 (1H, *m*)	127.7	7.14 (1H, *m*)	127.8	
4''	7.11 (1H, *m*)	126.6	7.13 (1H, *m*)	126.7	C-2'', C-6''
5''	7.11 (1H, *m*)	127.7	7.14 (1H, *m*)	127.8	
6''	6.94 (1H, *m*)	127.7	6.95 (1H, *dd*, *J* = 2.0, 7.7 Hz)	127.8	C-3
6-OCH_3	3.89 (3H, *s*)	55.7	3.89 (3H, *s*)	55.8	C-6
8-OCH_3	3.85 (3H, *s*)	55.0	3.92 (3H, *s*)	55.8	C-8
1-OH	1.96 (1H, *br s*)	-	1.91 (1H, *br s*)	-	C-1
$\underline{C}OOCH_3$	-	170.4	-	170.6	
$COO\underline{C}H_3$	3.67 (3H, *s*)	52.0	3.69 (3H, *s*)	52.0	$\underline{C}OOCH_3$
O-$\underline{CH_2}$-O	5.86 (2H, *s*)	100.8	5.89 (2H, *s*)	100.9	C-3', C-4'

* Cui *et al.*, 1997 ($CDCl_3$, 300 MHz)

** Cui *et al.*, 1997 ($CDCl_3$, 125 MHz)

3. Bioactivity Evaluation of Compounds Isolated from *Chisocheton penduliflorus* and cf. *Aglaia erythrosperma*

In the search for biologically active constituents of *Chisocheton penduliflorus* and cf. *Aglaia erythrosperma*, the hexane, $CHCl_3$ and MeOH extracts of the leaves, wood and stems bark of *C. penduliflorus*, as well as the leaves, pericarp and seeds of cf. *Aglaia erythrosperma*, were subjected to *in vitro* screening for their activity against three cancer cell lines (KB, BC and NCI-H187), antimalarial activity against *Plasmodium falciparum*, antituberculosis activity against *Mycobacterium tuberculosis* and anti-herpes simplex virus type 1 activity.

3.1 Bioactive Compounds from *Chisocheton penduliflorus*

The hexane extract of *C. penduliflorus* leaves displayed antituberculosis activity with MIC value of 200 μg/ml and also showed weak anti HSV-1 activity, whereas the $CHCl_3$ extract of the leaves was weakly active against BC cell line with IC_{50} value of 12.10 μg/ml and displayed antituberculosis activity with MIC value of 100 μg/ml. The hexane extract of *C. penduliflorus* wood was weakly active against BC cell line with IC_{50} value of 11.10 μg/ml and displayed antituberculosis activity with MIC value of 50 μg/ml. The $CHCl_3$ extract of the wood was weakly active against BC cell line with IC_{50} value of 11.25 μg/ml and was moderately active against NCI-H187 cell line with IC_{50} value of 9.85 μg/ml. It also displayed antituberculosis activity with MIC value of 25 μg/ml. In addition, the $CHCl_3$ extract of *C. penduliflorus* stem bark was weakly active against BC cell line with IC_{50} value 10.50 μg/ml. This extract also exhibited antituberculosis activity with MIC value of 25 μg/ml.

Subsequent extraction of the hexane leaf extract led to the isolation of a hexanortriterpenoid, hollongdione, and a dammarane triterpenoid, dammaradienone. Another dammarane triterpenoid, cabraleadiol, was isolated from the $CHCl_3$ extracts of the leaves, wood and stem bark and from the hexane wood extract. Finally, three aromadendrane sesquiterpenoids, 14-hydroxyviridiflorol, (-)-10*β*,13,14-trihydroxy-*allo*-aromadendrane and 14-hydroxyepiviridiflorol in a 2:3 mixture with 14-hydroxyviridiflorol) and three other dammarane triterpenoids, eichlerialactone, cabralealactone and cabraleahydroxylactone, were isolated from the $CHCl_3$ extract of the wood. All compounds were evaluated for their biological activities, the results of which are summarized in **Table 35**.

Table 35. Bioactivities of compounds isolated from *Chisocheton penduliflorus*

Compound	Anti HSV-1 IC_{50} (μg/ml)	Anti TB MIC (μg/ml)	Cytotoxicity IC_{50} (μg/ml)			
			NCI-H187	KB	BC	Vero cell
Hollongdione (CP-1)	inactive	inactive	inactive	inactive	inactive	>50
Dammaradienone (CP-2)	inactive	200	inactive	inactive	inactive	>50
Cabraleadiol (CP-3)	inactive	50	inactive	inactive	17.51	>50
14-Hydroxyviridiflorol (CP-4)	ND	100	inactive	inactive	inactive	>50
mixture of 14-hydroxy-viridiflorol and 14-hydroxyepiviridiflorol (CP-5)	ND	50	inactive	inactive	inactive	ND
Eichlerialactone (CP-6)	inactive	25	inactive	inactive	12.52	>50
Cabralealactone (CP-7)	inactive	50	7.64	inactive	16.92	>50
Cabraleahydroxylactone (CP-8)	3.2	50	inactive	inactive	18.01	>50
(-)-10*β*,13,14-Trihydroxy-*allo*-aromadendrane (CP-13)	ND	50	inactive	inactive	inactive	ND
Rifampicin	-	0.0047	-	-	-	-
Kanamycin sulfate	-	2.5	-	-	-	-
Isoniazid	-	0.05	-	-	-	-
Ellipticine	-	-	0.606	0.413	0.125	-
Doxorubicin	-	-	0.026	0.103	0.127	-
Acyclovir	0.9-1.9	-	-	-	-	-

ND = not determined

3.1.1 Cytotoxic activity

Most of the isolated dammarane-type triterpenoids, i.e. cabraleadiol, eichlerialactone, cabralealactone and cabraleahydroxylactone, exhibited weak cytotoxicity against BC cell line with ICs_{50} values of 17.51, 12.52, 16.92 and 18.01 μg/ml, respectively. Only cabralealactone displayed moderate cytotoxicity against NCI-H187 cell line with IC_{50} value of 7.64 μg/ml, suggesting the importance of carbonyl group at C-3 and carbonyl ester of γ-lactone ring substitution for this activity.

14-Hydroxyviridiflorol, which has been reported as displaying antineoplastic activity against human larynx carcinoma (Hep_2) cells with IC_{50} value of 11.6 μg/ml (Matos *et al.*, 2006), was inactive against the three cancer cell lines used in this study.

3.1.2 Antituberculosis activity

Dammaradienone, cabraleadiol, 14-hydroxyviridiflorol and a mixture of this sesquiterpenoid with 14-hydroxyepiviridiflorol, eichlerialactone, cabralealactone, cabraleahydroxylactone and (-)-10*β*,13,14-trihydroxy-*allo*-aromadendrane displayed antituberculosis activity against *Mycobacterium tuberculosis* $H_{37}Ra$ with MIC values of 200, 50, 100, 50, 25, 50, 50 and 50 μg/ml, respectively.

3.1.3 Anti HSV-1 activity

In the case of the hexane extract of this plant leaves, a dammarane triterpenoid, dammaradienone, represented the anti HSV-1 constituent of the extract. It exhibited weak anti HSV-1 activity.

Although only the hexane leaf extract showed weak anti HSV-1 activity, when other isolated constituents were subjected to the assay, cabraleahydroxylactone was shown to be against active HSV-1 virus with IC_{50} of 3.2 μg/ml.

3.2 Bioactive compounds from cf. *Aglaia erythrosperma*

Both the hexane and $CHCl_3$ extracts of the pericarp and seeds of cf. *Aglaia erythrosperma* were strongly active against NCI-H187 cell line with ICs_{50} values of 4.30, 1.27, 2.09 and 0.02 μg/ml, respectively, whereas the MeOH extract of the seeds and both the hexane and $CHCl_3$ extracts of leaves were moderately active against NCI-H187 cell line with IC_{50} values of 5.08, 5.85 and 6.11 μg/ml, respectively. The $CHCl_3$ pericarp extract and both the hexane and $CHCl_3$ seed extracts were strongly active against BC cell line with IC_{50} values of 2.30, 1.45 and 0.21 μg/ml, respectively. However, the $CHCl_3$ leaf extract was only weakly active against BC cell line (IC_{50} = 14.42 μg/ml). The $CHCl_3$ pericarp extract was moderately active against KB cell line (IC_{50} = 7.02 μg/ml), while both the hexane and $CHCl_3$ seed extracts were weakly (IC_{50} = 14.05 μg/ml) and strongly active against KB cell line (IC_{50} = 1.31 μg/ml), respectively. Both the hexane and $CHCl_3$ extracts of the leaves and seeds and the hexane pericarp extract showed antituberculosis activity with MIC values of 6.25, 25, 200, 50 and 100 μg/ml, respectively. In addition, both the hexane and $CHCl_3$ leaf extracts also exhibited anti HSV-1 activity with IC_{50} values of 3.9 and 12.1 μg/ml, respectively, whereas the $CHCl_3$ pericarp extract and both the $CHCl_3$ and MeOH seed extracts were moderately active against HSV-1. Only the $CHCl_3$ seed extract displayed antimalarial activity with IC_{50} value of 0.11 μg/ml.

Chemical investigation of the $CHCl_3$ leaf extract led to the isolation of 5,6-desmethylenedioxy-5-methoxy-aglalactone. Two 3,4-secodammarane triterpenoids, ethyl eichlerianoate and aglinin A, were isolated from the hexane and $CHCl_3$ pericarp extracts, respectively. The $CHCl_3$ and MeOH seed extracts afforded a flavagline derivative, 4′-demethoxy-3′,4′-methylenedioxy-methyl rocaglate. The results of the bioactivity evaluation of these isolated compounds are summarized in **Table 36**.

3.2.1 Cytotoxicity activity

Flavagline derivatives have been reported as exhibiting cytotoxicity against several cancer cell lines The flavagline 4′-demethoxy-3′,4′-methylenedioxy-methyl rocaglate has been reported potent by cytotoxic against U373 (human glioblastoma) and BC1 (human breast cancer) cell lines, with ED_{50} values of 0.0008 and 0.0009 μg/ml, respectively (Cui *et al.*, 1997; Lee *et al.*, 1998). 4′-Demethoxy-3′,4′-methylenedioxy-methyl rocaglate represents the cytotoxic constituent of the $CHCl_3$ extract of this plant seeds.

Table 36. Bioactivities of compounds isolated from cf. *Aglaia erythrosperma*

Compound	Anti HSV-1 IC_{50} (µg/ml)	Anti TB MIC (µg/ml)	Antimalarial activity EC_{50} (µg/ml)	Cytotoxicity IC_{50} (µg/ml)			
				NCI-H187	KB	BC	Vero cell
5,6-Desmethylenedioxy-5-methoxy-aglalactone (AE-4)	moderately active	25	inactive	7.22	inactive	14.37	>50
Ethyl eichlerianoate (AE-5)	inactive	25	inactive	5.10	inactive	inactive	>50
Aglinin A (AE-7)	moderately active	inactive	inactive	5.25	8.72	6.75	>50
4′-Demethoxy-3′,4′-methylenedioxy-methyl rocaglate (AE-10)	moderately active	50	7.30	2.17	2.10	0.11	1.5
Rifampicin	-	0.0047	-	-	-	-	-
Kanamycin sulfate	-	2.5	-	-	-	-	-
Isoniazid	-	0.05	-	-	-	-	-
Ellipticine	-	-	-	0.606	0.413	0.134	-
Doxorubicin	-	-	-	0.026	0.103	0.140	-
Dihydroartemisin	-	-	0.0043	-	-	-	-
Acyclovir	0.9-1.9	-	-	-	-	-	-

However, judging from the stronger activity of the extract, there might be some other active constituents not isolated in this study. The flavagline displayed strong cytotoxic activity against NCI-H187, KB and BC cell lines (IC_{50} = 2.17, 2.10 and 0.11 μg/ml, respectively). However, it was also cytotoxic against Vero cells (IC_{50} = 1.5 μg/ml), therefore its prospect for use as an anticancer agent might be limited.

5,6-Desmethylenedioxy-5-methoxy-aglalactone displayed moderate cytotoxic activity against NCI-H187 cell line (IC_{50} = 7.22 μg/ml) and weak cytotoxic activity against BC cell line (IC_{50} = 14.37 μg/ml).

The 3,4-secodammarane triterpenoid, ethyl eichlerianoate, exhibited moderate cytotoxic activity against NCI-H187 cell line (IC_{50} = 5.10 μg/ml), whereas another similar triterpenoid, aglinin A, was also moderate by active against NCI-H187, KB and BC cell lines (ICs_{50} = 5.25, 8.72 and 6.75 μg/ml, respectively).

The structures of ethyl eichlerianoate, aglinin A and eichlerialactone are rather similar except for the different substitution at C-24 and carboxyl group at C-3. For aglinin A, a hydroxy group and carboxylic acid are located at C-24 and C-3, respectively, while a hydrogen and an ethyl ester group are placed at C-24 and C-3, respectively in ethyl eichlerianoate. For eichlerialactone, carboxylic acid group and carbonyl ester of the γ-lactone ring are located at C-3 and C-24, respectively, suggesting the importance of hydroxyl at C-24 and carboxylic acid at C-3 substitution for this activity.

3.2.2 Antituberculosis activity

Nearly all the compounds (except aglinin A) isolated from this plant and subjected to this assay were found to be more or less active. Ethyl eichlerianoate exhibited antituberculosis activity with MIC value of 25 μg/ml while aglinin A was inactive, indicating the effect of the C-24 and C-3 substitutions on this activity. 5,6-Desmethylenedioxy-5-methoxy-aglalactone and 4′-demethoxy-3′,4′-methylenedioxy-methyl rocaglate also displayed antituberculosis activity with MIC values of 25 and 50 μg/ml, respectively.

3.2.3 Anti HSV-1 activity

5,6-Desmethylenedioxy-5-methoxy-aglalactone, aglinin A and 4′-demethoxy-3′,4′-methylenedioxy-methyl rocaglate showed moderate anti HSV-1 activity.

3.2.4 Antimalarial activity

4′-Demethoxy-3′,4′-methylenedioxy-methyl rocaglate displayed antimalarial activity with EC_{50} value of 7.3 μg/ml. This flavagline represented the antimalarial constituent of the the $CHCl_3$ extract of this plant seeds. However, judging from the stronger activity of the extract, there might be some other active constituents not isolated in this study.