CHAPTER IV

RESULT AND DISCUSSION

Dried ground wood of *Artocarpus altilis* (Park.) Fosb. (4 kg) was macerated with 95% ethanol. The ethanol extract was then partitioned according to the process shown in previous chapter. Three compounds, codenamed AA-1, AA-2, and AA-3, were isolated from the methanol fraction. Srtucture elucidation of these compounds will be discussed in this chapter.

1. Structure Elucidation of AA-1

AA-1 was obtained as yellow amorphous powder from F-01 (Table 10) The EIMS of AA-1 (Figure 2) revealed the molecular ion peak at *m/z* 418 (48%), which analysed for the molecular formula of $C_{25}H_{22}O_6$. The IR spectrum of AA-1 (Figure 3) disclosed the maximum absorption bands due to hydroxyl (3508-3400 cm^{-1}), carbonyl of α,β unsaturated ketone (1655, 1623 cm^{-1}), ether linkage (1260 cm^{-1}), and conjugated carbon moieties (1556-1467 cm^{-1}).

The absorption maxima at 371 and 308 nm observed from UV spectrum (Figure 4), indicated that compound AA-1 had a flavone chromophore. Furthermore, the bathochromic shift of the UV spectrum in the presence of $AlCl_3$/HCl caused by the effect of an acid-stable hydroxy-keto complex (Markham, 1982), this was an indicator of 5-OH flavone.

Compound AA-1 was assigned as a known pyranoflavone, isocyclomorusin by analyses of its ^1H and ^{13}C-NMR spectra and comparison with the literature values.

The ^{1}H-NMR spectrum of AA-1 (Figure 5) displayed 1 hydroxyl proton at 13.26 ppm, 8 olefinic protons between 5.5-8 ppm, and 4 methyl protons at 1.45 (6H), 1.67 and 1.93 ppm. The 1 sp^3 methine carbons, 7 sp^2 carbons, 12 quaternary carbons, 1 carbonyl and 4 methyl groups were seen in ^{13}C-NMR (Figure 8) and the DEPT spectra (Figure 9-10).

The chelated hydroxyl proton of C-5 position showed up at δ 13.26 ppm in ^{1}H-NMR spectrum (Figure 6). The characteristic signals of two vinyl protons at δ 6.64 ppm (d, J = 10.1 Hz, H-14) and δ 5.75 ppm (d, J = 10.1 Hz, H-15) and a six-proton signal at 1.45 ppm (H-17, H-18) indicated the presence of a 2,2-dimethylchromene group (Sultanbawa and Surendrakumar, 1989). The singlet signals at 6.45 ppm (H-8) and 13.26 ppm (5-OH) suggested that substituents were at C-5, C-6 and C-7.

The doublet signal at 6.19 ppm coupled to another signal at 5.47 ppm with coupling constant of 9.5 Hz were assigned to H-9 and H-10, respectively. The three aromatic proton signals, at 7.69 ppm, 6.63 ppm, and 6.42 ppm,assigned to the protons located at C-6′, C-5′ and C-3′. These signal indicated the *ortho* coupling between H-5′ and H-6′ with coupling constant 8.6 Hz and *meta* coupling (or long-range coupling *via* w-coupling) of H-5′ and H-3′ with a coupling constant of 2.4 Hz.

With the aid of HMQC spectrum (Figure 11-13), the oxygenated methine carbon signal at 70.2 ppm was assigned to C-9. Because of the steric effect from this position, the upfield methyl group (δ 18.6 ppm) could be assigned to C-12 and the lowerfield one (δ 25.8 ppm) to C-13. According to the methyl protons of H-12 at 1.67 ppm and H-13 at 1.93 ppm could be assigned the signals at . These two methyl groups showed long-range coupling with coupling constant 1.2 Hz. The other protonated carbon were C-15 at 129.3, C-6′ at 126.3, C-10 at 122.0 ppm, C-14 at 115.7 ppm, C-5′ at 110.8, C-3′ at 106.1, C-8 at 95.7 ppm, ppm, C-17 and C-18 at 28.3 ppm.

The assignments of quaternary carbons of AA-1 were made from the ^{1}H-detected heteronuclear multiple bond coherence (HMBC) spectra (Figure 17-22) as well as their chemical shifts. The signal at 179.2 ppm was assigned to carbonyl carbon of C-4. The oxygen connected sp^3 carbon at 78.7 ppm was assigned to C-16, which also showed long-range correlation with H-15 (δ 5.75 ppm) (Figure 20). The other quaternary carbons assigned were summarized in Table 11.

The unambiguous proton and carbon assignments with long-range correlations between carbons and protons observed from HMBC spectrum and comparison with the data previously reported (Chen *et al.*, 1993) were summarized in Table 11. Thus, compound AA-1 was determined as isocyclomorusin.

Table 11. The carbon and proton assignments of AA-1 with long-range correlations observed in HMBC spectrum.

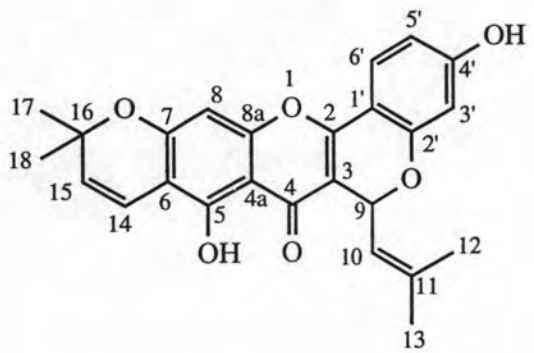
position	C, δ (ppm) Isocyclomorusin in DMSO-d_6	C, δ (ppm) in acetone-d_6 AA-1	H, δ (ppm) (multiplicity), *J* (Hz)	HMBC (^{1}H coupled)
1	-	-	-	-
2	157.9	157.3	-	H-6′, H-9
3	106.2	106.0	-	-
4	178.0	179.2	-	H-9
4a	104.8	104.8	-	5-OH, H-8
5	155.8	157.2	-	5-OH, H-8, H-14
6	108.3	108.3	-	-
7	163.9	159.9	-	H-14, H-8
8	95.4	95.7	6.45 (s)	-
8a	163.9	159.1	-	H-8
9	69.2	70.2	6.19 (d), 9.5	-
10	121.0	122.0	5.47 (br d), 9.5	H-12, H-13

Table 11. (continued)

position	C, δ (ppm) Isocyclomorusin in DMSO-d_6	C, δ (ppm) in acetone-d_6 AA-1	H, δ (ppm) (multiplicity), *J* (Hz)	HMBC (^{1}H coupled)
11	138.7	138.8	-	H-9, H-12, H-13
12	18.7	18.6	1.93 (d), 1.2	H-13
13	17.9	25.8	1.67 (d), 1.2	H-12
14	114.5	115.7	6.64 (d), 10.1	-
15	128.8	129.3	5.75 (d), 10.1	-
16	78.3	78.7	-	H-15
17	27.7	28.3	1.45 (s)	H-14, H-15
18	27.6	28.3	1.45 (s)	H-14, H-15
1′	110.2	109.9	-	H-9
2′	156.1	156.6	-	H-9
3′	103.7	106.1	6.42 (d), 2.4	-
4′	158.8	164.1	-	H-6′, H-3′
5′	110.2	110.8	6.63 (dd), 8.5, 2.4	-
6′	125.4	126.3	7.69 (d), 8.6	-
5-OH	-	-	13.26 (br s)	-

Note : The 4′-OH was not seen in the spectrum..

The structure of AA-1 is shown below



The structure of AA-1 was also confirmed by analyses of its mass fragmentation pattern in EIMS spectrum (Scheme 8). The base peak at *m/z* 403 [M^+-15] resulted from α-cleavage leading to loss of a methyl group.

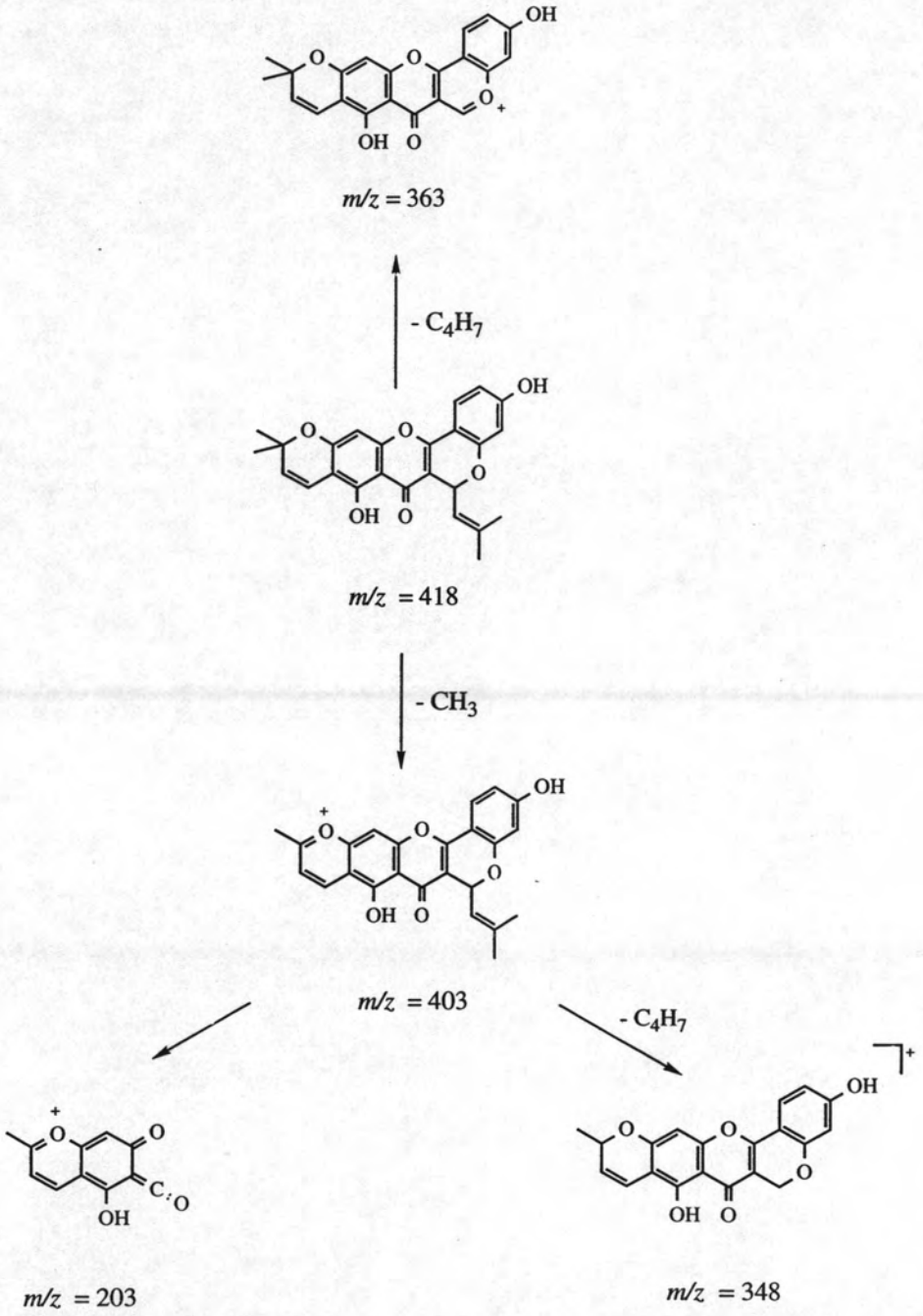
2. Structure Elucidation of AA-2

AA-2, obtained as pale yellow needles from acetone, was isolated from the same F-01 fraction as the previously mentioned flavone isocyclomorusin (AA-1).

The EIMS spectrum (Figure 23) exhibited the molecular ion $[M]^+$ peak at *m/z* 434 and established the molecular formula of this compound as $C_{26}H_{26}O_6$. Its IR spectrum (Figure 24) suggested the presence of hydroxyl group (3309 cm^{-1}), carbonyl group (1651, 1622 cm^{-1}), olefinic carbons (1583-1480 cm^{-1}), and ether linkage (1211 cm^{-1}).

The UV absorption bands at λ_{max} 368 nm (log ε 4.43) and 303 nm (log ε 3.90) represented the characteristic of a flavone chromophore. The bathochromic shift on UV spectrum in the presence of $AlCl_3$/HCl (Figure 25) was the result of an acid-stable formed complex between hydroxyl group and neighbouring carbonyl group (Markham, 1982).

Compound AA-2 was identified as a known prenylated flavone, cycloartocarpin, by analyses of its ^1H and ^{13}C-NMR spectra. Analysis of ^1H-NMR



Scheme 8 Proposed mass fragmentation of AA-1

spectrum (Figure 26) revealed the presence of signals of 2 hydroxyl groups, 7 olefinic protons, 2 aliphatic protons, 1 methoxyl group, and 4 methyl groups. The ^{13}C-NMR spectrum (Figure 29) and DEPT (Figure 30) suggested the presence of 1 carbonyl group, 11 quaternary carbons, 7 sp^2 carbons, 2 sp^3 methine carbons, 1 methoxyl group and 4 methyl groups.

The downfield region of its ^{1}H-NMR spectrum (Figure 26) revealed the presence of chelated hydroxyl proton at δ 13.63 ppm, due to intramolecular hydrogen bonding between hydroxyl group of C-5 to carbonyl group of C-4. Another signal at δ 9.32 ppm was the hydroxyl proton of C-4′. A one proton singlet (δ 6.74 ppm) was assigned to the only aromatic proton of ring A at position 8. Further analysis of ^{1}H NMR in more upfield area (Figure 27) revealed the signals at 6.42 ppm (1H, d, J = 2.14 Hz), 6.61 ppm (1H, dd, J= 8.6, 2.1 Hz), and 7.71 ppm (1H, d, J = 8.6 Hz) were assigned to positions of 3', 5', and 6', respectively. The pattern of these signals indicated the *ortho* coupling between H-5' and H-6' with a coupling constant of 8.6 Hz and *meta* coupling of H-5' and H-3' with a coupling constant of 2.1 Hz. This was confirmed by the correlation observable in the two dimensional ^{1}H-^{1}H COSY spectrum (Figure 31-32).

The coupled olefinic protons H-14 at δ 6.57 ppm (1H, d, J = 16.2 Hz) and H-15 at 6.67 ppm (1H, dd, J = 16.2, 7.0 Hz) exhibited the large coupling constant indicative of *trans* relationship. A doublet signal, integrated for one proton at δ 6.22 ppm coupled to the proton (1H, d) at δ 5.46 ppm with coupling constant 9.5 Hz could be, respectively, assigned to H-9 and H-10 of isoprenyl side-chain.

The one proton multiplet signal at 2.42 ppm in expanded ^{1}H-NMR spectrum (Figure 28), was preferably assigned to H-16. This splitting pattern was caused by the vicinal coupling to its neighbouring protons.

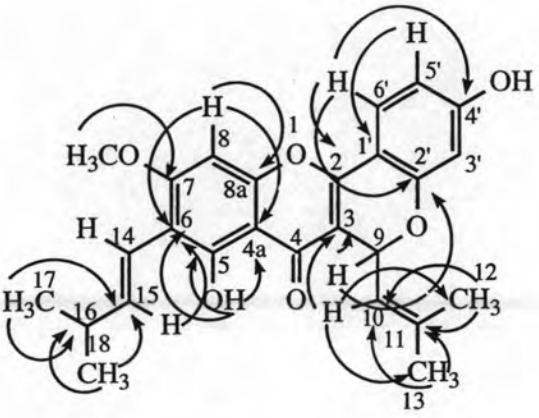
The ^{13}C-NMR spectrum supported the proposed structure. Their assignments were mainly based on the ^{13}C-^{1}H COSY experiment (Figure 33-35). The carbonyl carbon atom was assigned to the position 4. The protonated sp^2 carbons of flavone nucleus were C-8 at 91.0 ppm, C-3' at 104.90 ppm , C-5' at 110.8 ppm, and C-6' at 126.2 ppm. The protonated sp^2 carbons of isoprenyl side-chains were C-10 at 122.1 ppm, C-14 at 116.8 ppm, and C-15 at 142.5 ppm. The signal at δ 56.6 ppm belonged to the oxygenated sp^3 carbon. The remaining signals were protonated sp^3 carbons of isoprenyl sidechain, C-16 at 33.9 ppm, C-17 and C-18 at 23.0 ppm.

The signals of methyl groups in upfield area at δ 18.6 ppm and 25.8 ppm could not be readily assigned by ^{13}C-^{1}H COSY spectrum. However, determination by comparing their chemical shifts taken into account the γ-effect or steric effect due to C-9 at δ 70.4 ppm, the highfield signal at 18.6 ppm should be assigned to C-12 and the lowfield one at 25.8 ppm to C-13. Hence, the ^{13}C-^{1}H COSY spectrum (Figure 35) could be used to correlate the methyl proton signals at 1.95 ppm to C-13 and at 2.03 ppm to C-12.

The quaternary carbons were assigned according to the long-range coupling observed from the HMBC spectrum (Figure 36-41) and the information from their chemical shifts.The lowest-field signal (δ 179.3 ppm) was of carbonyl function. The quaternary carbon signals of flavone nucleus could be assigned as C-2 at 163.6 ppm, C-3 at 110.1 ppm, C-4a at 105.9 ppm, C-5 at 159.8 ppm, C-6 at 110.4 ppm, C-7 at 164.0 ppm, C-8a at 156.2 ppm, C-1' at 108.3 ppm, C-4' at 156.5 ppm, and C-2' at 159.1 ppm. The quaternary carbon of isoprene side-chain (C-11) was assigned the peak signal at δ 138.9 ppm which correlated to both methyl groups at δ 18.6 and 25.8 ppm.

In addition, the oxygen-connected methine proton at 6.22 ppm (H-9) showed two-bond coupling to C-3 at 110.1 ppm observable from the HMBC spectrum (Figure 39), indicating one isoprene side-chain (isopentenyl) as attached to the flavone

nucleus at C-3 and cyclized to form a pyran ring with the hydroxyl group at C-2'. These assigment could be supported by the correalation between proton of position 9 to the carbon at 159.1 ppm (C-2'). The other isoprenoid unit could be either at C-6 or C-8. The HMBC spectrum indicated the position of the isoprenoid (γ,γ dimethylallyl) to be at the former position, as evidence by the cross peak between the proton at 6.74 ppm (H-8) and the carbon signals of C-4a at 105.9 ppm and C-8a at 156.2 ppm, and the correlation between the proton at 116.8 ppm (H-14) to carbon at 159.8 ppm (C-5). These information confirmedthat one isoprenyl side-chain attached to C-3 and another one at C-6.



The explicit assignments of carbon and proton signals with long-range correlations were summarized in Table 12.

Table 12. The carbon and proton assignments of AA-2 with long-range correlations observed in HMBC spectrum.

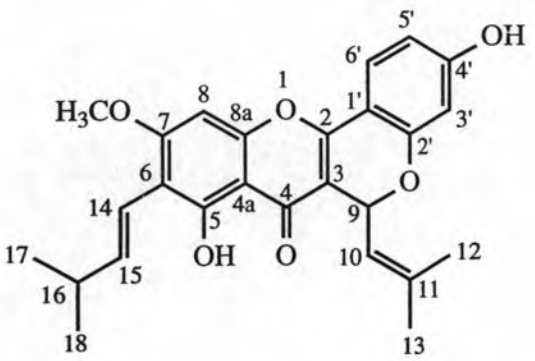
position	δ C (ppm)	δ H (ppm) (multiplicity), *J* (Hz)	HMBC (^{1}H coupled)
1	-	-	-
2	163.6	-	H-6'
3	110.1	-	H-9, H-10
4	179.3	-	-
4a	105.9	-	5-OH, H-8

Table 12. (continued)

position	δ C (ppm)	δ H (ppm) (multiplicity), J (Hz)	HMBC (^{1}H coupled)
5	159.8	-	5-OH, H-14
6	110.4	-	5-OH, H-8, H-15
7	164.0	-	O-CH_3
8	91.0	6.74 (s)	-
8a	156.2	-	H-8
9	70.4	6.22 (d), 9.5	-
10	122.1	5.46 (d), 9.5	H-12, H-13
11	138.9	-	H-12, H-13
12	18.6	2.03 (s)	H-10
13	25.8	1.95 (s)	H-10
14	116.8	6.57 (d), 16.2	-
15	142.5	6.67 (d,d),16.2, 7.0	H-14, H-17, H-18
16	33.9	2.42 (m)	H-17, H-18
17	23.0	1.07 (d), 0.9	-
18	23.0	1.07 (d), 0.9	-
1'	108.3	-	H-5'
2'	159.0	-	H-6', H-9
3'	104.9	6.42 (d), 2.4	-
4'	156.5	-	H-6'
5'	110.8	6.61 (d,d), 8.6, 2.1	-
6'	126.2	7.71 (d), 8.55	-
5-OH	-	13.63 (s)	-
4'-OH	-	9.32 (s)	-
O-CH_3	56.6	3.98 (s)	-

The proposed structure of AA-2 was eventually supported by the analyses of mass fragmentation from its EIMS spectrum (Scheme 9). A fragment at *m/z* 379 , suggested the present of dimethyl allyl groups. The based peak at *m/z* 335 was caused by the cleavage of methyl group, carbonyl function and ether linkage.

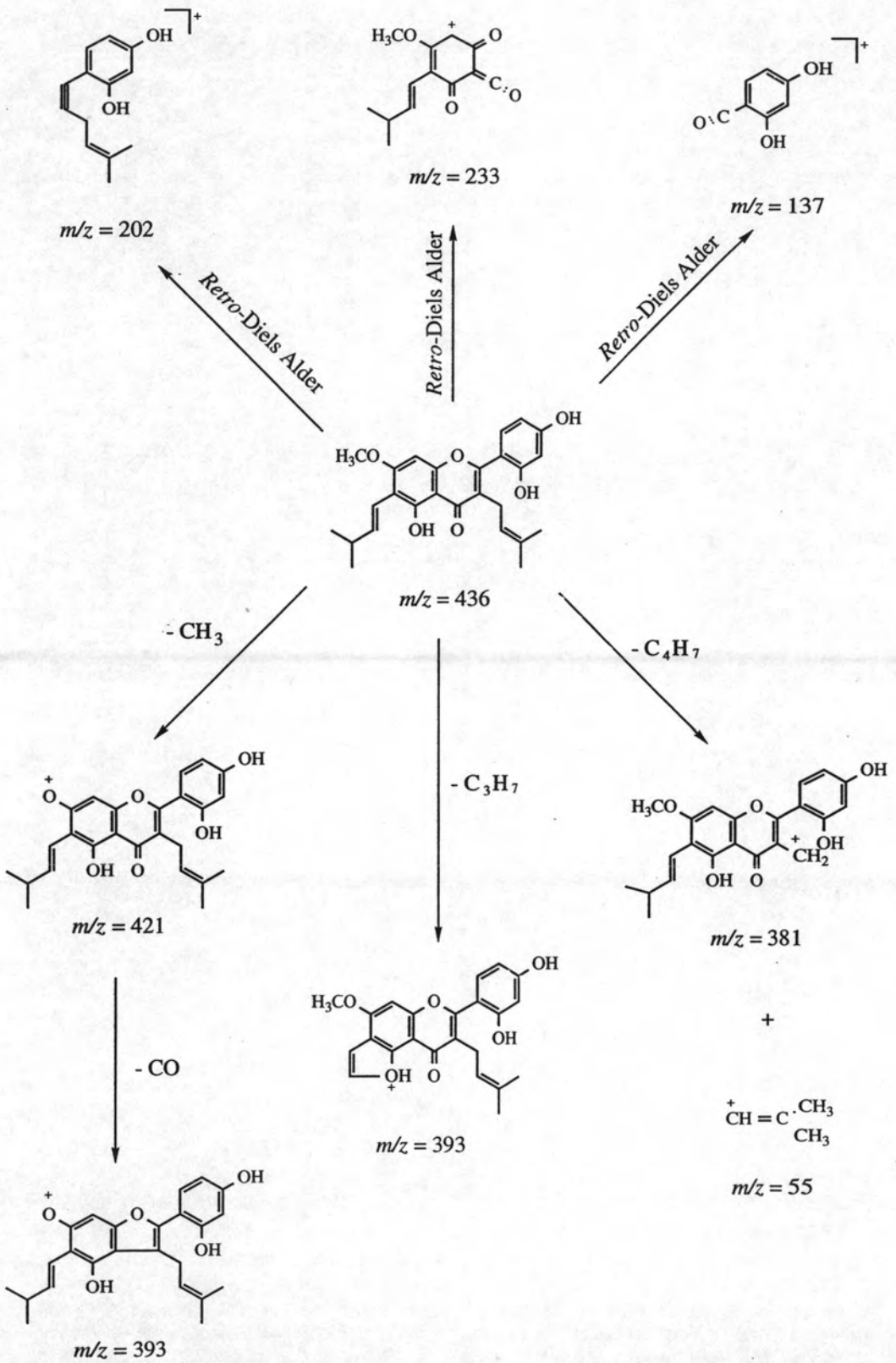
The structure of AA-2 is shown below.



3. Structure Elucidation of AA-3

AA-3 was obtained as yellow prism from the fraction F-02 (Table 10). The IR spectrum of AA-3 (Figure 43) exhibited absorption peaks of carbonyl group of α,β unsaturated ketone at 1651, 1620 cm^{-1}, hydroxyl group at 3415-3335 cm^{-1}, olefinic carbon at 1451-1351 cm^{-1}, and ether linkage at 1206 cm^{-1}.

Further important information of the molecular structure came from its EIMS spectrum (figure 42). The molecular ion peak [M^+] at *m/z* 436 corresponded to the molecular formula of $C_{26}H_{28}O_6$. The UV absorption spectrum of AA-3 (Figure 44) showing absorption maxima at 324 and 265 (sh) nm indicated a typical characteristic of flavone chromophore. The presence of $AlCl_3$/HCl induced the bathochromic shift as the effect of the acid stable hydroxyl-keto complexes.



Scheme 10 Proposed mass fragmentation of AA-3

Compound AA-3 was identified as a known prenylated flavone, artocarpin. The complete proton and carbon assignments were obtained by the analyses of ^{1}H and ^{13}C chemical shifts and their correlations observed in 2D-NMR spectra.

The ^{1}H-NMR spectrum (Figure 45) showed signals for 7 olefinic protons, 3 aliphatic protons, 1 methoxyl group, 4 methyl groups and 3 hydroxyl protons. The signals observed in its ^{13}C-NMR (Figure 48) and DEPT spectra (Figure 49) revealed 1 carbonyl carbon, 7 sp^2 carbons, 1 sp^3 methine carbon, 1 sp^3 methylene carbon, 11 quaternary carbons, 1 methoxyl group, and 4 methyl groups.

The lowest-field singlet signal at 13.94 ppm obtained in the ^{1}H-NMR spectrum (Figure 45) represented the chelated hydroxyl proton of C-5. The other hydroxyl signal with intensity of 2 protons at δ 8.76 ppm were assignable to positions 4' and 2' of the B-ring.

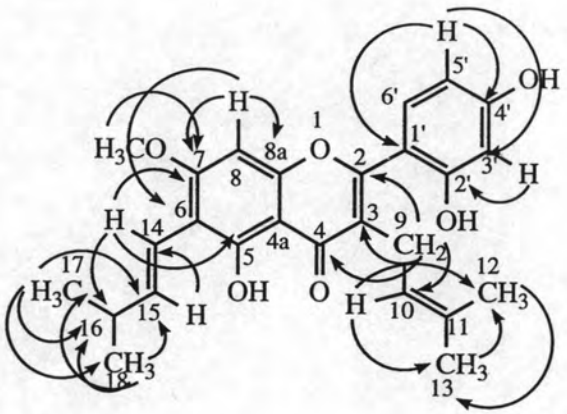
Expansion of the ^{1}H-NMR spectrum (Figure 46-47) exhibited signals indicative of 2',4'-substituted aromatic proton system. The proton at position 5' (δ 6.51 ppm, dd, J =8.2, 2.1 Hz) was *ortho*-coupled to H-6' (δ 7.20 ppm, d, J = 8.2 Hz) and *meta*-coupled to H-3' (δ 6.56 ppm, d, J = 2.1 Hz). These relationshipswere ccnfirmed by the cross-peaks of H-6', H-5', and H-3' discernible in the expansion of ^{1}H-^{1}H COSY spectrum (Figure 55). A singlet signal at 6.53 ppm was assignable to H-8.

The H-15 (δ 6.72 ppm, dd, J = 16.2, 7.3 Hz) showed *trans* coupled to the signsl at 6.59 ppm (dd, J = 16.2, 0.9 Hz) which, therefore could be assigned to H-14. The multiplet signal centered at 2.43 ppm (Figure 47) was assigned to H-16. Based on the cross peaks in ^{1}H-^{1}H COSY spectrum (Figure 54) and their splitting patterns, the methylene proton at 3.11 ppm (d, J = 7.0 Hz) coupled to the signal at 5.12 ppm (br t, J = 7.0 Hz) could be respectively assigned to H-9 and H-10.

The protonated carbons could then be assigned, based on the information achieved by ^{13}C-^{1}H COSY experiment (Figure 50-52), as followed : C-15 (142.2 ppm), C-6' (132.3 ppm), C-10 (122.5 ppm), C-14 (117 ppm), C-5' (108.0 ppm), C-3' (103.8 ppm), C-8 (90.5 ppm), C-16 (33.9 ppm) and C-9 (24.6 ppm). The methyl groups at 23.1 ppm were assigned to positions 17 and 18.

The assignment of the other 2 methyl groups at position 12 and 13 could be resolved owing to their correlations to H-10, observable in ^{1}H-^{1}H COSY experiment (Figure 54).As previously discussed, the signal at δ 17.6 ppm could be assigned to C-12 (H-12 at 1.42 ppm) and δ 25.7 ppm to C-13 (H-13 at 1.55 ppm).

The quaternary carbon assignments were done by investigation of carbon and proton long-range coupling signals in COLOC spectrum (Figure 56- 61) as well as their chemical shifts. The signal at 183.3 ppm could be easily assigned to carbonyl carbon of C-4. The methylene proton at position 9 (δ 3.11 ppm) showed correlation with carbonyl carbon carbon signal at δ 162.4, 121.9 and 157.2 ppm which could be assigned as C-2, C-3 and C-8a respectively. The correlation of C-3 and H-9 also confirmed isopentenyl side-chain attached to C-3. The C-5 signal (δ 159.8 ppm) correlated to H-14 (δ 6.59 ppm). The signal of C-11 at 132.2 ppm exhibited correlation with both methyl protons of position 12 and 13 (δ 1.42 and 1.55, respectively).In the B-ring C-4' (157.4 ppm) showed correlation to H-5' (6.51 ppm), whilst C-2' (161.5 ppm) and C-1' (112.8 ppm) correlated to H-3' (6.56 ppm). The correlation between C-7 (163.8 ppm) and the proton of position 8 and methoxyl proton supported the connection of γ,γ dimethylallyl side-chain at C-6.



The unequivocal proton and carbon assignments with long-range correlations were summarized in Table 13.

Table 13. The carbon and proton assignments of AA-3 with long-range correlations observed in COLOC spectrum.

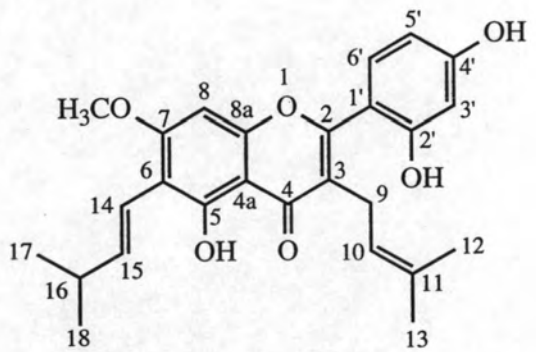
position	δ C (ppm)	δ H (ppm) (multiplicity), *J* (Hz)	COLOC (^{1}H coupled)
1	-	-	-
2	162.4	-	H-9
3	121.9	-	H-9
4	183.3	-	H-9
4a	105.6	-	-
5	159.8	-	H-14
6	112.8	-	H-8
7	163.8	-	O-CH_3, H-8, H-14
8	90.5	6.53 (s)	-
8a	157.2	-	H-8
9	24.6	3.11 (d), 7.0	-
10	122.5	5.12 (br t), 7.0	H-9
11	132.2	-	-
12	17.6	1.42 (s)	-

Figure 1 *Artocarpus altilis* (Park.) Fosb., (Ng, 1978)

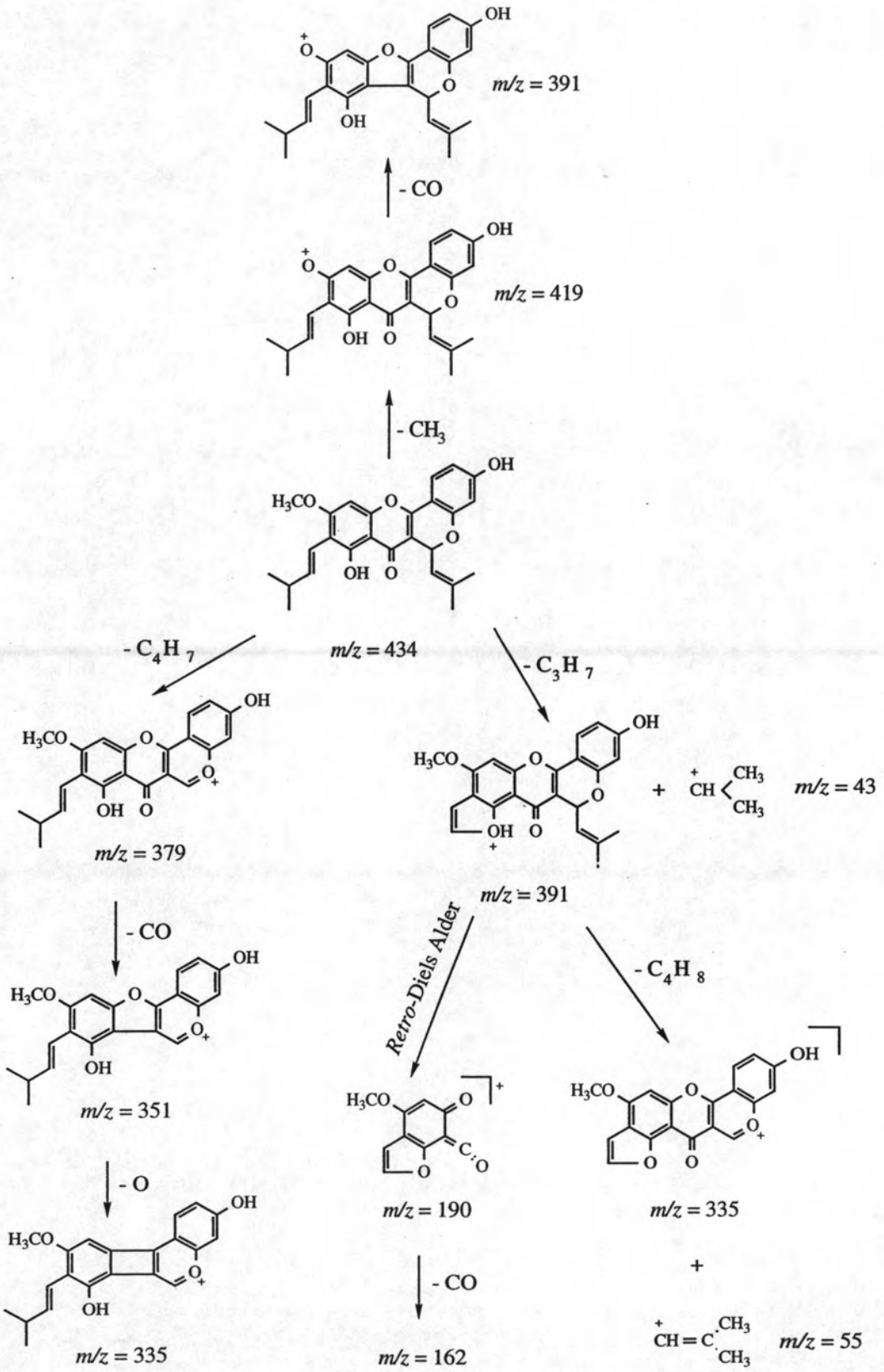
Table 13. (continued)

position	δ C (ppm)	δ H (ppm) (multiplicity), *J* (Hz)	COLOC (^{1}H coupled)
13	25.8	1.55 (s)	-
14	117.0	6.59 (dd), 16.2, 0.9	H-15
15	142.2	6.72 (dd), 16.2, 7.3	H-17, H-18
16	33.9	2.43 (m)	H-14, H-17, H-18
17	23.1	1.07 (s)	H-18
18	23.1	1.08 (s)	H-17
1'	109.7	-	H-5'
2'	161.5	-	H-3'
3'	103.8	6.56 (d), 2.1	H-5'
4'	157.4	-	H-3', H-5'
5'	108.1	6.51 (dd) 8.2, 2.1	-
6'	132.3	7.20 (d), 8.2	-
5-OH	-	13.94 (s)	-
4'-OH	-	8.76 (br s)	-
6'-OH	-	8.76 (br s)	-
O-CH_3	56.6	3.95	-

The structure of AA-3 is shown below.



The tentative structure of AA-3 was finally confirmed by analysis of its EIMS mass fragmentation pattern (Scheme 10).



Scheme 9 Proposed mass fragmentation of AA-2