CHAPTER IV

RESULTS AND DISCUSSION

The dried heartwood of *Artocarpus lakoocha* Roxb. (200 g) was extracted with methanol. The methanol extract was then separated by several chromatographic techniques to afford two pure compounds (AL1 and AL2).

The dried roots of *Artocarpus gomezianus* Wall. ex Tre′c. (8.5 kg) were extracted with petroleum ether, ethyl acetate and methanol to give a petroleum ether extract (25 g), an ethyl acetate extract (486 g) and a methanol extract (1300 g), respectively. After repetitive chromatography of the pet. ether and methanol extracts, eleven compounds were identified.

The structure determinations of all of the isolates were performed by interpretation of their UV, IR, NMR and MS data, and then confirmed by comparison with literature values.

1. Structure Determination of Isolated Compounds

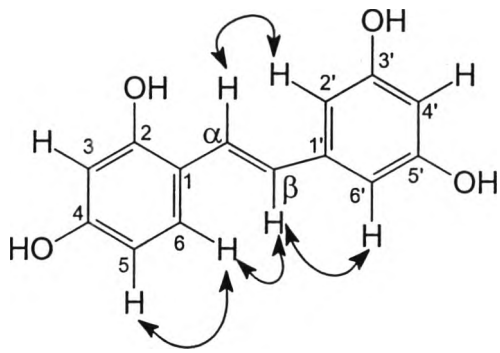
1.1 Structure Determination of Compound AL1

Compound AL1 was obtained as a white powder. The UV spectrum (Figure 4) showed a maximal absorption at λ_{max} 282 nm, and exhibited IR bands (Figure 5) at 3472 (OH), 1605 (>C=C<), 1510 (aromatic ring) and 975 (trans –CH=CH-) cm^{-1}. The EI mass spectrum (Figure 6) revealed a $[M]^+$ ion at *m/z* 244, consistent with the molecular formula $C_{14}H_{12}O_4$. Other significant peaks were found at *m/z* 226 (loss of H_2O) and 123 $[M\text{-}C_7H_7O_2]^+$.

Compound AL1 was identified as oxyresveratrol or 2,4,3′,5′-tetrahydroxystilbene [**141**] by analysis of its NMR spectral data. Oxyresveratrol [**141**] has been isolated from several plants in the families Moraceae and Liliaceae (Venkataraman, 1972) and the fungus-infected xylem tissue of mulberry shoots (Takasuki, Munoz and Masamune, 1978). The ^{13}C NMR data of oxyresveratrol have not been reported. Its structure has been elucidated by analysis of the ^1H NMR spectra of its methylation (Takasuki *et al.*, 1978) and acetylation (Gerber, 1986) products.

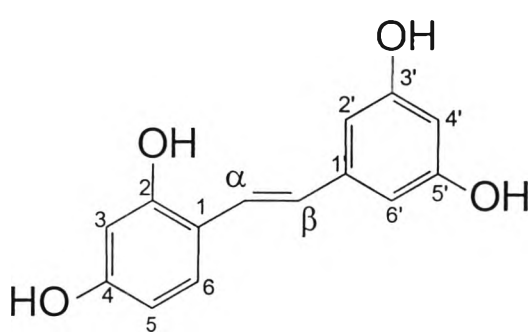
The ^1H NMR spectrum of compound AL1 (Figure 7) showed signals for trans olefinic proton at δ 6.77 and 7.15 (each 1H, d, J = 16.5 Hz) which could be assigned to H-β and H-α. Six aromatic protons appearing at 6.08 (1H, brs), 6.25 (1H, dd, J = 8.4,

2.4 Hz), 6.33 (1H, d, *J* = 2.4 Hz), 6.35 (2H, d, *J* = 1.8 Hz) and 7.34 (1H, d, *J* = 8.4 Hz) were assigned to H-4′, H-5, H-3, H-2′, H-6′ and H-6, respectively. The ^{1}H-^{1}H COSY experiment (Figure 8) revealed coupling for these aromatic protons. The NOE interactions observed in the NOESY spectrum (Figure 9) of compound AL1 are summarized below.



The ^{13}C NMR spectrum showed 14 carbons which could be classified by examination of the DEPT 90 and DEPT 135 spectra (Figure 10). These spectra suggested the presence eight methine and six quarternary carbons. The HETCOR spectrum (Figure 11) revealed correlation between the directly coupled ^{1}H and ^{13}C nuclei. According to the HETCOR correlations, all protonated carbons were assigned.

Assignments of C-α and C-β could be confirmed by examination of the COLOC spectrum (Figure 12). The carbon at α position (δ 123.3) showed long-range correlation with the aromatic proton at δ 7.34 (H-6), and the carbon at β position (δ 124.7) showed three-bond coupling with the aromatic protons at δ 6.35 (H-2′ and H-6′). Quarternary carbons could be assigned from the ^{1}H-^{13}C long-range couplings displayed in the COLOC spectrum, as shown in Table 11.



[**141**]

Table 11 ^{1}H and ^{13}C spectral data of compound AL1 (in DMSO-d_6) with long-range correlations observed in the COLOC spectrum

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	Correlation with proton
1	-	115.4	H-3, H-5 and H-β
2	-	156.1	H-3*, H-6 and H-α
3	6.33 (d, 2.4)	102.7	H-5
4	-	158.2	H-6
5	6.25 (dd, 8.4, 2.4)	107.4	H-3
6	7.34 (d, 8.4)	127.3	H-α
1′	-	140.1	H-α
2′	6.35 (d, 1.8)	104.2	H-4′, H-6′and H-β
3′	-	158.5	H-2′* and H-4′*
4′	6.08 (s)	101.5	H-2′ and H-6′
5′	-	158.5	H-4′* and H-6′*
6′	6.35 (d, 1.8)	104.2	H-2′, H-4′ and H-β
α	7.15 (d, 16.5)	123.3	H-6
β	6.77 (d, 16.5)	124.7	H-2′ and H-6′

* Two-bond coupling.

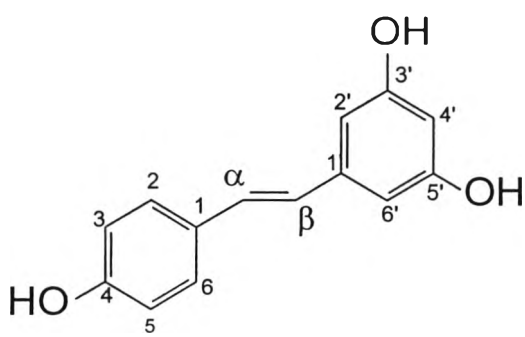
1.2 Structure Determination of Compound AL2

Compound AL2 was obtained as a yellow powder. The UV spectrum (Figure 13) showed absorption maxima at 241 and 306 nm. Its IR spectrum (Figure 14) exhibited absorption bands at 3289 (OH), 1603 (>C=C<), 1512 (aromatic ring) and 965 (trans –CH=CH-) cm^{-1}. The EI mass spectrum (Figure 15) displayed a molecular ion peak at *m/z* 228, suggesting the molecular formula $C_{14}H_{22}O_3$. Other major peaks showed at 227 $[M-H]^+$ and 211 $[M-O]^+$. The difference in the molecular weights of AL1 and AL2 indicated that AL2 had only three hydroxyl groups.

The ^{1}H NMR spectrum (Figure 16) of compound AL2 exhibited nine proton signals in the olefinic and aromatic proton regions, among which a pair of doublet signals at δ 6.81 and 6.93 were assigned to the trans olefinic protons H-β and H-α (*J* = 16.5 Hz). The ortho-coupled doublets (*J* = 8.4 Hz) at δ 6.75 (2H, H-3 and H-5) and 7.39

(2H, H-2 and H-6) revealed the presence of *p*-substituted benzene ring, and this was confirmed by the correlation peaks in the ^{1}H-^{1}H COSY spectrum (Figure 17). The other proton signals at δ 6.11 (1H, br s) and 6.38 (2H, d, J = 1.8 Hz) showed a coupling pattern similar to that of compound AL1. By comparing the above spectral information with reported ^{1}H NMR data (Nakajima *et al.*, 1978), compound AL2 identified as resveratrol or 4,3′,5′-trihydroxystilbene [**144**]. Resveratrol [**144**] has been isolated from plants in the families Pinaceae, Leguminosae, Myrtaceae, Moraceae, Fagaceae and Liliaceae (Venkataraman, 1972). With regard to its ^{13}C NMR properties, no study has been reported.

In the present investigation, the ^{13}C NMR, DEPT 135 and DEPT 90 spectra (Figure 18) provided signals for nine methine and five quarternary carbons. Complete ^{13}C NMR assignments were then obtained by analysis of the HETCOR (Figure 19) and COLOC (Figure 20) spectra, as summarized in Table 12.



[**144**]

Table 12 ^{1}H and ^{13}C spectral data of compound AL2 (in DMSO-d_6) and ^{1}H spectral data of resveratrol (in acetone-d_6) with long-range correlations observe in COLOC spectrum

Position	Compound AL2		Resveratrol	Correlation with proton
	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
1	-	127.8	-	H-3, H-5 and H-β
2	7.39 (d, 8.4)	127.6	7.40 (d, 9.0)	H-6 and H-α
3	6.75 (d, 8.4)	115.4	6.82 (d, 9.0)	-
4	-	156.9	-	H-2 and H-6
5	6.75 (d, 8.4)	115.4	6.82 (d, 9.0)	-
6	7.39 (d, 8.4)	127.6	7.40 (d, 9.0)	H-2 and H-α
1′	-	139.0	-	H-α
2′	6.38 (d, 1.8)	104.1	6.55 (d, 2.0)	H-4′, H-6′ and H-β
3′	-	158.2	-	H-2′ and H-4′*
4′	6.11 (s)	101.6	6.28 (d, 2.0)	H-2′ and H-6′
5′	-	158.2	-	H-4′* and H-6′*
6′	6.38 (d, 1.8)	104.1	6.55 (d, 2.0)	H-2′, H-4′ and H-β
α	6.39 (d, 16.5)	127.6	6.76 (d, 17.0)	H-2 and H-6
β	6.81 (d, 16.5)	125.4	7.08 (d, 17.0)	H-2′ and H-6′

* Two-bond coupling.

1.3 Structure Determination of Isolate AG1

Isolate AG1 was obtained as colorless needles. A Libermann-Burchard test gave a green color, indicative of a steroidal skeleton. Two molecular ions at *m/z* 412 and 414 were observed in the EIMS (Figure 21). Isolate AG1 was identified as a mixture of β-sitosterol [**140**]and Stigmasterol [**160**] by comparison of its ^{1}H and ^{13}C NMR data with reported values (Khalil and Idler, 1980; Iribarren and Pomilio, 1985; Heupel *et al.*, 1986 and Wright *et al.*, 1978).

In the ^{1}H NMR spectrum (Figures 22a and 22b), the signals at δ 5.03 (0.2H, dd, *J* = 15.2, 8.4 Hz), 5.17 (0.2H, dd, *J* = 15.2, 8.4 Hz) and 5.63 (1H, d, *J* = 4.8 Hz) were assigned to H-22 and H-23 of stigmasterol, and H-6 of β-sitosterol and stigmasterol.

The integration steps of H-6, H-22 and H-23 were approximately in the ratios of 1:0.2:0.2. Therefore, it could be deduced that AG1 was a mixture of β-sitosterol and stigmasterol in the ratio of 8:2 or 4:1.

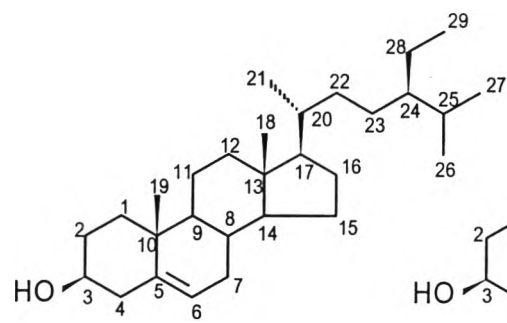
The ^{13}C NMR spectrum (Figure 23) of AG1 displayed 46 signals. Comparison of these data with reported values of β-sitosterol and stigmasterol (Wright *et al.*, 1978) was shown in Table 13.

Table 13 ^{13}C NMR spectral data of β-sitosterol, stigmasterol, (NMR 500 MHz, in $CDCl_3$) and isolate AG1 (in $CDCl_3$)

Carbon	Chemical shift (ppm)		
	β-sitosterol	Stigmasterol	Isolate AG1
1	37.31	37.31	37.66
2	31.57	31.67	32.07
3	71.69	71.81	72.21
4	42.45	42.35	42.70, 42.69
5	140.76	140.80	141.17
6	121.59	121.69	122.12
7	31.92	31.94	32.31
8	31.92	31.94	32.31
9	50.17	50.20	50.54
10	36.51	36.56	36.91
11	21.11	21.11	21.48
12	39.81	39.74	40.18, 40.09
13	42.33	42.35	42.71
14	56.79	56.91	57.17, 57.27
15	24.32	24.39	24.70, 24.76
16	28.26	28.96	28.65, 29.31
17	56.11	56.06	56.46, 56.36
18	11.87	12.07	12.38, 12.45
19	19.40	19.42	19.79

Table 13 (continued)

Carbon	Chemical shift (ppm)		
	β-sitosterol	Stigmasterol	Isolate AG1
20	36.17	40.54	36.95, 40.88
21	18.82	21.11	19.18, 21.48
22	33.95	138.37	34.35, 138.72
23	26.13	129.32	26.49, 129.68
24	45.85	51.29	46.24, 51.64
25	29.18	31.94	29.56, 32.31
26	19.84	21.26	20.22, 21.61
27	19.04	19.02	19.43, 19.38
28	23.09	25.44	23.47, 25.8
29	12.32	12.27	12.64, 12.45



β-Sitosterol

[140]

Stigmasterol

[160]

1.4 Structure Determination of Compound AG2

Compound AG2, a light brown powder, showed UV absorption (Figure 24) at 254 nm. The IR spectrum (Figure 25) of compound AG2 exhibited absorption bands for N-H stretching at 3393 cm^{-1}, C-N stretching at 1308 cm^{-1} and aromatic ring at 1416 and 1600 cm^{-1}. Its high resolution mass spectrum (Figure 26) revealed a molecular ion at *m/z* 219.1012 (calcd. 219.1049), corresponding to the molecular fomula $C_{16}H_{13}N$.

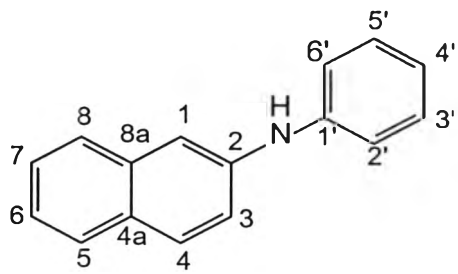
The ^{1}H NMR spectrum (Figure 27), showed twelve aromatic proton signals at δ 6.79 (dddd, *J* = 7.3,7.3,1.2,1.2 Hz), 7.15 (2H, dd, *J* = 8.5,1.2 Hz), 7.22 (dd, *J* = 8.8, 2.1 Hz), 7.28 (ddd, *J* = 8.2, 7.0, 1.2 Hz), 7.29 (2H, dd, *J* = 7.3, 8.5 Hz), 7.39 (ddd, *J* = 8.2, 7.0, 1.2 Hz), 7.43 (br d, *J* = 2.1 Hz), 7.63 (br dd, *J* = 8.2, 0.6), 7.72 (br dd, *J* = 8.8, 2.1 Hz) and 7.73 (br d, *J* = 8.8 Hz).

The ^{13}C NMR, DEPT 90 and DEPT 135 spectra (Figure 28) exhibited signals for twelve methine and four quarternary carbons, and this was supported by the correlations in the HMQC spectrum (Figure 29). At this stage, a naphthalene skeleton with aniline ring was proposed for the structure of AG2. For the naphthalene nucleus, the proton at δ 7.34 (H-1) was meta-coupled to a proton at δ 7.22 (H-3). This proton (H-3) showed ortho-coupling with H-4 at δ 7.73. These observations indicated that AG2 was a β-naphthalene derivative. The naphthalene structure was connected to the aniline ring through the N atom since five aromatic protons of the aniline ring were detected in the ^{1}H NMR spectrum. A NOESY experiment was then performed to confirm the structure.

From the NOESY spectrum (Figure 30), the H-7 (δ 7.39) signal showed NOE interactions with the resonances of H-8 (δ 7.63) and H-6 (δ 7.28). H-6 (δ 7.28) also showed NOE interaction with H-5 (δ 7.72). As expected, H-4 (δ 7.73) showed NOE interaction with H-3 (δ 7.22). In the aniline ring, the H-2′ (H-6′) at δ 7.15 signals showed NOE interactions with the resonances of H-3′ (H-5′) at δ 7.29 and H-4′ (δ 6.97).

The HMBC spectrum (Figure 31) showed correlations of long-range H-C coupling. All quarternary carbons of AG2 were assigned. The quarternary carbon at C-2 position was correlated to the proton at H-4, while C-1′ was correlated to H-3′ and H-5′. The HMBC correlations and the complete proton and carbon assignments of compound AG2 are summarized in Table 14. From all of the above spectral data, compound AG2 was identified as phenyl-β-naphthylamine [**161**]. This compound has been isolated from some Russian plants namely, *Aconitum karacolicum*

(Ranunculaceae), *Reseda lutea* and *R. luteola* (Resedaceae) (Sultankhodzhaev, 1976). The present study provided the first ^{1}H and ^{13}C NMR reports for this compound.



[161]

Table 14 ^{1}H and ^{13}C spectral data of compound AG2 (in $CDCl_3$) with long-range correlations observed in HMBC spectrum

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	Correlation with proton
1	7.43 (br d, 2.1)	111.6	H-3
2	-	140.8	H-4
3	7.22 (dd, 8.8 2.1)	120.0	H-1
4	7.73 (br d, 8.8)	129.2	H-3
4a	-	129.2	H-1, H-3, H-6 and H-8
5	7.72 (br dd, 8.2, 0.6)	127.6	H-4 and H-7
6	7.28 (ddd, 8.2, 7.0, 1.2)	123.5	H-8
7	7.39 (ddd, 8.2, 7.0, 1.2)	126.4	H-5
8	7.63 (br dd, 8.2, 0.6)	126.4	H-1 and H-6
8a	-	134.6	H-4, H-5 and H-7
1′	-	142.9	H-3′ and H-5′
2′	7.15 (dd, 8.5, 1.2)	118.3	H-4′ and H-6′
3′	7.29 (dd, 7.3, 8.5)	129.4	H-2′*
4′	6.97 (dddd, 7.3, 7.3, 1.2, 1.2)	121.4	H-2′
5′	7.29 (dd, 7.3, 8.5)	129.4	H-6′*
6′	7.15 (dd, 8.5, 1.2)	118.3	H-2′, H-4′

* Two-bond coupling.

1.5 Structure Determination of compound AG3

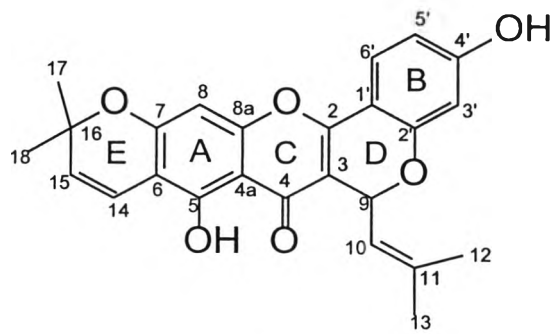
Compound AG3 was obtained as a yellowed powder. The UV spectrum (Figure 32) showed the pattern of a flavone chromophore (Markham, 1982) with maximal absorptions at 241, 294 and 370 nm. The IR spectrum (Figure 33) of compound AG3 indicated absorption bands for a hydroxyl group at 3414 cm^{-1}, aromatic rings at 1472-1625 cm^{-1}, ether linkage at 1187 cm^{-1} and a carbonyl functional group at 1655 cm^{-1}.

The EI mass apectrum (Figure 34) exhibited a molecular ion peak at *m/z* 418, consistent with a molecular formula of $C_{25}H_{22}O_6$. Two significant peaks at 403 $[M-Me]^+$ and 363 $[M-C_4H_7]^+$ were observed.

The ^1H NMR spectrum (Figure 35) showed two singlets for two chromene methyl groups at δ 1 39 and 1.40, and two doublets (J = 9.9 Hz) for cis-olefenic protons at δ 5.77 and 6.57, characteristics of a 2,2-dimethyl chromene (Sultanbawa and Surendrakuma, 1989). The chelated hydroxyl proton of C-5 position showed a sharp singlet signal δ 13.16, and H-8 appeared at δ 6.48, suggesting that the chromene ring was positioned at C-6 and C-7 The two singlets (3H each) at δ 1.83 (H_3-12) and 1.62 (H_3-13), together with two doublets (J = 9.3 Hz) at δ 6.10 (H-9) and 5.40 (br d, H-10), indicated that the 2′-hydroxyl group of ring B in the flavonoid skeleton has been oxidatively cyclized with the allylic methylene of a prenyl chain in the 3-position (Lin and Shieh, 1991). The three aromatic proton signals at δ 6.35 (br s), 6.55 (m) and 7.62 (d, J=8.1 Hz) could be assigned to H-3′, H-5′ and H-6′ respectively, according to their coupling constants. In the ^{13}C NMR (Figure 36), DEPT 135 and DEPT 90 spectra (Figure 37), AG3 exhibited twenty-five signals representing four methyl carbons, eight methine carbons and thirteen quaternary carbons. The most down-field carbon signal was assigned to the C-4 carbonyl carbon. From HETCOR spectrum (Figures 38a and 38b) all protonated carbons could be assigned.

The ^1H and ^{13}C NMR signals of C-12 and C-13 positions could be classified, according to the steric effects (or γ-effect). The up-field methyl carbon at δ 19.2 was assigned to C-12 and the down-field signal at δ 26.3 was assigned to C-13. As a result, the methyl protons of C-12 and C-13 could be assigned at δ 1.83 and 1.62, respectively. Analysis of the HMBC spectrum (Figures 39a-39f) indicated that the structure of AG3 was isocyclomorusin **[25]** It was first isolated from *Artocarpus altilis* (Moraceae) (Chen *et al.*, 1993). Important correlations were the 3-bond connectivities between C-7 and H-14, C-5 and H-14, C-4 and H-9 and C-2 and H-6′. Correlations of C-2′ with H-9 indicated that the pyran ring was attached at C-3 and C-2′ positions of the flavone

skeleton. The results obtained from HMBC spectral data suggested that the assignment of C-13 in a previous report (Chen *et al.*, 1993) should be revised and all quaternary carbons should be re-assigned. The ^{1}H and ^{13}C NMR data of AG3 with long-range correlations are summarized in Table 15.



[25]

Table 15 ^{1}H and ^{13}C NMR spectral data of compound AG3 (in DMSO-d_6) and Isocyclomorusin (in acetone-d_6) with long-range correlations observed in HMBC spectrum

Position	Compound AG3		Isocyclomorusin		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
2	156.4	-	157.9	-	H-9 and H-6′
3	109.2	-	106.2	-	H-9*
4	178.5	-	178.0	-	H-9
4a	105.7[a]	-	104.8	-	H-8
5	156.4	-	155.8	-	H-14
6	105.6[a]	-	108.3	-	H-8, H-15
7	159.2	-	163.9	-	H-8* and H-14
8	95.5	6.50 (s)	95.4	6.28 (s)	-
8a	156.5	-	163.9	-	H-8*
9	69.7	6.10 (d, 9.3)	69.2	6.13 (br d)	-
10	121.9	5.40 (br d, 8.7)	121.0	5.36 (br d)	H-9*
11	139.1	-	138.7	-	H-9, H-12 and H-13

Table 15 (continued)

Position	Compound AG3		Isocyclomorusin		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
12	19.2	1.83 (br s)	18.7	1.88 (s)	H-10 and H-13
13	26.3	1.62 (br s)	17.9	1.61 (s)	H-10 and H-12
14	115.4	6.57 (d, 9.9)	114.5	6.60 (d, 10.1)	-
15	129.8	5.77 (d, 9.9)	128.8	5.53 (d, 10.1)	H-17 and H-18
16	78.8	-	78.3	-	H-14 and H-15*
17	28.7[b]	1.40 (br s)	27.7	1.37 (s)	H-15
18	28.6[b]	1.40 (br s)	27.6	1.37 (s)	H-15
1′	107.2	-	110.2	-	H-3′ and H-5′
2′	158.4	-	156.1	-	H-9, H-3′* and H-6′
3′	104.6	6.35 (d, 2.1)	103.7	#	H-5′
4′	164.5	-	158.8	-	H-3′* and H-6′
5′	111.1	6.55 (dd, 8.1, 2.1)	110.2	#	H-3′
6′	126.3	7.62 (d, 8.1)	125.4	7.55 (d, 8.4)	-
5-OH	-	13.16 (br S)	-	13.03 (s)	

[a,b] Interchangable within the same column

[#] No reported data

*Two-bond coupling

1.6 Structure Determination of Compound AG4

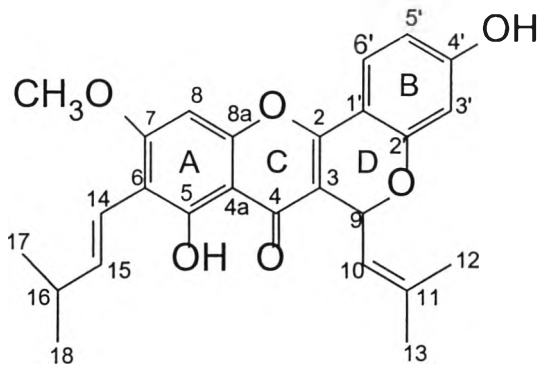
Compound AG4 was obtained as a yellow powder. It showed UV absorption maxima at 240, 292, and 368 nm (Figure 40), suggesting a flavone skeleton (Markham, 1982). The IR spectrum (Figure 41) exhibited a hydroxyl group at 3417, an aromatic ring at 1456-1615 and a chelated carbonyl functionality at 1653 cm^{-1}. The EIMS of compound AG4 (Figure 42) revealed a molecular ion peak at *m/z* 434, corresponding to the molecular formula $C_{26}H_{26}O_6$. It also showed other major peaks at 391 $[M\text{-}CH_3\text{-}CO]^+$ or $[M\text{-}C_3H_7]^+$, 379 $[M\text{-}C_4H_7]^+$ and 335 $[M\text{-}C_3H_7\text{-}C_4H_8]^+$. By analyses of the ^1H and ^{13}C NMR data, compound AG4 was identified as cycloartocarpin [**28**], as narrated below.

The ^{1}H NMR data (Figure 43) showed signals for a pyrano-γ,γ-dimethylallyl moiety, with H-9 and H-10 resonating at δ 6.12 (d, *J* = 9.3 Hz) and 5.41 (d, *J* = 9.0 Hz), and vinyl methyl protons appearing at δ1.83 and 1.62. Similar to AG3, compound AG4 displayed aromatic proton signals for H-3′, H-5′ and H-6′ at δ 6.36 (br s), 6.56 (br d, *J* = 8.1 Hz) and 7.66 (d, *J* = 8.4 Hz), respectively. Couplings between these protons were confirmed by a ^{1}H-^{1}H COSY experiment (Figure 45). At the C-6 and C-7 positions of ring A, two substituents were present, as indicated by the chelated hydroxyl group of C-5 at δ 13.54 and the singlet proton signal of H-8 at δ 6.75. The proton signal at δ 3.90 and the carbon signal at δ 57.3 represented a methoxyl group. The doublet and the doublet of doublets with trans-coupling at δ 6.44 (1H, d, *J* = 16.5 Hz) and 6.62 (1H, dd, *J* = 16.5, 5.7 Hz), together with the singlet at δ 2.42 (1H, m) and the doublet at δ 1.04 (6H, d, *J* = 6.6 Hz) indicated that compound AG4 contained a Δ^{1}-isopentenyl group (Lu and Lin, 1994). This was also supported by the signals at δ 23.6 ppm in the ^{13}C NMR spectrum (Figure 44). The HMBC correlations, as will be described later, placed the methoxyl group at C-7 and the Δ^{1}-isopentenyl group at C-6. Assignments of all of the carbon signals were then made by analysis of the DEPT (Figure 44), HETCOR (Figures 46a and 46b) and HMBC spectra (Figures 47a-47f). From the HMBC spectrum, a chelated hydroxyl group (δ13.54) showed two-bond coupling with H-5 at δ 158.8, and three-bond coupling with C-6 and C-4a at δ109.6 and 105.4. The C-6 and C-4a were distinguished by the correlation between C-6 and H-15. The correlation between C-5 and H-14 indicated that the Δ^{1}-isopentenyl group was situated at C-6. H-9 showed correlations with C-2, C-3, C-4 and C-2′, indicating the location of the pyran ring at C-3 and C-2′ positions. The long-range correlations of all protons and carbons of compound AG4, together with complete NMR assignments, are summarized in Table 16.

Table 16 ^{1}H and ^{13}C spectral data of compound AG4 (in DMSO-d_6) with long-range correlations observed in HMBC spectrum

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	HMBC (Correlation with proton)
2	-	156.1	H-9 and H-6′
3	-	109.3	H-9*
4	-	178.4	H-9
4a	-	105.4	H-8 and 5-OH
5	-	158.4	H-14 and 5-OH*
6	-	109.6	H-8, H-15 and 5-OH
7	3.91 (br s)	162.9	H-8*, H-14 and OCH_3
8	6.75 (s)	91.5	-
8a	-	155.4	H-8*
9	6.12 (d, 9.3)	69.8	-
10	5.41 (br d, 9.0)	121.9	H-9*, H-12 and H-13
11	-	138.9	H-9
12	1.89 (3H, br s)	19.4	H-10 and H-13
13	1.66 (3H, br s)	26.4	H-10 and H-12
14	6.44 (d, 16.5)	116.5	H-15* and H-16
15	6.62 (dd, 16.5, 5.7)	142.2	H-14*, H-17 and H-18
16	2.42 (m)	33.5	H-14, H-15*, H-17* and H-18*
17	1.04 (3H, d, 6.6)	23.6	H-15
18	1.04 (3H, d, 6.6)	23.6	H-15
1′	-	107.2	H-3′ and H-5′
2′	-	158.3	H-9, H-3′ and H-6′
3′	6.36 (br s)	104.6	H-5′
4′	-	164.2	H-3′* and H-6′
5′	6.56 (d, 8.1)	111.0	H-3′
6′	7.66 (d, 8.4)	126.2	-
5-OH	13.54 (br s)	-	-
7-OCH_3	3.91 (br s)	57.3	-

* Two-bond coupling



[28]

Cycloartocarpin **[28]** is a prenylated flavone which has been found in almost all species of the genus *Artocarpus* (See Historical section). In addition, it has been isolated from *Peltogyne porphyrocardia*, *Colophospermum mupane* and *Morus* spp. (Venkataraman, 1972). The ^{1}H and ^{13}C NMR data of this compound have not been reported.

1.7 Structure Determination of Compound AG5

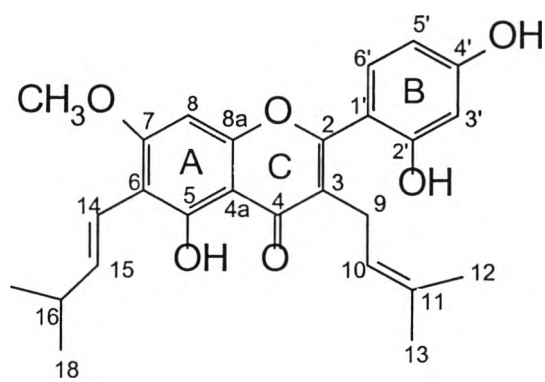
Compound AG5, a yellow powder, showed UV absorption maxima at 228, 240, 292 and 369 nm. (Figure 48). The IR spectrum (Figure 49) showed absorption bands at 3417 (hydroxyl group), 1456-1615 (aromatic ring) and 1647 cm^{-1} (carbonyl group). The EI mass spectrum (Figure 50) of compound AG5 exhibited a molecular ion peak at *m/z* 436, consistent with a molecular formula of $C_{26}H_{28}O_6$. Other major fragment ions appeared at *m/z* 421 $[M\text{-}Me]^+$, 321 $[M\text{-}C_4H_7\text{-}C_3H_7\text{-}OH]^+$ and 365 $[M\text{-}C_3H_7\text{-}CO]^+$.

Compound AG5 was identified as artocarpin [**4**] by analyses of the ^{1}H (Figure 51) and ^{13}C NMR data (Figure 52), and comparison of its ^{13}C NMR properties with previously published values (Lin, Lu and Huang, 1995). Artocarpin [**4**] has been isolated from several species of the genera *Artocarpus* and *Morus* (Venkataraman, 1972).

The structure of compound AG5 was partially similar to that of compound AG4, having a Δ^{1}-isopentenyl group at C-6 of ring A. The ^{1}H NMR spectrum (Figure 51) showed signals for this moiety at δ 1.04 (3H, d, J = 6.6 Hz, H_3-18), 1.06 (3H, d, J = 6.6 Hz, H_3-17), 2.44 (1H, m, H-16), 6.50 (d, J = 16.5 Hz, H-14) and 6.64 (dd, J = 16.5, 6.9 Hz, H-15). The chelated hydroxyl group of C-5 appeared at δ 13.89, while H-8 and 7-OCH_3 showed at δ 6.65 and 3.89. The aromatic protons at δ 6.36 (dd, J = 8.1, 2.1 Hz),

6.45 (d, J = 1.8 Hz) and 7.12 (d, J = 8.4 Hz) could be assigned to H-5′, H-3′ and H-6′. Their relationships were confirmed by the ^{1}H-^{1}H COSY correlations (Figure 54). AG5 possessed another prenyl group at C-3, as indicated by the methylene protons at δ 3.02 (2H, a J = 6.7 Hz, H-9), a broad triplet (J = 7.0 Hz, H-10) and two methyl singlets at δ 1.38 (H_3-12) and 1.55 (H_3-13). Differentation between the two methyl groups was done by conducting a NOESY experiment. From the NOESY spectrum (Figure 56), H-9 (δ 3.02) showed NOE interaction with H_3-12 (δ 1.38), whereas H-10 (δ 5.05) showed NOE interaction with H_3-13 (δ 1.55).

The ^{13}C NMR properties of AG5 were also studied using the DEPT (Figures 53a and 53b), HETCOR (Figure 55) and HMBC techniques. This led to the complete assignments of all of the carbon resonances (Table 17). From the HMBC spectrum (Figures 57a-57g), the intramolecular H-bonded hydroxyl group showed long-range correlations with C-5, C-6 and C-4a at δ 158.9, 109.1 and 105. The correlations of C-2′ with H-3′, and C-4′ with H-6′ confirmed the hydroxyl positions. The resonances C-2′ and C-4′ could not be distinguished by examination of the HMBC correlation peaks. However, their assignments were made by comparison with those of AG7, of which the HMBC correlation between H-5′ and C-4′ was clearly detected.



[4]

Table 17 ^{1}H and ^{13}C spectral data of compound AG5 (in DMSO-d_6) and ^{13}C spectral data of artocarpin (in acetone-d_6) with long-range correlations observed in HMBC spectrum

Position	Compound AG5		Artocarpin	Correlation with proton
	δ_C (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_C (ppm)	
2	-	162.9	160.0	H-9 and H-6′
3	-	121.1	122.3	H-9*
4	-	182.7	183.7	H-9
4a	-	105.0	106.0	5-OH and H-8
5	-	158.9	157.8[a]	5-OH* and H-14
6	-	109.1	113.6	5-OH, H-8 and H-15
7	-	163.4	164.2	H-8*, H-14 and OCH_3
8	6.65 (s)	91.0	90.8	-
8a	-	158.6	162.8	H-8*
9	3.02 (d, 6.7)	24.5	25.0	-
10	5.05 (br t, 7.0)	122.3	122.9	H-9*, H-12 and H-13
11	-	132.1	132.6	H-9, H-12* and H-13*
12	1.38 (br s)	18.2	18.0	H-10 and H-13
13	1.55 (br s)	26.3	26.2	H-10 and H-12
14	6.50 (d, 16.5)	116.7	117.4	H-15* and H-16
15	6.64 (dd, 16.5, 6.9)	142.0	142.6	H-14*, H-17 and H-18
16	2.44 (m)	33.4	34.3	H-14, H-15*, H-17* and H-18*
17	1.06 (d, 6.6)[c]	23.5	23.5	H-15 and H-16*
18	1.04 (d, 6.6)[c]	23.5	23.5	H-15 and H-16*
1′	-	111.7	110.2	H-3′ and H-5′
2′	-	157.3	157.6[a]	H-3′* and H-6′
3′	6.45 (d, 1.8)	103.5	104.2	H-5′
4′	-	161.3	161.9	H-3′* and H-6′
5′	6.36 (dd, 8.1, 2.1)	107.6	108.5	H-3′
6′	7.12 (d, 8.4)	131.9	132.7	-
5-OH	13.89 (br s)		-	-
7-OCH_3	3.89 (br s)		57.0	-

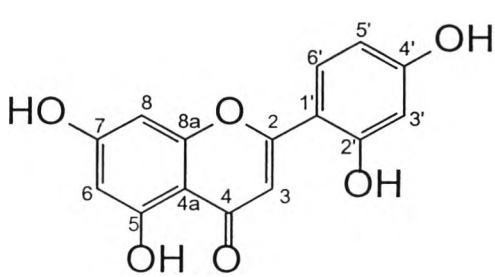
[a,c] These signal may be reversed in each column * Two-bond coupling.

1.8 Structure Determination of Compound AG6

Compound AG6 was obtained as a yellow prism. The UV spectrum (Figure 58) showed maximal absorptions at 227, 241, 289 and 346 nm, suggesting a flavone chromophore (Markham, 1982). It showed a hydroxyl group at 3338 cm^{-1}, an aromatic ring at 1571 and 1623 cm^{-1}, and a chelated carbonyl group at 1663 cm^{-1} in its IR spectrum. (Figure 59). The EI mass spectrum (Figure 60) exhibited a molecular ion $[M]^+$ at *m/z* 286, suggesting the molecular formula $C_{15}H_{10}O_6$. Other important peaks appeared at 269 $[M\text{-}OH]^+$, 258 $[M\text{-}CO]^+$, 153 $[M\text{-}C_8O_2H_5]^+$ and 134 $[M\text{-}C_7H_4O_4]^+$, attributed to the retro-Diels-Alder fragmentation (Markham, 1982).

The ^1H NMR (Figure 61) showed six aromatic and olefinic protons at δ 6.1-7.9 and a chelated hydroxyl group at δ 13.06. The meta-coupled doublet signals (J = 1.8 Hz) at δ 6.18 and 6.44 could be assigned to H-6 and H-8. The ABM-type aromatic proton signals appearing at δ 6.50 (br s), 6.44 and 7.76 (d, J = 8.7 Hz) were due to H-3′, H-5′ and H-6′, respectively. The proton signal of H-5′ was overlapped with H-8, but showed correlation with H-6′ in ^1H-^1H COSY spectrum (Figure 64). The singlet proton signal at δ 7.00, was assigned to H-3.

The ^{13}C NMR (Figure 62), DEPT 90 and DEPT 135 spectra (Figure 63), suggested the presence of six methine carbons and nine quarternary carbons. The most downfield signal at δ 182.5 was assigned to C-4. By examination of the HETCOR spectrum (Figure 65), all of the protonated carbons could be assigned. Examination of the COLOC spectrum (Figure 66) provided complete assignments for all of the quaternary carbons. The carbons at 5, 4a and 6 positions could be assigned by correlation with 5-OH. The correlation of C-2′ with H-6′ and the correlations of C-4′ with H-5′ and H-6′ suggested that the hydroxyl group was located at C-2′ and C-4′ positions. The correlation of 5-OH with C-6 and the correlations of C-7 with H-6 and H-8 confirmed the positions of the hydroxyl groups at C-5 and C-7 positions. The assignments of all of the quaternary carbons are summarized in Table 18.



[43]

Table 18 ^{1}H and ^{13}C NMR spectral data of compound AG6 (in DMSO-d_6) and ^{13}C NMR spectral data of norartocarpetin (in CD_3OD+acetone-d_6) with long-range correlations observed in COLOC spectrum

Position	Compound AG6		Norartocarpetin	Correlation with proton
	δ_C (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_C (ppm)	
2	-	162.4	163.1	H-3* and H-6
3	7.00 (s)	107.2	109.1	-
4	-	182.5	184.2	-
4a	-	104.3	105.2	H-3, H-6, H-8 and 5-OH
5	-	162.1	160.2	H-6* and 5-OH*
6	6.18 (d, 1.8)	99.4	99.8	H-8 and 5-OH
7	-	164.6	165.6	H-6* and H-8*
8	6.44 (d, 1.8)	94.6	94.8	H-6
8a	-	158.0	163.3	H-8*
1′	-	109.4	110.7	H-3 and H-5′
2′	-	159.5	159.4	H-6′
3′	6.50 (br s)	104.0	104.2	H-5′
4′	-	162.4	163.9	H-5′* and H-6′
5′	6.44 (m)	108.9	106.4	H-3′
6′	7.76 (d, 8.7)	130.5	131.0	-
5-OH	13.06 (br s)	-	-	

* Two-bond coupling

From all of the above spectral data, compound AG6 was identified as norartocarpetin [**43**]. It was first isolated from *Artocarpus heterophyllus* (Radhakrishnan, Rao and Venkataraman, 1965) and later found in almost all species of *Artocarpus*. (Table 1).

1.9. Structure Elucidation of Compound AG7

Compound AG7 was obtained as a yellow powder. It showed characteristics of a flavone skeleton with absorption maxima at 241, 274 and 306 nm in its UV spectrum (Figure 67). The EI mass spectrum (Figure 69) exhibited a molecular ion peak at *m/z* 422 corresponding to $C_{25}H_{26}O_6$, with other peaks at 379 $[M-C_3H_7]^+$ and 367 $[M-C_4H_7]^+$. The IR spectrum (Figure 68) of compound AG7 showed absorption bands for a hydroxyl group at 3321 cm^{-1}, an aromatic C=C streching at 1456-1626 cm^{-1} and a chelated carbonyl functional group at 1651 cm^{-1}.

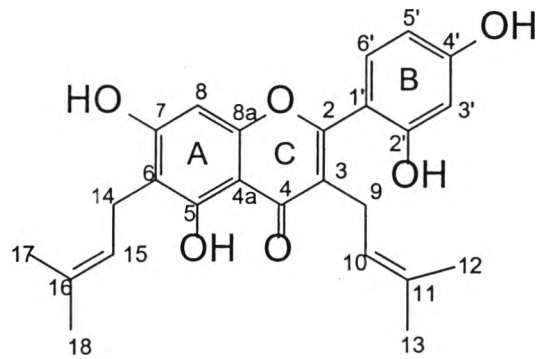
Compound AG7 was identified as cudraflavone C **[162]** by analyses of its ^1H (Figure 70) and ^{13}C NMR (Figure 71) spectral data and comparison with reported ^{13}C NMR data (Hano *et al.*, 1990). Cudraflavone C **[162]** was first isolated from *Cudrania tricuspidata* (Moraceae) (Hano *et al.*, 1990).

The aromatic proton signals of compound AG7 were similar to those of compound AG6, except for the absence of H-3 and H-6 resonances. The signals at δ 6.34 (s), 6.34, 6.42 (d, J = 2.1 Hz) and 7.06 (d, J = 8.4 Hz) were due to H-8, H-5′, H-3′ and H-6′, respectively. H-6′ showed ortho-coupling (J = 8.4 Hz) with H-5′. Similar to AG6, the proton signal of H-5′ of AG7 was overlapped with H-8, showing meta-coupling with H-5′ (J = 2.1 Hz). Other ^1H NMR signals belong to the two γ,γ-dimethylallyl groups. The assignment of each proton was accomplished by analysis of the ^{13}C NMR (Figure 71), DEPT 90, DEPT 135 (Figures 71 and 72), HETCOR (Figure 73) and HMBC (Figures 74a-74h) spectra. The four up-field signals at δ 1.36, 1.54, 1.62 and 1.73 were assigned to methyl protons of H_3-12, H_3-13, H_3-17 and H_3-18, respectively while the two doublet signals at δ 2.97 and 3.22 were assigned to H_2-9 and H_2-14. The triplet proton signals at δ 5.02 and 5.17 could be assigned to H-10 and H-15.

The ^{13}C NMR spectrum, together with the DEPT 90 and DEPT 135 data, indicated the presence of one carbonyl group, four methyl groups, six methine carbons, two methylene carbons and twelve quaternary carbons. The signal of C-12 (δ 18.2) appeared at a more up-field position than that of C-13 (δ 26.3) due to γ-effect or steric effect. A similar observation was found between C-17 and C-18.

The HMBC spectrum showed correlations of the long-range coupled ^1H and ^{13}C nuclei, permitting unambiguous ^{13}C NMR assignment. The correlations of C-6 with 5-OH, H-8 and H-15, confirmed the location of the isoprenyl group at C-6, and the correlations of H-9 with C-3 and C-4 confirmed the position of the other prenyl group at C-3. The correlations of C-2′ with H-3′ and H-6′, and C-4′ with H-3′, H-5′ and H-6′

suggested that C-2′ and C-4′ were substituted. The HMBC spectrum showed correlations of 5-OH with C-5, C-6 and C-4a respectively. The C-6 and C-4a signals could be differentiated by the correlation of C-6 with H-14. The complete ^{1}H and ^{13}C NMR assignments, and the HMBC correlations are summarized in Table 19.



[162]

Table 19 ^{1}H and ^{13}C NMR spectral data of compound AG7 (in DMSO-d_6) and Cudraflavones C (in acetone-d_6) with long-range correlations in HMBC spectrum

Position	Compound AG7		Cudraflavones C		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, *J* in Hz)	
2	162.3	-	162.0	-	H-9 and H-6′
3	120.5	-	121.5	-	H-9*
4	182.3	-	183.0	-	H-9
4a	104.1	-	105.0	-	5-OH and H-8
5	159.1	-	160.0	-	5-OH* and H-14
6	111.3	-	111.8	-	5-OH
7	162.6	-	162.3	-	H-8* and H-14
8	93.5	6.34 (s)	93.5	6.40 (s)	-
8a	156.3	-	157.0	-	H-8*
9	24.5	2.97 (d, 6.6)	24.6	3.12 (d, 7.0)	H-10*
10	122.5	5.02 (t, 6.6)	122.7[a]	5.14 (m)	H-12 and H-13
11	131.9	-	132.0[b]	-	H-9, H-12* and H-13*
12	18.2	1.36 (br s)	17.9[c]	1.43 (br s)	H-10 and H-13
13	26.3	1.54 (br s)	25.8[d]	1.57 (br s)	H-10 and H-12
14	21.9	3.22 (d, 6.9)	22.0	3.41 (d, 7.0)	H-15
15	123.2	5.17 (t, 6.6)	123.3[a]	5.29 (m)	H-14*, H-17 and H-18
16	131.4	-	131.4[b]	-	H-14, H-17* and H-18*
17	18.6	1.73 (br s)	17.6[c]	1.78 (br s)	H-15
18	26.3	1.62 (br s)	25.9[d]	1.65 (br s)	H-15
1′	111.9	-	113.1	-	H-3′ and H-5′
2′	157.2	-	157.1	-	H-3′* and H-6′
3′	103.5	6.42 (d, 2.1)	103.9	6.57 (d, 2.0)	H-5′
4′	161.2	-	161.3	-	H-3′*, H-5′* and H-6′
5′	107.6	6.34 (m)	108.1	6.52 (dd, 8.0, 2.0)	H-3′
6′	132.0	7.06 (d, 8.4)	132.2	7.19 (d, 8.0)	-
5-OH	-	13.31 (br s)	-	13.43 (s)	-

[a, b, c, d] Assignments may be reveraed in each column * Two-bond coupling.

1.10 Structure Determination of Compound AG8

AG8 was identified as resveratrol [**144**], having the same structure as AL2, by comparison of its MS and ^{1}H NMR data with those of AL2.

1.11 Structure Determination of Compound AG9

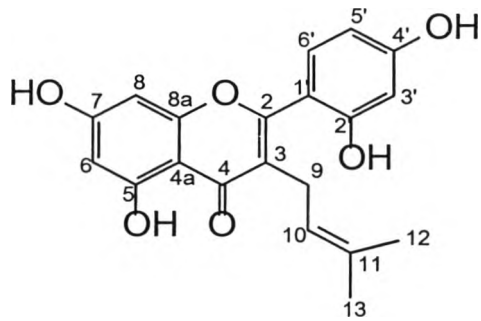
Compound AG9, a yellow powder, showed IR (Figure 76) absorption bands for a hydroxyl group at 3377 cm^{-1}, an aromatic ring at 1456-1611 cm^{-1} and a chelated carbonyl at 1655 cm^{-1}. The UV spectrum (Figure 75) exhibited maximal absorptions at 239 and 320 nm. The EI mass spectrum (Figure 77) of compound AG9, showed a molecular ion peak at *m/z* 354, suggesting the molecular formula $C_{20}H_{18}O_6$. Other significant peaks at *m/z* 311 $[M-C_3H_7]^+$ and retro-Diels Alder fragmentation ions at *m/z* 153 and 202 (Markham, 1982) were also observed.

Comparison of its ^{1}H and ^{13}C NMR spectral data with reported values (Ferrarie *et al.*, 1989) suggested that compound AG9 was identical with albanin A [**163**]. Albanin A [**163**] is also a component of *Brosimopsis oblongifolia* (Ferrarie *et al.*, 1989).

The ^{1}H NMR spectrum (Figure 78) of compound AG9 displayed aromatic meta-coupled doublet signals (J = 1.8 Hz) at δ 6.17 and 6.26, indicating that it was unsubstituted at the C-6 and C-8 positions. These proton signal were assigned to H-6 and H-8. The aromatic proton signals of ring B appeared at δ 6.42 (d, J=1.8 Hz), 6.34 (dd, J=8.1, 1.8 Hz) and 7.08 (d, J=8.1 Hz), which could be assigned to H-3′, H-5′ and H-6′, respectively. The ^{1}H NMR spectrum also showed the presence of a γ,γ-dimethylallyl group at δ 1.36 (br s), 1.54 (br s), 2.97 (d, J = 6.6 Hz) and 5.02 (t, J = 6.6 Hz), due to H_3-12, H_3-13, H-9 and H-10 respectively. The ^{13}C NMR spectrum (Figure 79), together with DEPT 90 and DEPT 135 data, revealed the presence of one carbonyl carbon, two methyl carbons, six methine carbons, one metylene carbon and ten quaternary carbons. All protonated carbons were then assigned by analysis of the HETCOR spectrum (Figure 80).

The long-range C-H correlations of compound AG9 could be observed from the HMBC spectrum (Figures 81a-81e). The intra hydrogen bonded hydroxyl group at C-5 was correlated with carbons at δ 162.3, 99.3 and 104.3, which were assigned to C-5, C-6 and C-4a, respectively. The correlations of C-3 and C-4 with H-9 confirmed that the γ,γ-dimethylallyl side-chain was attached to C-3. C-4a showed correlations with H-6 and H-8, suggesting that AG9 was unsubstituted at the C-6 and C-8 positions. The

carbon signals of C-2′ and C-4′ were assigned by comparison with those of AG7. The results from the HMBC experiment and the ^{1}H and ^{13}C NMR data of compound AG9 and albanin A are summarized in Table 20.



[163]

Table 20 ^{1}H and ^{13}C NMR spectral data of compound AG9 (in DMSO-d_6) and albanin A (in acetone-d_6) with long-range correlation observed in COLOC spectrum

Position	Compound AG9		Albanin A		Correlation with proton
	δ_C (ppm)	δ_H (ppm) (multiplicity, J in Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity, J in Hz)	
2	162.6	-	159.2[b]	-	H-9 and H-6′
3	120.7	-	121.6	-	H-9*
4	182.3	-	182.9	-	H-9
4a	104.3	-	105.3	-	5-OH, H-6 and H-8
5	162.3	-	162.0[b]	-	5-OH* and H-6*
6	99.3	6.17 (d, 1.8)	99.1	6.25 or 6.32 (d, 2.0)	5-OH and H-8
7	165.0	-	164.6	-	H-6* and H-8*
8	94.2	6.26 (d, 1.8)	94.0	6.25 or 6.32 (d, 2.0)	H-6
8a	158.6	-	157.1[b]	-	H-8*
9	24.5	2.97 (d,6.6)	24.4	3.10 (d, 7.0)	H-10*
10	122.1	5.02 (t, 6.6)	122.5	5.13 (t, 7.0)	H-12 and H-13
11	132.0	-	131.9	-	H-9, H-12* and H-13*
12	18.2	1.36 (br s)	17.5	1.43 (s)	H-10 and H-13
13	26.3	1.54 (br s)	25.6	1.57 (s)	H-10 and H-12
1′	111.9	-	113.0	-	H-3′ and H-5′
2′	157.2[a]	-	161.3[b]	-	H-3′* and H-6′
3′	103.5	6.42 (d, 1.8)	103.9	6.56 (d, 2.5)	H-5′
4′	161.3[a]	-	163.3[b]	-	H-3′* and H-6′
5′	107.6	6.34 (dd, 8.1, 1.8)	108.0	6.51 (dd, 8.0, 2.5)	H-3′
6′	132.0	7.08 (d, 8.1)	132.1	7.18 (d, 8.0)	-
5-OH	-	13.05 (s)	-	13.10 (s)	-

[a, b] Within the same column these signals may be interchanged.

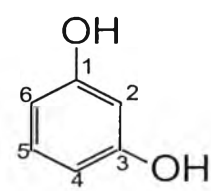
* Two-bond coupling.

1.12 Structure Determination of Compound AG10

Compound AG10 was obtained as colorless needles. The UV spectrum (Figure 82) showed absorptions at λ_{max} 226 and 276 nm. The IR spectrum (Figure 83) displayed absorption bands for a hydroxyl group at 3257 cm^{-1} and an aromatic C=C at 1607 cm^{-1}. The EI mass spectrum (Figure 84) of compound AG10 showed a molecular ion peak at *m/z* 110 corresponding to the molecular formula $C_6H_6O_2$. It was identified as resorcinol [**142**] as described below.

The ^1H NMR spectrum (Figure 85) showed three signals for four aromatic protons, indicating that the molecular was symmetrical. The protons (2H) at δ 6.18 were assigned to H-4 and H-6. The other aromatic protons at δ 6.20 and 6.91 were assigned to H-2 and H-5. Each of these proton signals could be classified by the coupling pattern. H-4 and H-6 appeared as a doublet of doublets with ortho and meta-coupling (*J* = 6.6 and 2.1 Hz) to H-5 and H-2. H-5 showed a triplet signal with ortho-coupling constants due to its coupling with H-4 and H-6. H-2 was a broad singlet because of its meta-coupling with H-4 and H-6. The correlations of all of these aromatic protons were confirmed by a ^1H-^1H COSY experiment (Figure 87).

The ^{13}C NMR, DEPT 90 and DEPT 135 (Figure 86), HETCOR (Figure 88) and COLOC techniques (Figure 89) were used to assign all of the ^{13}C NMR signals (Table 21)



[**142**]

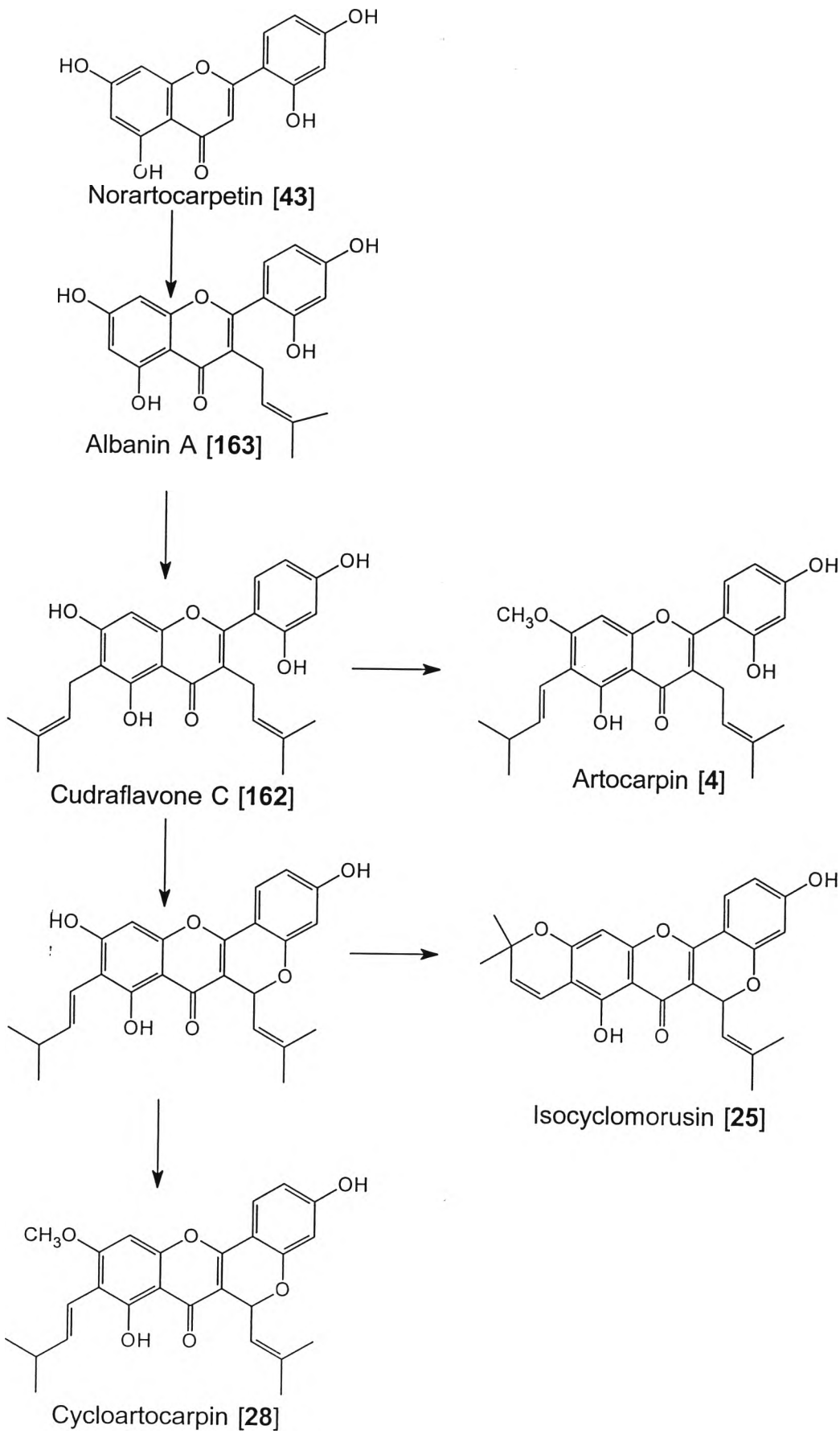
Table 21 ^{1}H and ^{13}C spectral data of compound AG10 (in DMSO-d_6) with long-range correlations observed in COLOC spectrum

Position	δ_H (ppm) (multiplicity, *J* in Hz)	δ_C (ppm)	COLOC (Correlation with proton)
1	-	159.3	H-2*, H-4*, H-5 and H-6*
2	6.20 (br s)	103.3	H-4 and H-6
3	-	159.3	H-2*, H-4*, H-5 and H-6*
4	6.18 (dd, 6.6, 2.1)	107.1	H-2 and H-6
5	6.91 (dd, 6.6, 6.6)	130.6	-
6	6.18 (dd, 6.6, 2.1)	107.1	H-2 and H-4

* Two-bond coupling

2. Proposed Biogenetic pathway of flavones

The biogenetic relationships of the six isolated flavones [**4**, **25**, **28**, **43**, **162** and **163**] could be proposed as follows (Scheme 2). Norartocarpetin [**43**] is formed through the normal acetate-shikimate pathway. Prenylation of **43** at C-3 gives albanin A [**163**], which is then transformed to cudraflavone C [**162**] by the second prenylation at C-6. Artocarpin [**4**] is formed by 7-*O*-methylation of **162**. The reaction between the 2′-OH oxygen and the isoprene unit of **162** results in the formation of an intermediate which could be either converted to isocyclomorusin [**25**] after cyclization of the other prenyl unit or methylated to furnish cycloartocarpin [**28**].



Scheme 2 Proposed Biogenetic pathway of flavones

3. Tyrosinase inhibitory activity of pure compounds

Tyrosinase is a copper monooxygenase enzyme widely distributed in nature. It has been found in plants, fungi, insects and amimals. A number of physiological functions of this enzyme have been studied (Gelder *et al.*, 1997). Tyrosinase is one of the key enzymes involved in the molting process of insects (Kubo *et al.*, 1995). A search for its inhibitors may therefore lead to the discovery of insect control agents. In plants, tyrosinase has been found to be responsible for browning, especially in fruits and vegetables. (Gelder *et al.*, 1997). In mammals and humans, the function of tyrosinase in the biosynthesis of the skin pigment melanin is well established (Gelder *et al.*, 1997). The biosynthesis of melanin has been studied extensively by Raper (Britton, 1983) and Mason (Britton, 1983), leading to the Raper-Mason scheme of melanogenesis (Scheme 1). Thus, the study of tyrosinase inhibitors should be useful for the treatment of localized hyperpigmentation in human such as nevus, lentigo, post-inflammatory state, ephelis and melanoma of pregnancy. Moreover, tyrosinase inhibitors are becoming more important for the development of cosmetic products (Kubo *et al.*, 1995).

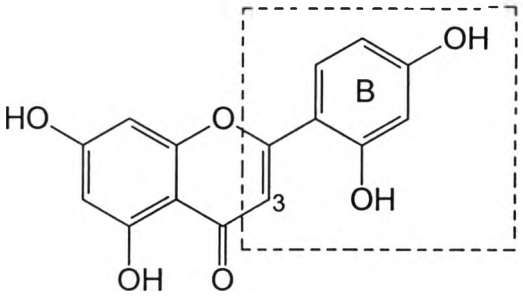
In this study, the tyrosinase inhibitory activity of each pure compound and each crude extract was determined by the dopachrome method. It was modified from the procedures described by Masamoto (Masamoto *et al.*, 1980), Iida (Iida *et al.*, 1995) and Morita (Morita *et al.*, 1994). The MeOH extract of *A. lakoocha* showed 92.17% of tyrosinase inhibition. The EtOAc and MeOH extracts of *A. gomezianus* showed 82.52% and 87.38% of tyrosinase inhibition respectively, but the pet. ether extract no have activity (crude extract 30 mg in MeOH 10 ml) The results of pure compounds were illustrated as IC_{50} values (concentration of 50% inhibition) in comparison with kojic acid, a well known inhibitor of tyrosinase, as summarized in Table 22 and Figure 90.

Table 22 Comparison of IC_{50} values for pure compounds on tyrosinase inhibitory activity

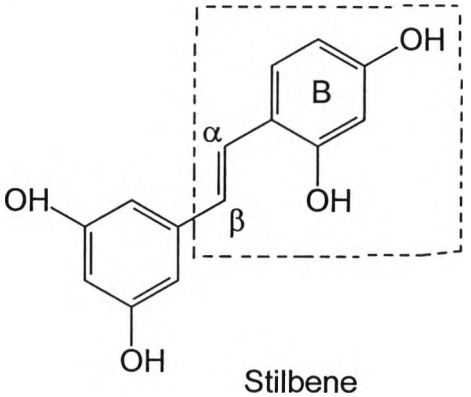
Compound	IC_{50} (μM)
AL1	1.5
AL2 or AG8	14.4
AG1	-
AG3	>260
AG4	>260
AG5	>260
AG6	19.4
AG7	>260
AG9	>260
AG10	>260
Kojic acid	26.8

Among the tested compounds, AL1 **[141]**, a stilbene derivative, was the most potent tyrosinase inhibitor with an IC_{50} value of 1.5 μM, much stronger than kojic acid (IC_{50} 26.8 μM). AL2 (AG8) **[144]**, the other stilbene analogue, was also more active than Kojic acid, displaying an IC_{50} value of 14.4 μM. Its activity, however, appeared to be about 9 times weaker than AL1. This may suggest the importance of the 2-OH group for the stilbene compounds to exert their tyrosinase inhibitory activity. As for the flavones, only AG6, an analogue without C-3 substitution, displayed potent inhibitory effect on tyrosinase (IC_{50} 19.4 μM). On the other hand, all of the other flavones, which possessed a prenyl unit at C-3, showed no activity at all. From these observations, it could be hypothesized that tyrosinase inhibitory activity of these flavones is attenuated by the presence of a substituent at C-3. This is supported by, and may account for, the weak tyrosinase inhibitory activity earlier observed for several flavonols and their derivatives, whose IC_{50} values (60-330 μM) were higher than that of kojic acid (20 μM) (Kubo, 1997). When compared with L-DOPA, the C_3-C_6 phenylpropane unit of the flavone skeleton seemed to be essential for the activity. Comparison of the structures of stilbenes and flavones in this study revealed this partial structural similarity. It could be noted that the styryl moiety which was present in both types of compounds, may play a major role in demonstrating tyrosinase inhibitory activity. These data should provide

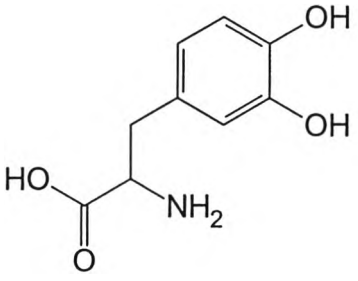
useful information for the development of strong tyrosinase inhibitors based on the flavone and stilbene skeleta.



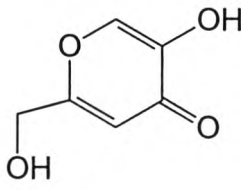
Flavone



Stilbene



L-DOPA



Kojic acid