CHAPTER IV

RESULT AND DISCUSSION

During the investigation of the chemical constituents of the leaves of *Aglaia chittagonga* Miq., three compounds ACH1, ACH2 ,and ACH3, were isolated from the hexane extract. These compounds gave positive results with Liebermann Burchard test, suggesting their terpenoid nature.

The identification and structure elucidation of the compounds were based on the interpretation of their spectral data, and further confirmed by comparision with those reported values in the literature.

Compound ACH1

Compound ACH1 was crystallized as white needles from methanol. Its IR spectrum (Figure 8) exhibited a broad band at 3464cm $^{-1}$, indicating the presence of hydroxyl group in the molecule.

The ^{1}H NMR (Figure10-11) spectrum of ACH1 is too complex to allow direct assignment of most methylene and methine chemical shifts in the structure. Experiment indicated the signal of 7 methyl protons at δ 0.70, 0.75, 0.8, 0.9, 0.95,(6H), and 1.0 ppm. The presence of downfield singlet of exomethylene proton at δ 4.5 and 4.7 ppm.

The ^{13}C NMR spectrum (Figure12-13) showed the signals of 30 carbons atom, supporting the assignment of ACH1 as a triterpenoid derivative. DEPT (Figure14) experiment indicated the signals of 7 methyl carbons at δ 14.7, 15.5, 16.1, 16.3, 18.1, 19.4, and 28.1 ppm, 11 methylene carbons at δ 18.5, 21.0, 25.3, 27.5, 27.6, 29.9, 34.4, 35.6, 38.8, 40.1, and 109.2 ppm, 6 methine carbons at δ 38.1, 48.4, 48.1, 50.5, 55.3, and 79.0 ppm, and

6 Quaternary carbons at δ 37.3, 38.9, 40.9, 42.9, 43.1 and 150.8 ppm.

The EIMS of ACH1 (Figure 9) displayed a prominent molecular ion peak at *m/z* 426 ($C_{30}H_{50}O$). Intense EIMS fragment peaks at *m/z* 189, 191,and 218 were important in showing ACH1 as having a skeleton structure of the lupane-type triterpenoid.(Budzikiewicz *et al.*, 1964 ; Ogunkoya, 1981) (Figure 3)

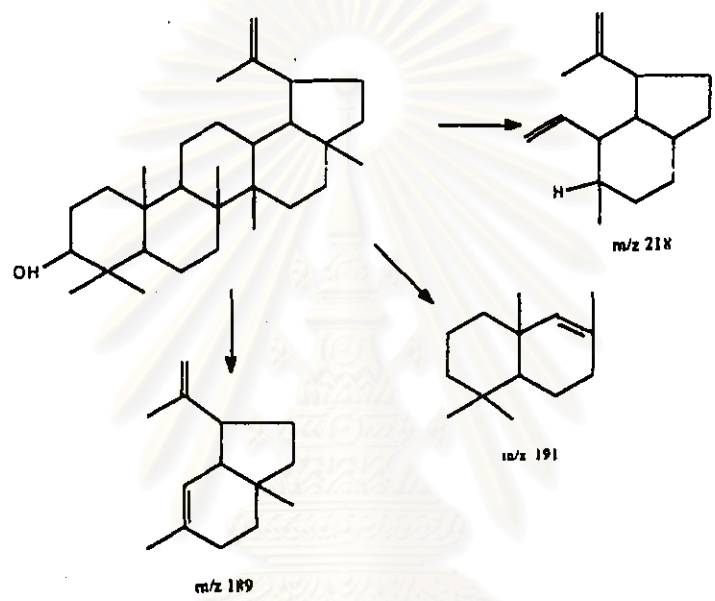


Figure 3 Mass fragmentation of ACH1

Therefore, the identity of ACH3 was mainly deduced by comparison of its carbon chemical shifts with those of a known triterpenoid, lupeol (Sholichin *et al.*, 1980).

The 7 methyl carbon signals at 28.1, 15.5, 16.3, 16.1, 14.7, 18.1, and 6.0 ppm could then be assigned as those of C-23, C-24, C-25, C-26, C-27, C-28, and C-29 position, respectively, whereas the 11 methylene carbon signals at δ 38.8, 27.6, 18.5, 34.4, 21.0, 25.2, 27.5, 35.6, 29.9, 40.0, and 109.2 ppm were assigned as those of C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-21, C-22 and C-30 position, respectivetively. Similarly, the 6 methine

carbon signal at δ 79.0, 55.3, 50.5, 38.1, 48.1 and 48.4 ppm were assigned as those of C-3, C-5, C-9, C-13, C-18 and C-19, respectively, and the 6 quaternary carbon signals at δ 38.9, 40.9, 37.3, 42.9, 43.1 and 150.8 ppm could then be assigned as those of C-4, C-8, C-10, C-14, C-17 and C-20, respectively.

The complete carbon chemical shift assignment of ACH1 was found to be fully in agreement with those of lupeol (Table 6).

Table 6 Comparison of ^{13}C-NMR chemical shifts of ACH1 and lupeol

position	ACH1	lupeol	position	ACH1	lupeol
1	38.8	38.7	16	35.6	35.6
2	27.6	27.5	17	43.1	43.0
3	79.0	79.0	18	48.1	48.0
4	38.9	38.9	19	48.4	48.3
5	55.3	55.3	20	150.8	150.9
6	18.5	18.3	21	29.9	29.9
7	34.4	34.3	22	40.1	40.0
8	40.9	40.9	23	28.1	28.0
9	50.5	50.5	24	15.5	15.3
10	37.3	37.2	25	16.3	16.1
11	21.0	21.0	26	16.1	16.0
12	25.2	25.2	27	14.7	14.6
13	38.1	38.1	28	18.1	18.0
14	42.9	42.9	29	19.4	19.3
15	27.5	27.5	30	109.2	109.3

* Solvent $CDCl_3$; NMR Spectra Measurements : A JEOL PFT-100

Therefore, it was concluded that ACH1 is lupeol, the structure of which is shown below.

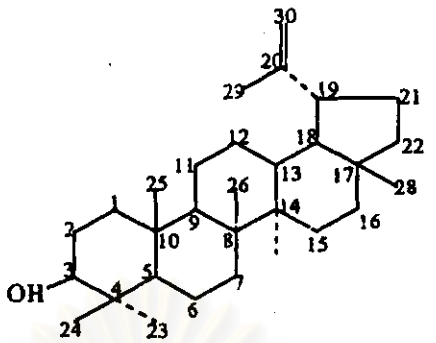


Figure 4 The structure of ACH1 (MW. 426)

Lupeol is a known terpenoid previously isolated from several species of meliaceous plants, ie. *Guarea trichilioides* (Furlan , Roque and Filho, 1993) , *Trichilia claussenii* (Pupo *et al* .,1996) and *Aglaia harmsiana* (Inada *et al.*,1995). Previous pharmacological studies of lupeol revealed antimalarial (Alves *et al.*,1997), antitumor (Moriarity *et al.*, 1998 ; Miles *et al.*,1976), and anti inflammatory activities (Geetha *et al.*, 1998 ; Akihisa *et al.*,1996).

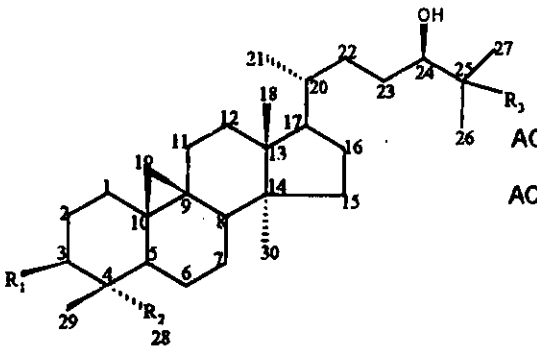
Compound ACH2

Compound ACH2 was crystallized as white needles from methanol. The molecular formular of $C_{31}H_{54}O_3$ was suggested for this compound based on its EIMS molecular ion peak at *m/z* 474, and the $[M\text{-}OH]^+$,$[M\text{-}H_2O]^+$,$[M\text{-}OH\text{-}CH_3]^+$, $[M\text{-}H_2O\text{-}CH_3]^+$ at *m/z* 457, 456, 442, 441, respectively. (Figure 15)

The presence of hydroxyl groups in the molecule was also confirmed by a very intense IR absorption band at 3470 $cm.^{-1}$ (Figure 16)

The ^1H NMR spectrum of ACH2 (Figure19) showed a pair of upfield thylene proton doublets at δ 0.31 and 0.53 ppm (1H each, J= 4 Hz). These two doublets are characteristic of non- equivalent protons of a cyclopropyl methylene group in the cycloartane skeleton. (Inada *et al.*,1997) .

The complete ^{13}C-NMR chemical shift assignments of ACH2 were shown in Table 6.



(75) R_1 =α-OH, β-H; R_2 =CH_3; R_3 = OH

(76) R_1 =β-OH, α-H; R_2 =CH_3; R_3 = OH

ACH2 R_1 =β-OH, α-H; R_2 = CH_3; R_3 = OCH_3

ACH3 R_1 =β-OH, α-H; R_2 =H; R_3= OCH_3

Figure 5 Structure of (24R)- cycloartane -3α, 24, 25-triol (75), (24R)-cycloartane-3β, 24, 25-triol (76) , ACH2 and ACH3

Table 7 The comparison of ^{13}C-NMR spectral data

Position	75	76	ACH2	ACH3	Position	75	76	ACH2	ACH3
1	27.5	32	30.2	30.8	16	26.3	26.5	26.5	25.2
2	28.8	30.4	30.4	34.8	17	52.3	52.4	52.4	52.3
3	77.1	78.9	78.8	76.6	18	18.0	18.1	18.1	17.8
4	39.6	40.5	40.5	44.6	19	29.8	29.9	29.9	27.3
5	41.1	47.2	47.1	43.3	20	36.4	36.4	36.4	36.4
6	21.1	21.1	21.1	24.6	21	18.5	18.5	18.4	18.4
7	28.1	28.2	28.1	28.1	22	33.6	33.6	33.7	33.7
8	48.1	48.0	40.8	46.9	23	28.6	28.8	28.3	28.3
9	19.9	20.0	20.0	23.5	24	79.7	79.7	77.6	77.6
10	26.5	26.1	26.1	29.5	25	73.2	73.2	77.6	77.6
11	26.3	26.0	26.0	26.9	26	23.3	23.3	18.8	18.8
12	33.6	33.0	32.9	32.9	27	26.6	26.6	20.8	20.8
13	45.3	45.3	45.3	45.3	28	25.9	25.5	25.4	-
14	48.9	48.8	48.8	48.9	29	21.3	14.0	14.0	14.4
15	35.5	35.6	35.5	35.3	30	19.3	19.4	19.3	19.1
					OCH_3			49.0	49.0

The ^{1}H NMR spectrum of ACH2 also showed the presence of eight methyl protons at δ 0.78 (s), 0.86 (d,*J*= 6 Hz), 0.87 (s), 0.94 (6H,s), 1.07 (s), 1.11 (s) and 3.2 (s).

The ^{13}C-NMR (Figure23) and DEPT(Figure24-25) spectra of compound ACH2 exhibited the signal 7 methyl carbons at δ 18.1, 18.4 , 18.8, 20.8, 25.4, 14 , 19.3 and one methoxyl carbon at δ 49.0 ppm, 11 methylene carbons at δ 32, 30.4, 21.1, 28.1, 26.0, 32.9, 35.5, 26.5, 29.9, 33.7, and 28.3 ppm, 6 methine carbons at δ 78.8, 47.1, 48.0, 52.4, 36.4 and 77.6ppm, and 6 quaternary carbons at δ 40.5, 20.0, 26.1, 45.3, 48.8 and 77.6 ppm .

The elucidation of ACH2 structure was chiefly done by comparison of ^{13}C NMR chemical shifts of this triterpenoid with those corresponding signals of (24R)- cycloartane -3α, 24, 25-triol (75) and (24R)-cycloartane-3β, 24, 25-triol (76) (Inada *et al.*, 1997b), both of which possess the same basic structure as ACH2. Methine proton signals of H-3 and H-24 at δ 3.26 (dd,*J*= 7.8, 4.4 Hz) and 3.34 (dd,*J*= 10, 1.83 Hz), respectively, indicated the orientation of hydroxyl groups at both position to be beta.

The structure of ACH2 was also confirmed by the HMBC experiment (Figure 34-39). The Me-29 proton signal at δ 0.79 ppm displayed three-bond correlations with C-28 (δ 25.4 ppm), C-5 (δ 47.1 ppm), and C-3 (δ78.8 ppm), confirming its position as attached at C-4. Another methyl proton signal at δ 0.95 ppm (Me-28) showed three-bond coupling with C-29 (δ 14.0 ppm), C-5 (δ 47.1 ppm), and C-3 (δ 78.8 ppm), indicating its attachment as at C-4 position. The Me-18 proton signal at δ 0.95 ppm displayed three-bond correlation with C-12 (δ 32.9 ppm), C-14 (δ 48.8 ppm), and C-17 (δ 52.4 ppm) confirming its position as attached at C-13. Correlations could also be observed between Me-30 signal at δ 0.87 ppm and C-8

(δ 48.0), C-13 (δ 45.3 ppm), C-15 (δ 35.5 ppm), as well as between Me-26 (δ 1.07ppm) and Me-27(δ 1.11ppm) proton signals and C-24 (δ 77.6 ppm), confirming the assignments of all these positions. The long-range coupling of Me-21 proton signal at δ 0.86 ppm with C-17 (δ 52.4 ppm) and C-22 (δ 33.7ppm) indicated the position of this methyl group at C-20 in side-chain. The methoxyl proton at δ 3.2 ppm showed long -rang coupling with C-25. Intense EIMS peak at *m/z* 73 , which corresponded to the formation of $[(CH_3)_2 C=^{+}OCH_3]$ fragment., confirming the presence of methoxyl group at C-25 in side-chain.

A set of major cross-peaks observed between the cyclopropyl methylene proton signals at δ 0.31 and (H-19) 0.53 ppm (H-19) and C-1 (δ 32.0 ppm), C-5(δ 47.1ppm), C-8(δ 48.0ppm), C-11 (δ 26.0ppm) indicated that the position of the cyclopropane ring was between C-9 and C-10.

Therefore it was concluded that ACH2 is a cycloartane – type triterpenoid with a β-hydroxyl group at C-3 in ring A and 24β-hydroxyl , 25 methoxyl in side-chain (25 – Methoxylcycloartane - 3β, 24 - diol). (Figure 6)

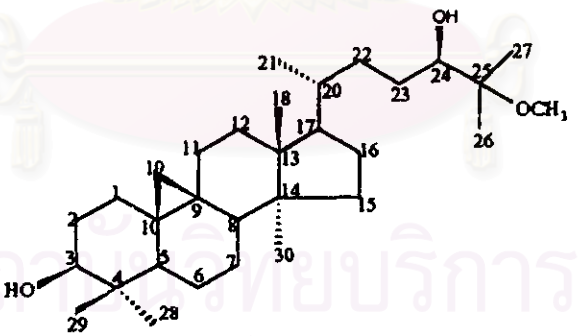


Figure 6 25- Methoxylcycloartane - 3β, 24 - diol

Compound ACH3

Compound ACH3 was crystallized as white needles from methanol. The molecular formular of $C_{30}H_{52}O_3$ was suggested for this compound based on EIMS molecular ion peak at *m/z* 460, and the $[M-OH]^+$, $[M-H_2O]^+$, $[M-OH-CH_3]^+$,

$[M-H_2O-CH_3]^+$ at *m/z* 443, 442, 428, 427, respectively. (Figure 40)

The presence of hydroxyl groups in the molecule was also confirmed by a very intense IR absorption band at 3402 $cm.^{-1}$(Figure 41)

The ^1H NMR spectrum of ACH3 (Figure 43) showed a pair of upfield methylene proton doublets at δ 0.12 and 0.36 ppm (1H each, *J*= 4 Hz). These two doublets are characteristic of non- equivalent protons of a cyclopropyl methylene group in the cycloartane skeleton. (Inada *et al.*,1997) The ^1H NMR spectrum of ACH3 also showed the presence of seven methyl protons at δ 0.87(d,*J*= 6Hz), 0.87 (s), 0.95 (s), 0.96 (d,*J*= 6Hz), 1.07(s), 1.1(s), and 3.2(s).

The ^{13}C-NMR (Figure 48) and DEPT (Figure 49-50) spectra of compound ACH3 exhibited the signals of 6 methyl carbons at δ 14.5, 17.8, 18.4, 18.9, 19.1, 20.8, and one methoxyl carbon at δ 49.0 ppm, 11 methylene carbons at δ 24.6, 25.2, 26.9, 27.3, 28.1, 28.3, 30.8, 32.9, 33.7, 34.8, and 35.3 ppm , 7methine carbons at δ 36.4, 43.3 , 44.6, 46.9, 52.3, 76.6, and 77.6 ppm and 5 quatemary carbons at δ 23.5, 29.5, 45.3, 48.9, and 77.6 ppm. When compared to ACH2, the compound ACH3 exhibited molecular ion peak at *m/z* 460 (14 mass unit less than ACH2) and one more methyl carbon while showing one less methine and quatemary carbon, (Table 7). In addition, the elucidation of the struture of ACH3 was mainly accomplished by comparison of the ^{13}C NMR chemical shift data (Table 7), those of ACH3.

Table 8 Comparison of ACH2 and ACH3

Character	ACH2	ACH3
Molecular weight	474	460
Methyl carbon	8	7
Methylene carbon	11	11
Methine carbon	6	7
Quartemary carbon	6	5

The structure of ACH3 was also confirmed by the HMBC experiment. (Figure 58-62) The Me-29 proton signals at δ 0.96 ppm displayed three-bond correlations with both C-3 (δ 76.6 ppm) and C-5 (δ 44.3 ppm), confirming its position as attached at C-4. Another methyl proton signal at δ 0.95 ppm (Me-18) showed three- bond coupling with C-12 (δ 32.9ppm), C-14 (δ 48.9ppm), and C-17 (δ 52.3 ppm), indicating its attachment as at C-13 position. Correlations could alsi be observed between Me-30 signal at δ 0.87 ppm and C-8 (δ 46.9), C-13 (δ 45.3 ppm), C-15 (δ 35.3ppm), as well as Me-26 (δ 1.07ppm) and Me-27 (δ 1.11ppm) proton signals and C-24 (δ 77.6 ppm), confirming the assignments of all these positions. The long-range coupling of Me-21 proton signal at δ 0.87 ppm with C-17(δ 52.3ppm) and C-22 (δ 33.7ppm) indicated the position of this methyl group at C-20 in side-chain. The methoxyl proton at δ 3.2ppm (OCH_3) showed long-range coupling with C- 25 and intense EIMS peak at *m/z* 73, which corresponded to the formation of [$(CH_3)_2C=^{+}OCH_3$] fragment, confirming the presence of methoxyl group at C-25 in side-chain.

A set of major cross-peaks observed between the cyclopropyl methylene proton signals at δ 0.12 and 0.36 ppm (H-19) and C-1(δ30.8 ppm), C-5(δ 43.3ppm), C-8(δ 46.9ppm), C-11(δ 26.9ppm) indicated that the position of the cyclopropane ring was between C-9 and C-10 .

Therefore it was concluded that the structure ACH3 loss Me-28 in a cycloartane skeleton with a β-hydroxyl group at position-3 and 24β hydroxyl 25 methoxyl groups in the side - chain (25- Methoxyl-28-norcycloartane- 3β, 24 – diol). (Figure 7)

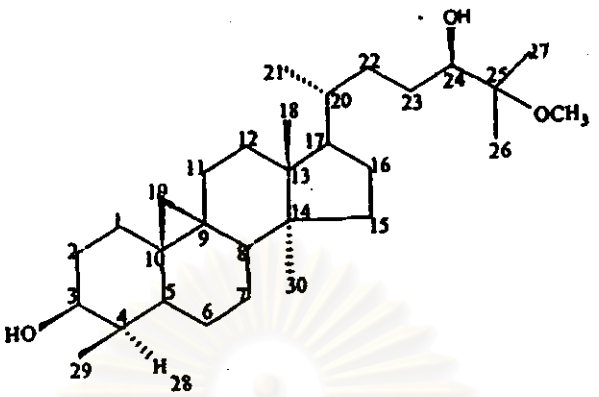


Figure 7 25- Methoxyl-28-norcycloartane- 3β, 24 – diol