CHAPTER III

EXPERIMENTAL

1. Sources of Plant Meterials

The heartwood of *Artocarpus lakoocha* Roxb. was bought from Wetchapong Osot drugstore in June 1997. The roots of *Artocarpus gomezianus* Wall ex Tre′c. were collected from Trang province, Thailand in May 1998. The plant was identified by Dr. Thawatchai Santisuk of the Botanical Section, Royal Forest Department, Ministry of Agriculture and Co-operatives, Bangkok, Thailand.

2. General Techniques

2.1 Analytical Thin-Layer Chromatography (TLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	6 cm
Temperature	:	Laboratory temperature (30-35 ^{o}C)
Detection	:	1. Ultraviolet light at wavelengths of 254 and 365 nm 2. 10% Sulfuric acid in ethanol and heated at 105 ^{o}C for 10 min.

2.2 Preparative Thin-Layer Chromatography (PLC)

Technique	:	One dimension, ascending
Adsorbent	:	Silica gel 60 F_{254} (E. Merck) precoated plate
Layer thickness	:	0.2 mm
Distance	:	15 cm
Temperature	:	Laboratory temperature (30-35 ^{o}C)
Detection	:	Ultraviolet light at wavelengths of 254 and 365 nm

2.3 Column Chromatography

2.3.1 Quick Column Chromatography

Adsorbent	:	Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)
Packing method	:	Dry packing
Sample loading	:	The sample was dissolved in a small amount of organic solvent, mixed with a small quantity of adsorbent, triturated, dried and then placed gently on top of the column.
Detection	:	Fractions were examined by TLC observing under UV light at the wavelengths of 254 and 365 nm

2.3.2 Flash Column Chromatography

Adsorbent	:	Silica gel 60 (No. 7734) particle size 0.063-0.200 nm (70-230 mesh ASTM) (E. Merck)
Packing method	:	Wet packing
Sample loading	:	The sample was dissolved in a small amount of eluant and then applied gently on top of the column.
Detection	:	Fractions were examined in the same manner as described in section 2.3.1

2.3.3 Gel Filtration Chromatography

Gel filter	:	Sephadex LH 20 (Phamacia)
Packing method	:	Gel filter was suspended in the eluant and left standing to swell for 24 hours prior to use. It was then poured into the column and allowed to set tightly.
Sample loading	:	The sample was dissolved in a small volume of eluant and applied on top of the column.

2.4 Spectroscopy

2.4.1 Ultraviolet (UV) Absorption Spectra

UV (in methanol and chloroform) spectra were obtained on a Shimadzu UV-160A UV/vis spectrophotometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.4.2 Infrared (IR) Absorption Spectra

IR spectra (KBr disc and film) were recorded on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer (Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University) and a Perkin Elmer FT-IR 1760X spectrometer (Sciencetific and Technological Research Equipment Center, Chulalongkorn University).

2.4.3 Mass Spectra

Electron Impact mass spectra (EIMS) were measured on a Fison Micromass VG Platform II mass spectrometer (Phamaceutical Research Instrument Center, Faculty of Phamaceutical Sciences, Chulalongkorn University). The High Resolution Fast-Atom Bombardment mass spectrum (HR-FAB-MS) was measured with a Hitachi RMU-7M mass spectrometer.

2.4.4 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H and ^{13}C-NMR) Spectra

^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained with a Bruker Avance DPX-300 FT-NMR spectrometer, (Faculty of Pharmaceutical Sciences, Chulalongkorn University).

^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were obtained with a JEOL JMN-A 500 NMR spectrometer (Scientific and Technological Research Equipment Center, Chulalongkorn University).

Solvents for NMR spectra were deuterated dimethylsulfoxide (DMSO-d_6) and deuterated chloroform (chloroform-*d*). Chemical shifts were reported in ppm scale using the chemical shift of the solvent as the reference signal.

2.5 Physical Properties

2.5.1 Melting Points

Melting points were obtained on a Fisher/Johns melting point apparatus (Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University).

2.5.2 Optical Rotation

Optical rotations were measured on a Perkin Elmer 341 polarimeter (Pharmaceutical Research Instrument Center, Faculty of Phamaceutical Sciences, Chulalongkorn University).

2.6 Solvents

Throughout this work, all organic solvents were of commercial grade and were redistilled prior to use.

3. Extraction and Isolation

3.1 Extraction and Isolation of Compounds from *Artocapus lakoocha*

3.1.1 Extraction

The dried heartwood of *A. lakoocha* (200g) was extracted with methanol three times (3x200 ml). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 ^{o}C to yield a methanol extract (syrupy mass 38.5 g, 19.23% based on dried weight of heartwood).

3.1.2 Isolation

3.1.2.1 Isolation of Compound AL1

The methanol extract (5 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (No. 9385) and dried under reduced pressure. It was then fractionated by quick column chromatography using a sintered glass filter column of silica gel '60 (No. 9385) (100 g). Elution was performed in a polarity gradient manner with mixtures of chloroform and methanol as the solvents. The ratios and volumes of solvents used in this column chromatography are summarized in Table 4.

Table 4 The ratios and volumes of solvents for quick column chromatography of methanol extract (5 g) of *Artocarpus lakoocha*

Fraction	Ratio (%) of $CHCl_3$: MeOH	Volume of solvent (ml)
1	100 : 0	150
2	98 : 2	150
3	96 : 4	150
4-10	92 : 8	1050
11-13	90 : 10	450
14-15	89 : 11	300
16-17	88 : 12	300
18-20	87 : 13	450
21	86 : 14	150
22	85 :15	150
23	84 :16	150
24	83 : 17	150
25	82 : 18	150
26	81 : 19	150
27	80 : 20	150
28	79 : 21	150
29	78 :22	150
30	70 : 30	150
31	50 : 50	150
32	0 : 100	150

The eluants were examined by TLC using 15% chloroform in methanol as developing solvent. Fractions (32 fractions) with similar chromatographic pattern were combined to yield eleven fractions, as shown in Table 5.

Table 5 Combination of fractions from quick column chromatography of methanol extract (5 g) from *Artocarpus lakoocha*

Fraction	Weight (g)	Volume of solvent (ml)
1-5	0.010	750
6-7	0.036	300
8-9	0.068	300
10-15	0.073	900
16-17	0.019	300
18-22	0.151	750
23-24	0.036	300
25-28	0.137	600
29-30	0.161	300
31-32	4.1200	300

Fractions 18-22 were pooled and dried (151 mg). The residue was then fractionated on a column using silica gel(No.9385) (3 g) as the adsorbent. Gradient elution was performed using mixtures of chloroform and methanol. Fractions of 50 ml were collected. The eluates were examined by TLC using 15% chloroform in methanol as developing solvent. Fractions with similar chromatographic pattern were pooled to yield eight combined fractions. Fraction 2 of this column was further separated on a column using silica gel 60 (No. 9385) (3 g) as the adsorbent. Elution was performed in a polarity gradient manner with chloroform and methanol. Fractions of 50 ml were collected. Similar fractions were combined after examination with TLC using 15% chloroform in methanol as the developing system. The TLC chromatogram of fractions 5-6 showed a single spot under UV light at 254 nm, R_f 0.24 (Silica gel, 15% chloroform in methanol). Evaporation of this fraction under reduced pressure gave 52 mg of compound AL1 as a white powder (0.2% based on dried weight of heartwood). This compound was later identified as 2,4,3′,5′-tetrahydroxystilbene or oxyresveratrol [**141**].

3.1.2.2 Isolation of Compound AL2

The methanol extract (30 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (No.9385) and dried under reduced pressure. It was then fractionated by quick column chromatography using a sintered glass filter column of silica gel 60 (No.9385) (250 g). Elution was performed in a polarity gradient manner with chloroform and methanol as the solvents. The ratios and volumes of solvent used in this column chromatography are summarized in Table 6.

Table 6 The ratios and volumes of solvents for quick column chromatography of methanol extract (30 g) of *Artocarpus lakoocha*

Fraction	Ratio (%) of $CHCl_3$: MeOH	Volume of solvent (ml)
1-4	100 : 0	600
5-10	90 : 10	900
11	88 : 12	150
12	86 : 14	150
13-16	84 : 16	600
17	82 : 18	150
18	80 : 20	150
19	78 : 22	150
20	76 : 24	150
21-22	70 : 30	300
23	50 : 50	150
24	0 : 100	150

The eluates were examined by TLC using 15% chloroform in methanol as developing solvent. Fractions (24 fractions) with similar chromatographic pattern were combined to yield ten fractions. Fraction 2 (632 mg) was subsequently fractionated on a column using silica gel 60 (No.9385) (10 g) as the adsorbent. Gradient elution was performed using mixtures of chloroform and methanol. Fractions of 50 ml were collected. The eluates were examined by TLC using 15% chloroform in methanol as developing solvent. Fractions (12 fractions) with similar chromatographic pattern were combined to yield five fractions. Fraction 3 (82 mg) from this column was

further separated on a column using silica gel 60 (No.9385) (3 g) as the adsorbent. Elution was performed in a polarity gradient manner with chloroform and methanol. Twelve fractions of 50 ml were collected. Fractions 7-8 (12 mg) were combined and dried. Further separation was done by gel filtration chromatography using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with methanol as eluent. Eight fractions, approximately 50 ml each, were collected. The TLC chromatogram of fraction 5 using 15% methanol in chloroform showed a single spot under UV light at 254 nm, R_f 0.4 (15% methanol in chloroform). Evaporation of this fraction under reduced pressure gave 9 mg of compound AL2 as a yellow powder (0.005% based on dried weight of heartwood). It was later identified as 4,3′,5′-trihydroxystilbene or resveratrol **[144]**.

3.2 Extraction and Isolation of Compounds from *Artocarpus gomezianus*

3.2.1 Extraction

The dried roots of *Artocarpus gomezianus* (8.5 kg) were chopped and blended into small pieces. They were extracted with petroleum ether two times (2 x 30 L). The filtrates were pooled and evaporated under reduced pressure at temperature not exceeding 40 ^{o}C to yield a petroleum extract (25 g, 0.29% based on dried weight of roots).

The marc (after extracted with petroleum ether) was extracted three times with ethyl acetate (3 x 30 L). The obtained extract was evaporated under reduced pressure to yield an ethyl acetate extract (486 g, 5.72% based on dried weight of roots).

The marc (after extracted with petroleum ether and ethyl acetate, respectively) was extracted two times with methanol (2 x 30 L). Removal of the organic solvent gave a methanol extract (1,300 g, 15.29% based on dried weight of roots).

3.2.2 Isolation

3.2.2.1 Isolation of Compounds from Petroleum Ether Extract

The petroleum ether extract was divided into three portions: A (7.4 g), B (8.8 g) and C (8.8 g). Each was dissolved in a small amount of petroleum ether, triturated with silica gel 60 (No. 7734) and dried under vacuum, and then fractionated by quick column chromatography using a sintered glass filter column of silica gel 60 (No. 7734) (240 g). Elution was performed in a polarity gradient manner with petroleum ether and acetone. The ratios and volumes of solvents used in this quick column chromatography are summarized in Table 7.

Table 7 The ratios and volumes of solvents for quick column chromatography of petroleum ether extract of *Artocarpus gomezianus*

Portions	Fractions	Ratio (%) of Pet. ether : Acetone	Volume of solvent (ml)
A	1-2	98 : 2	400
	3	94 : 6	400
	4	92 : 8	400
	5	90 : 10	400
	6	88 : 12	400
	7-11	86 : 14	2000
	12-13	85 : 15	800
	14-22	84 : 16	3600
	23	80 : 20	400
	24	78 : 22	400
	25	76 : 24	400
	26-29	72 : 28	1600
	30	70 : 30	400
	31-32	0 : 100	800
B	1	98 : 2	400
	2	96 : 4	400
	3	94 : 6	400
	4	92 : 8	400
	5	90 : 10	400
	6	88 : 15	400
	7-13	86 : 14	2800
	14	84 : 16	400
	15	82 : 18	400
	16	76 : 24	400

Table 7 (continued)

Portions	Fractions	Ratio (%) of Pet. ether : Acetone	Volume of solvent (ml)
	17-21	72 : 28	2000
	22	70 : 30	400
	23-24	0 : 100	800
C	1	96 : 4	400
	2	94 : 6	400
	2	92 : 8	400
	4	90 : 10	400
	5	88 : 12	400
	6-14	86 : 14	3600
	15-19	84 : 16	2000
	20-25	72 : 28	2400
	26	70 : 30	400
	27	60 : 40	400
	28-29	0 : 100	800

The eluates obtained from each column were examined by TLC using acetone : petroleum ether (1:5) as the developing system. Fractions with similar chromatographic pattern were combined to yield 15 fractions, as shown in Table 8.

Table 8 Combination of fractions from quick column chromatography of petroleum ether extract from *Artocarpus gomezianus*

Combined fractions	Fractions	Total Weight (g)	Volume of solvent (ml)
P-I	1-3 A 1-3 B 1-3 C	0.035	3600
P-II	4 A	2.130	400
P-III	4 B 4 C	8.090	800
P-IV	5 A	0.815	400
P-V	5 B	0.798	400
P-VI	6 A	0.576	400
P-VII	7 A 6-9 B 5-8 C	2.705	3600
P-VIII	8-9 A	0.413	800
P-IX	9-10 C	0.550	800
P-X	10-16 B 11-19 C	1.256	6400
P-XI	10-14 A	0.475	2000
P-XII	15-22 A 17-19 B 20-27 C	1.915	7600
P-XIII	23-30 A 20-22 B	0.342	4400
P-XIIII	31 A 23 B 28 C	1.860	1200
P-XV	32 A 24 B 29 C	1.905	1200

3.2.2.1.1 Isolation of Isolate AG1

Isolate AG1 (2.25 g) was obtained as colorless needles from fraction P-VII through recrystallization from methanol. The yield was 0.027% based on dried weight of the root [R_f 0.4, acetone : pet. ether (1.5 : 5), silica gel, detected by anisaldehyde]. The isolate was identified as a mixture of β-sitosterol [**140**] and stigmasterol [**160**].

3.2.2.1.2 Isolation of Compound AG2

Fraction P-VI (576 mg) was fractionated on a column using silicagel 60 (No. 7734) (220 g) as the adsorbent. Elution was performed in a polarity gradient manner with chloroform and petroleum ether. Sixty-two fractions, approximately of 50 ml, were collected. The eluates were examined by TLC using acetone : pet. ether (1.5 : 5) as the developing solvent. Fractions showing similar chromatographic pattern were combined. Fractions 21-39 (15.8 mg) were combined and further separated by gel filtration chromatography, using a column of sephadex LH 20 with methanol as the eluant. Twenty four fractions were collected (20 ml per fraction) and examined by TLC using acetone : pet. ether (1.5 : 5) as the developing system. The TLC chromatogram of fractions 6-8 showed only one blue fluorescent spot under UV light at 365 nm, R_f 0.39 [Silica gel, acetone : pet. ether (1.5 : 5)]. Evaporation of this fraction under reduced pressure gave 4 mg of compound AG2 as a light brown powder (0.00004% based on dried weight of the root). It was later identified as phenyl-β-naphthylamine [**161**].

3.2.2.1.3 Isolation of Compound AG3

Fraction P-VIII (413 mg) was separated by gel filtration chromatography, using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with methanol as the eluant. Twenty-one fractions, approximately 25 ml each, were collected and examined by TLC, using acetone : pet. ether (1.5 : 5) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield seven major fractions: Fractions 1-4 (110 mg), fraction 5 (112 mg), fractions 6-7 (14 mg), fractions 8-12 (15 mg), fractions 13-14 (12 mg), fractions 15-16 (10 mg) and fractions 17-21 (3 mg).

The TLC chromatogram of fractions 15-16 showed only one spot under UV light at 254 nm, [R_f 0.34, Silica gel, acetone : pet. ether (1.5 : 5)]. Evaporation of this fraction under reduced pressure gave 10 mg of compound AG3 as a yellow powder.

Fractions 13-14 (12 mg) was further separated by gel filtration, using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with methanol as eluant. Nine fractions were collected (10 ml per fraction) and examined by TLC using acetone : pet. ether (1.5 : 5) as the developing solvent. The TLC chromatogram of fractions 5-9 showed only one spot under UV light at 254 nm, R_f 0.34 [Silica gel, acetone : pet. ether (1.5 : 5)]. Evaporation of this fraction under reduced pressure gave 8 mg of compound AG3 as a yellow powder (0.0002% based on dried weight of the root). It was later identified as isocyclomorusin **[25]**.

3.2.2.1.4 Isolation of Compound AG4

Fraction P-X (1256 mg) was separated by gel filtratration, using a column of sephadex LH 20 (100 g,2.5 x 80 cm) with methanol as the eluant. Nineteen fractions, approximately 30 ml each, were collected and examined by TLC, using acetone : pet. ether (1.5 : 5) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions: fractions 1-3 (612 mg), fractions 4-6 (304 mg), fractions 7-13 (124 mg), fractions 14-18 (126 mg) and fraction 19 (2 mg).

Fraction P-XI (475 mg) was separarted by gel filtration, using a column of sephadex LH 20 (100 g,2.5x80 cm) with methanol as eluant. Eighteen fractions were collected (30 ml per fraction) and examined by TLC, using acetone : pet. ether (1.5 : 5) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions: fractions 1-2 (75 mg), fractions 3-5 (59 mg), fraction 6 (20 mg), fractions 7-9 (151 mg) and fractions 10-18 (114 mg).

Fractions 14-18 (126 mg) from P-X and fractions 10-18 (114 mg) from P-XI were combined and further separated by preparative TLC on precoated silica gel 60 F_{254} (0.2 mm, 15x20 cm) plates with development in EtOAc : hexane (1 : 4). Two dark bands were observed under UV light at 254 nm, with the upper band [R_f 0.23, EtOAC:Hexane (1:4)] and the lower band [R_f 0.17, EtOAc:Hexane (1:4)]. Extraction of the upper band with acetone gave compound AG3 (18 mg)

Extraction of the lower band with acetone gave a yellow powder (55 mg) which was further purified by gel filtration, using a column of sephadex LH 20 (100 g, 2.5x80 cm) eluted with acetone. Twelve fractions were collected (50 ml per fraction) and examined by TLC using EtOAC : Hexane (1 : 2) as the developing solvent. The TLC chromatogram of fractions 1-3 showed a single spot under UV light at 254 nm,

R_f 0.28 [Silica gel, acetone : pet. ether (1.5 : 5)]. Evaporation of this fraction under reduced pressure gave 34 mg of compound AG4 as a yellow powder (0.0004% based on dried weight of the root). This compound was subsequently identified as cycloartocarpin [**28**].

3.2.2.1.5 Isolation of Compound AG5

Fraction P-XIII (342 mg) was separated by gel filtration chromatography, using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with methanol as the eluant. Nineteen fractions were collected (25 ml per fraction) and examined by TLC using EtOAc : hexane (2 : 3) as the developing solvent. Fractions with similar chromatographic pattern were combined to yield three fractions: fractions A (67 mg), B (89 mg) and C (115 mg).

Fraction C (115 mg) was further separated by gel filtration (sephadex LH 20) with methanol as the eluant. The eluates were examined by TLC, and fractions giving the same chromatographic pattern were combined to yield three fractions: fractions C-1 (104 mg), C-2 (7 mg) and C-3 (3 mg).

The TLC chromatogram of fraction C-2 showed only one spot under UV light at 254 nm, R_f 0.24 [Silica gel, acetone : pet. ether (1.5 : 5)]. Evaporation of this fraction under reduced pressure gave 7 mg of compound AG5 as a yellow powder. This compound was subsequently identified as artocarpin [**4**].

Fraction C-1 (104 mg) was further separated by gel filtration chromatographic technique (sephadex LH 20) with acetone as the eluant. The eluates were examined by TLC, and fractions giving the same chromatograhpic pattern were combined to yield three fractions : fraction C-1A (83 mg), fraction C-1B (5 mg of compound AG5) and fraction C-1C (15 mg).

Fraction C-1A (83 mg) was separated by preparative TLC using precoated silica gel 60 F_{254} (0.2 mm, 10x20 cm) plate with double development in acetone : pet. ether (1 : 4) to give compound AG5 as a yellow powder (53 mg). (total weight 65 mg, 0.000754% based on dried weight of the root).

3.2.2.2 Isolation of Chemical Compounds from Ethyl Acetate Extract

The ethyl acetate extract was not investigated because its showed a TLC pattern similar to that of MeOH extract except for the absence of minor constituents.

3.2.2.3 Isolation of Chemical Compounds from Methanol Extract

The methanol extract (100 g) was dissolved in a small amount of methanol, triturated with silica gel 60 (No. 7734) and dried under reduced pressure. It was then fractionated by quick column chromatography using a sintered glass filter column of silica gel (No. 7734, 400 g). Elution was performed in a polarity gradient manner with hexane and ethyl acetate as the solvents. The ratios and volumes of solvents used in this column chromatography are summarized in Table 9.

Table 9 The ratios and volumes of solvents for quick column chromatography of methanol extract (100 g) from *Artocarpus gomezianus*

Fraction	Ratio (%) of Hexane : EtOAc	Volume of solvent (ml)
1	100 : 0	500
2-3	99 : 1	1000
4-5	95 : 5	1000
6-7	90 : 10	1000
8	80 : 20	500
9	70 : 30	500
10	60 : 40	500
11-16	50 : 50	3000
17-21	45 :55	3000
22-25	40 : 60	2000
26-31	30 : 70	3000
32-35	25 : 75	2000
36-37	20 : 80	1000
38-42	0 : 100	2500

The eluates were examined by TLC using EtOAc : hexane (2 : 1) as developing solvent. Fractions (42 fractions) with similar chromatographic pattern were combined to yield thirteen fractions, as shown in Table 10.

Table 10 Combination of fractions from quick column chromatography of methanol extract (100 g) from *Artocarpus gomezianus*

Combined fractions	Fractions	Weight (g)	Volume of solvent (ml)
M-1	1-2	0.026	1000
M-2	3-6	0.009	2000
M-3	7-8	0.067	1000
M-4	9	0.009	500
M-5	10	0.032	500
M-6	11	0.048	500
M-7	12-14	0.641	1500
M-8	15	0.338	500
M-9	16-17	0.956	1000
M-10	18-26	23.563	4500
M-11	27-32	4.644	3000
M-12	33-38	12.715	3000
M-13	39-42	65.000	2000

3.2.2.3.1 Isolation of Compound AG6

Compound AG6 (20.3 g) was obtained as yellow prisms from fraction M-10 through recrystallization from ethyl acetate. The yield was 3.11% based on dried weight of root [Silica gel, R_f 0.27 EtOAc : hexane (2 : 1)]. It was identified as norartocarpetin [**43**].

3.2.2.3.2 Isolation of Compound AG7

Fraction M-8 (338 mg) was equally divided into fourteen portions (a-n). Each was fractionated by gel filtration chromatography using a column of sephadex LH 20 (100 g, 25x80 cm) with acetone as the eluent. Eight fractions, approximately 50 ml each, were collected. The eluates were examined by TLC using acetone : toluene(1 : 3)

as the developing solvent. Fractions with similar chromatographic pattern were combined to give five fractions namely: M-8A (43 mg), M-8B (25 mg), M-8C (27 mg), M-8D (29 mg) and M-8E (157 mg).

Fraction M-8A (43 mg) was equally divided into two portions. Each was fractionated by gel filtration chromatography using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with acetone as the eluant. The eluates were collected 20 ml per fraction and examined by TLC using acetone : toluene (1 : 3) as the developing system. Fractions 1-5 of each portion showing only one spot on TLC under UV light at 254 nm, R_f 0.29 [Silica gel, acetone : toluene (1 : 3)] were combined. Evaporation of the combined fractions under reduced pressure gave 19 mg of compound AG7 as a yellow powder (0.003% based on dried weight of the root). This compound was later identified as cudraflavones C [**162**].

3.2.2.3.3 Isolation of compounds AG8 and AG9

Fraction M-8C (27 mg) was equally divided into two portions. Each was fractionated by gel filtration, using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with acetone as the eluant. The eluates was collected 20 ml per fraction and examined by TLC, using acetone : toluene (1 : 3) as the developing system. Fractions 5-10 (5 mg) of the first column and fractions 4-7 (3.9 mg) of the second column were combined, and then separated by gel filtration (sephadex LH 20). Elution was performed in a polarity gradient manner with chloroform and methanol (50 : 50 to 0 : 100) as the solvents. The eluates were collected 30 ml per fraction and examined by TLC using acetone : toluene (1 : 3) as the developing solvent. Fractions with similar chromatographic pattern were combined to give five fractions: fractions 1-4 (0.2 mg), fractions 5-8 (5.4 mg), fraction 9 (0.1 mg), fractions 10-12 (3 mg) and fraction 13 (0.1 mg).

Compound AG8 (3 mg) was obtained as a yellow powder from fractions 10-12 [R_f 0.22, silica gel, acetone : toluene (1 : 3), 0.0005% based on dried weight of the root]. It was later identified as 4,3′,5′-trihydroxystilbene or resveratrol [**144**].

Compound AG9 was separated as a yellow powder from fractions 5-8 (5.4 mg) by preparative TLC on precoated silica gel 60 F_{254} (0.2 mm, 15 x 20 cm) plate with development in acetone : toluene (1 : 3) [5 mg, R_f 0.20, silica gel, acetone : toluene (1 : 3), 0.0007% based on dried weight of the root]. It was subsequently identified as albanin A [**163**].

3.2.2.3.4 Isolation of Compound AG10

Fraction M-8E (157 mg) was separated by gel filtration, using a column of sephadex LH 20 (100 g, 2.5 x 80 cm) with gradient elution using chloroform in methanol (50:50 to 0:100), as eluants. Fractions of 30 ml were collected and examined with TLC on silica gel plate using acetone : toluene (1 : 3) as developing solvent. Fractions with similar chromatographic pattern were combined to yield five fractions. Fractions 9-10 was further purified by gel filtration two times to give 40 mg of compound AG10 as colorless needles [Silica gel, R_f 0.31 acetone : toluene (1 : 3), 0.006% based on dried weight of the root). This compound was subsequently identified as resorcinol **[142]**.

4. Physical and Spectra data of Isolated Compounds

4.1 Compound AL1

Compound AL1 was obtained as a white powder (52 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 6

224 (M^+, 100), 242 (26), 226 (41), 198 (29), 160 (19), 147 (32), 123 (71), 115 (25), 110 (65), 77 (29), 69 (76), 55 (60)

UV : λ_{max} nm (log ε), in methanol; Figure 4

282 (2.66)

IR : ν_{max} cm^{-1}, KBr disc; Figure 5

3472 (br), 1605, 1510, 1305, 1150, 975, 825

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 7

6.08 (1H, br s, H-4′), 6.25 (1H, dd, *J* = 8.4, 2.1 Hz, H-5), 6.33 (1H, d, *J* = 2.4 Hz, H-3), 6.35 (2H, d, *J* = 1.8 Hz, H-2′ and H-6′), 6.77 (1H, d, *J* = 16.5 Hz, H-β), 7.15 (1H, d, *J* = 16.5 Hz, H-α), 7.34 (1H, d, *J* = 8.4 Hz, H-6)

^{13}C NMR :δ ppm, 75 MHz, in DMSO-d_6; Figure 10

101.5 (d, C-4′), 102.7 (d, C-3), 104.2 (d, C-2′ and C-6′), 107.4 (d, C-5), 115.4 (s, C-1), 123.3 (d, C-α), 124.7 (d, C-β), 127.3 (d, C-6), 140.1 (s, C-1′), 156.1 (s, C-2), 158.2 (s, C-4), 158.5 (s, C-3′ and C-5′)

4.2 Compound AL2

Compound AL2 was obtained as a yellow powder (9 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 15

228 (M^+, 100), 227 (42), 211 (26), 181 (46), 157 (28), 152 (25), 115 (26), 91 (31), 76 (31), 76 (41), 69 (32), 55 (36)

UV : λ_{max} nm (log ε), in methanol; Figure 13

306 (4.22), 241 (4.19)

IR : ν_{max} cm^{-1}, KBr disc; Figure 14

3289 (br s), 2923, 1603, 1583, 1512, 1381, 1151, 965, 830

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 16

6.11 (1H, br s, H-4′), 6.38 (2H, d, *J* = 1.8 Hz, H-2′and H-6′), 6.75 (2H, d, *J* = 8.4 Hz, H-3 and H-5), 6.81 (1H, d, *J* = 16.5 Hz, H-β), 6.93 (1H, d, *J* = 16.5 Hz, H-α), 7.39 (2H, d, *J* = 8.4 Hz, H 2 and H-6)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure18

101.6 (d, C-4′), 104.1 (d, C-2′ and C-6′), 115.4 (d, C-3 and C-5), 125.4 (d, C-β), 127.6 (d, C-2, C-6 and C-α), 127.8 (s, C-1), 139.0 (s, C-1′), 156.9 (s, C-4), 158.2 (s, C-3′ and C-5′)

4.3 Isolate AG1

Isolate AG1 was obtained as colorless needles (2.25 g). It was soluble in chloroform.

EIMS : *m/z* (% relative intensity); Figure 21

414 (M^+, 48), 412 (M^+, 16), 399 (16), 396 (22), 381 (13), 329 (25), 303 (19), 273 (16), 255 (21), 231 (15), 213 (30), 161 (30), 159 (38), 145 (45), 133 (33), 119 (32), 105 (48), 95 (50), 81 (59), 69 (66), 55 (100)

^{13}C NMR : δ ppm, 75 MHz, in $CDCl_3$; Figure 23

See Table 13

4.4 Compound AG2

Compound AG2 was obtained as a light-brown powder (4 mg). It was soluble in chloroform.

HR-FAB-MS : *m/z* (% relative intensity); Figure 26

219 (M^+, 100), 218 (19), 154 (23), 136 (20), 115 (13), 77 (15), 69 (12), 55 (11), 41 (8)

UV : λ_{max} nm (log ε), in chloroform; Figure 24

254 (3.19)

IR : ν_{max} cm^{-1}, Film ; Figure 25

3393, 3051, 2924, 1629, 1500, 1416, 1308, 739

^{1}H NMR : δ ppm, 500 MHz, in $CDCl_3$; Figure 27

6.97 (1H, dddd, *J* = 7.3, 7.3, 1.2, 1.2 Hz, H-4′), 7.15 (2H, dd, *J* = 8.5 Hz, 1.2, H-2′ and H-6′), 7.22 (1H, dd, *J* = 8.8, 2.1 Hz, H-3), 7.28 (1H, ddd, *J* = 8.2, 7.0, 1.2 Hz, H-6), 7.29 (2H, dd, *J* = 7.3, 8.5 Hz, H-3′ and H-5′), 7.39 (1H, ddd, *J* = 8.2, 7.0, 1.2 Hz, H-7), 7.43 (1H, br d, *J* = 2.1 Hz, H-1), 7.63, (1H, br dd, *J* = 8.2, 0.6 Hz, H-8), 7.72 (1H, br dd, *J* = 8.2, 0.6 Hz, H-5), 7.73 (1H, br d, *J* = 8.8 Hz, H-4)

^{13}C NMR :δ ppm, 125 MHz, in $CDCl_3$; Figure 28

111.6 (d, C-1), 118.3 (d, C-2′ and C-6′), 120.0 (d, C-3), 121.4 (d, C-4′), 123.5 (d, C-6),126.4, (d, C-7, C-8), 127.6 (d, C-5), 129.2 (d, C-4), 129.2 (s, C-4a), 129.4 (d, C-3′, C-5′), 134.6 (s, C-8a), 140.8 (s, C-2), 142.9 (s, C-1′)

4.5 Compound AG3

Compound AG3 was obtained as a yellow powder (36 mg). It was soluble in methanol.

Melting Point : 265-267 °C

$[\alpha]^{20}_D$: +60.0° (c 0.01 g / 100ml, methanol)

EIMS : *m/z* (% relative intensity); Figure 34

418 (M^+, 41), 404 (20), 403 (100), 363 (59), 347 (46), 203 (21), 194 (45), 189 (16), 174 (55), 137 (15), 115 (11), 91 (11), 77 (19), 69 (21), 55 (13)

UV : λ_{max} nm (log ε), in methanol; Figure 32

241 (4.59), 294 (4.43), 370 (4.35)

IR :ν_{max} cm^{-1}, KBr disc; Figure 33

3414 (br), 2971, 2923, 1655, 1625, 1583, 1548, 1472, 1187, 1135, 1080, 985, 821 821

^{1}H NMR : δ ppm, 500 MHz, in DMSO-d_6; Figure 35

1.40 (6H, br s, H_3-17 and H_3-18), 1.62 (3H, br s, H_3-13), 1.83 (3H, br s, H_3-12), 5.40 (1H, br d, *J* = 8.7 Hz, H-10), 5.77 (1H, d, *J* = 9.9 Hz, H-15), 6.10 (1H, d, *J* = 9.3 Hz, H-9), 6.35 (1H, d,2.1 Hz, H-3′), 6.55 (1H, dd, *J* = 8.5, 2.1 Hz, H-5′), 6.57 (1H, d, *J* = 9.9 Hz, H-14), 7.62 (1H, d, *J* = 8.1 Hz, H-6′), 8.31 (1H, s, 4′-OH), 13.16 (1H, s, 5-OH)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure 36

19.2 (q, C-12), 26.3 (q, C-13), 28.6 and 28.7 (q each, C-17 and C-18), 69.7 (d, C-9), 78.8 (s, C-16), 95.9 (d, C-8), 104.6 (d, C-3′), 105.6 and 105.7 (s each, C-4a and C-6), 107.2 (s, C-1′), 109.2 (s, C-3), 111.1 (d, C-5′), 115.4 (d, C-14), 121.9 (d, C-10), 126.3 (d, C-6′), 129.8 (d, C-15), 139.1 (s, C-11), 156.4 (s, C-2 and C-5), 156.5 (s, C-8a), 158.4 (s, C-2′), 159.2 (s, C-7), 164.5 (s, C-4′), 178.5 (s, C-4)

4.6 Compound AG4

Compound AG4 was obtained as a yellow powder (34 mg). It was soluble in methanol.

Melting Point : 158-162 °C

$[\alpha]^{20}_{D}$: +149.7° (*c* 0.0147 g / 100ml)

EIMS : *m/z* (% relative intensity); Figure 42

434 (M^+, 21), 391 (11), 379 (22), 363 (21), 335 (100), 189 (22), 174 (10), 137 (15), 115 (15), 91 (22), 77 (24), 69 (30), 55 (38)

UV : λ_{max} nm (log ε), in methanol; Figure 40

240 (4.01), 292 (3.92), 368 (3.86)

IR : ν_{max} cm^{-1}, KBr disc; Figure 41

3401 (br), 2955, 2923, 1653, 1615, 1556, 1480, 1456, 1207, 1084, 989

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 43

1.04 (6H, d, *J* = 6.6 Hz, H_3-17 and H_3-18), 1.66 (3H, br s, H_3-13), 1.89 (3H, br s,H_3-12), 3.91 (3H, br s, 7-OCH_3), 5.41 (1H, d, *J* = 9.0 Hz, H-10), 6.12 (1H, d, *J* = 9.3 Hz, H-9), 6.36 (1H, br s, H-3′), 6.44 (1H, d, *J* = 16.5 Hz, H-14), 6.56 (1H, d, *J* = 8.1 Hz, H-5′), 6.62 (1H, dd, *J* = 16.5, 5.7 Hz, H-15), 6.75 (1H, s, H-8), 7.66 (1H, d, *J* = 8.4 Hz, H-6′), 13.54 (1H, br s, 5-OH)

^{13}C NMR :δ ppm, 75 MHz, in DMSO-d_6; Figure 44

19.4 (q, C-12), 23.6 (q, C-17 and C-18), 26.4 (q, C-13), 33.5 (d, C-16), 57.3 (q, 7-OCH_3), 69.8 (d, C-9), 91.5 (d, C-8), 104.6 (d, C-3′), 105.4 (s, C-4a), 107.2 (s, C-1′), 109.3 (s, C-3), 109.6 (s, C-6), 111.0 (d, C-5′), 116.5 (d, C-14), 121.9 (d, C-10), 126.2 (d, C-6′), 138.9 (s, C-11), 142.2 (d, C-15), 155.4 (s, C-8a), 156.1 (s, C-2), 158.3 (s, C-2′), 158.8 (s, C-5), 162.9 (s, C-7), 164.2 (s, C-4′), 178.4 (s, C-4)

4.7 Compound AG5

Compound AG5 was obtained as a yellow powder (53.2 mg). It was soluble in methanol.

Melting Point : 137-141 °C

EIMS : *m/z* (% relative intensity); Figure 50

436 (M^+, 1), 421 (8), 420 (25), 403 (7), 377 (11), 365 (22), 364 (12), 349 (12), 33 (7), 322 (21), 321 (100), 309 (15), 189 (12), 174 (7), 155 (11), 137 (8), 115 (6), 91 (7), 77 (8), 69 (17), 55 (16)

UV : λ_{max} nm (log ε), in methanol; Figure 48

228 (4.16), 240 (4.27), 292 (4.11), 369 (4.09)

IR : ν_{max} cm^{-1}, KBr disc; Figure 49

3417 (br), 2955, 2923, 1647, 1615, 1563, 1456, 1246, 1076, 985

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 51

1.06 (6H, d, *J* = 6.6 Hz, H_3-17 and H_3-18), 1.38 (3H, br s, H_3-12), 1.55 (3H, br s, H_3-13), 2.44 (1H, m, H-16), 3.02 (1H, d, *J* = 6.7 Hz, H-9), 3.89 (3H, br s, 7-OCH_3), 5.05 (1H, t, *J* = 7.0 Hz, H-10), 6.36 (1H, dd, *J* = 8.1, 2.1 Hz, H-5′), 6.45 (1H, d, *J* = 1.8 Hz, H-3′), 6.50 (1H, d, *J* = 16.5 Hz, H-14), 6.64 (1H, dd, *J* = 16.5, 6.9 Hz, H-15), 6.65 (1H, s, H-8), 7.12 (1H, d, *J* = 8.4 Hz, H-6′), 13.89 (1H, br s, 5-OH)

^{13}C NMR :δ ppm, 75 MHz, in DMSO-d_6; Figure 52

18.2 (q, C-12), 23.5 (q, C-17 and C-18), 24.5 (t, C-9), 26.3 (q, C-13), 33.4 (d, C-16), 57.2 (q, 7-OCH_3), 91.0 (d, C-8), 103.5 (d, C-3′), 105.0 (s, C-4a), 107.6 (d, C-5′), 109.1 (s, C-6), 111.7 (s, C-1′), 116.7 (d, C-14), 121.1 (s, C-3), 122.3 (d, C-10), 131.9 (d, C-6′), 132.1 (s, C-11), 142.0 (d, C-15), 156.8 (s, C-8a), 157.3 (s, C-2′ or C-4′), 158.9 (s, C-5), 161.3 (s, C-2′ or C-4′), 162.9 (s, C-2), 163.1 (s, C-7), 182.7 (s, C-4)

4.8 Compound AG6

Compound AG6 was obtained as yellow prisms (20.3 g). It was soluble in methanol.

Melting Point : 340-342 °C

EIMS : *m/z* (% relative intensity); Figure 60

286 (M^+, 92), 269 (8), 258 (8), 244 (5), 229 (4), 217 (4), 153 (100), 152 (14), 134 (46), 129 (24), 96 (12), 78 (30), 69 (59), 63 (14). 53 (18), 51 (27)

UV : λ_{max} nm (log ε), in methanol; Figure 58

346 (4.30), 289 (4.04), 241 (4.38), 227 (4.20)

IR : ν_{max} cm^{-1}, KBr disc; Figure 59

3338 (br), 1663, 1613, 1571, 1450, 1359, 1169, 1024, 853, 824

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 61

6.18 (1H, d, *J* = 1.8 Hz, H-6), 6.45 (1H, br d, *J* = 8.7 Hz, H-5′), 6.44 (1H, d, *J* = 1.8 Hz, H-8), 6.50 (1H, br s, H-3′), 7.00 (1H, s, H-3), 7.76 (1H, d, *J* = 8.7 Hz, H-6′), 13.06 (1H, br s, 5-OH)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure 62

94.6 (d, C-8), 99.4 (d, C-6), 104.0 (d, C-3′), 104.3 (s, C-4a), 107.6 (d. C-3), 108.9 (d, C-5′), 109.4 (s, C-1′), 130.5 (d, C-6′), 158.0 (s, C-8a),

159.5 (s, C-2′), 162.1 (s, C-5), 162.4 (s, C-2, C-4′), 164.6 (s, C-7), 182.5 (s, C-4)

4.9 Compound AG7

Compound AG7 was obtained as a yellow powder (19 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 69

422 (M^+, 13), 379 (14), 367 (10), 349 (6), 323 (14), 311 (5), 281 (4), 253 (3), 203 (8), 189 (9), 165 (24), 147 (15). 135 (4), 123 (21), 115 (18), 105 (13), 91 (27), 81 (21), 77 (22), 69(70), 56 (38), 55 (100)

UV : λ_{max} nm (log ε), in methanol; Figure 67

306 (4.21), 274 (4.17), 241 (4.72)

IR : ν_{max} cm^{-1}, KBr disc; Figure 68

3321 (br), 2923, 1651, 1626, 1575, 1456, 1357, 1183, 1020, 814

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 70

1.36 (3H, s, H_3-12), 1.54 (3H, s, H_3-13), 1.62 (3H, s, H_3-18), 1.73 (3H, s, H_3-17), 2.97 (2H, d, *J* = 6.6 Hz, H-9), 3.22 (2H, d, *J* = 6.6 Hz, H-14), 5.02 (1H, t, *J* = 6.6 Hz, H-10), 5.17 (1H, d, *J* = 6.6 Hz, H-15), 6.34 (1H, m, H-5′), 6.34 (1H, s, H-8), 6.42 (1H, d, *J* = 2.1 Hz, H-3′), 7.06 (1H, d, *J* = 8.4 Hz, H-6′), 9.78 (1H, s, 4′-OH), 9.86 (1H, s, 2′-OH), 10.74 (1H, s, 7-OH), 13.31 (1H, s, 5-OH)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure 71

18.2 (q, C-12), 18.6 (q, C-17), 21.9 (t, C-14), 24.5 (t, C-9), 26.3 (q, C-13 and C-18), 93.5 (d, C-8), 103.5 (d, C-3′), 105.0 (s, C-4a), 107.6 (d, C-5′), 111.3 (s, C-6), 111.9 (s, C-1′), 120.5 (s, C-3), 122.5 (d, C-10), 123.2 (d, C-15), 131.4 (s, C-16), 131.9 (s, C-11), 132.0 (d, C-6′), 156.3 (s, C-8a), 157.2 (s, C-2′), 159.1 (s, C-5), 161.2 (s, C-4′), 162.3 (s, C-2), 162.6 (s, C-7), 182.3 (s, C-4)

4.10 Compound AG8

Compound AG8 was obtained as a yellow powder (3 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 15

228 (M^+, 100), 227 (42), 211 (26), 181 (46), 157 (28), 152 (25), 115 (26), 91 (31), 76 (31), 76 (41), 69 (32), 55 (36)

UV : λ_{max} nm (log ε), in methanol; Figure 13

306 (4.22), 241 (4.19)

IR : ν_{max} cm^{-1}, KBr disc; Figure 14

3289 (br s), 2923, 1603, 1583, 1512, 1381, 1151, 965, 830

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 16

6.11 (1H, br s, H-4′), 6.38 (2H, d, *J* = 1.8 Hz, H-2′ and H-6′), 6.75 (2H, d, *J* = 8.4 Hz, H-3 and H-5), 6.81 (1H, d, *J* = 16.5 Hz, H-β), 6.93 (1H, d, *J* = 16.5 Hz, H-α), 7.39 (2H, d, *J* = 8.4 Hz, H 2 and H-6)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure 18

101.6 (d, C-4′), 104.1 (d, C-2′ and C-6′), 115.4 (d, C-3, C-5), 125.4 (d, C-β), 127.6 (d, C-2, C-6 and C-α), 127.8 (s, C-1), 139.0 (s, C-1′), 156.9 (s, C-4), 158.2 (s, C-3′ and C-5′)

4.11 Compound AG9

Compound AG9 was obtained as a yellow powder (5 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 77

354 (M^+, 32), 337 (5), 321 (7), 312 (17), 311 (100), 297 (12), 281 (6), 229 (1), 175 (7), 153 (16), 147 (7), 123 (10), 95 (11), 91 (4), 77 (4), 69 (7), 60 (13), 55 (18)

UV : λ_{max} nm (log ε), in methanol; Figure 75

320 (4.13), 239 (4.57)

IR : ν_{max} cm^{-1}, KBr disc; Figure 76

3377 (br), 2923, 1655, 1611, 1516, 1456, 1365, 1119, 822

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 78

1.36 (3H, s, H_3-12), 1.54 (3H, s, H_3-13), 2.97 (2H, d, J = 6.6 Hz, H-9), 5.02 (1H, t, J = 6.6 Hz, H-10), 6.17 (1H, d, J = 1.8 Hz, H-6), 6.26 (1H, d, J = 1.8 Hz, H-8), 6.34 (1H, dd, J = 8.1, 1.8 Hz, H-5′), 6.42 (1H, d, J = 1.8 Hz, H-3′), 7.08 (1H, d, J = 8.1 Hz, H-6′), 13.05 (1H, s, 5-OH)

^{13}C NMR : δ ppm, 75 MHz, in DMSO-d_6; Figure 79

18.2 (q, C-12), 24.5 (t, C-9), 26.3 (q, C-13), 94.2 (d, C-8), 99.3 (d, C-6), 103.5 (d, C-5′), 104.3 (s, C-4a), 107.6 (d, C-5′), 111.9 (s, C-1′), 120.7 (s, C-3), 122.4 (d, C-10), 132.0 (d, C-6′), 132.0 (s, C-11), 157.2 (s, C-2′), 158.6 (s, C-8a), 161.3 (s, C-4′), 162.3 (s, C-5), 162.6 (s, C-2), 165.0 (s, C-7), 183.2 (s, C-4)

4.12 Compound AG10

Compound AG10 was obtained as colorless needles (40 mg). It was soluble in methanol.

EIMS : *m/z* (% relative intensity); Figure 84

110 (M^+, 100), 95 (1), 83 (1), 82 (12), 81 (6), 63 (1), 54 (1), 53 (2)

UV : λ_{max} nm (log ε), in methanol; Figure 82

226 (3.66), 276 (3.29)

IR : ν_{max} cm^{-1}, KBr disc; Figure 83

3257 (br), 1607, 1490, 1382, 1297, 1167, 1150, 963, 842, 773, 739, 680

^{1}H NMR : δ ppm, 300 MHz, in DMSO-d_6; Figure 85

6.18 (2H, dd, J = 6.6, 2.1 Hz, H-4, H-6), 6.20 (1H, br s, H-2), 6.91 (1H, dd, J = 8.1, 8.1 Hz, H-5), 9.2 (2H, br s, 1-OH, 3-OH)

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$N_1V_1 = N_2V_2$

N_1 = Beginning concentration (μM)

V_1 = Beginning volume (μl)

N_2 = Final concentration (μM)

V_2 = Final volume (μl)

Thus, the final volume of AL1 solution = 1366 μM x 20 μl / 200 μl

= 136.6 μM

The final concentrations of other dilutions were calculated by the same method.

5.2 Measurement of activity

The reaction mixture (200μl) was measured in four wells (A, B, C and D). In each well, the substance was added in the order of mixing, as follows;

A (control)	20 μl of mushroom tyrosinase solution (48 unit/ml)
	140 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of methanol
B (blank of A)	160 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of methanol
C (test sample)	20 μl of mushroom tyrosinase solution (48 unit/ml)
	140 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of test sample in methanol
D (blank of C)	160 μl of 20 mM phosphate buffer (pH 6.8)
	20 μl of test sample in methanol

After each well was mixed and pre-incubated at 25 °C for 10 minutes, 20 μl of 0.85 μM L-DOPA was added and incubated at 25 °C for 20 minutes. The absorbance of each well was measured at 492 nm with the microplate reader both before and after incubation.

5.3 Calculation of the percent inhibition of tyrosinase enzyme

The percent inhibition of tyrosinase reaction was calculated as follows.

$$\% \text{ Tyrosinase inhibition} = \left[\frac{(A-B)-(C-D)}{A-B} \right] X\ 100$$

A : The difference of optical density before and after incubation at 492 nm without test sample

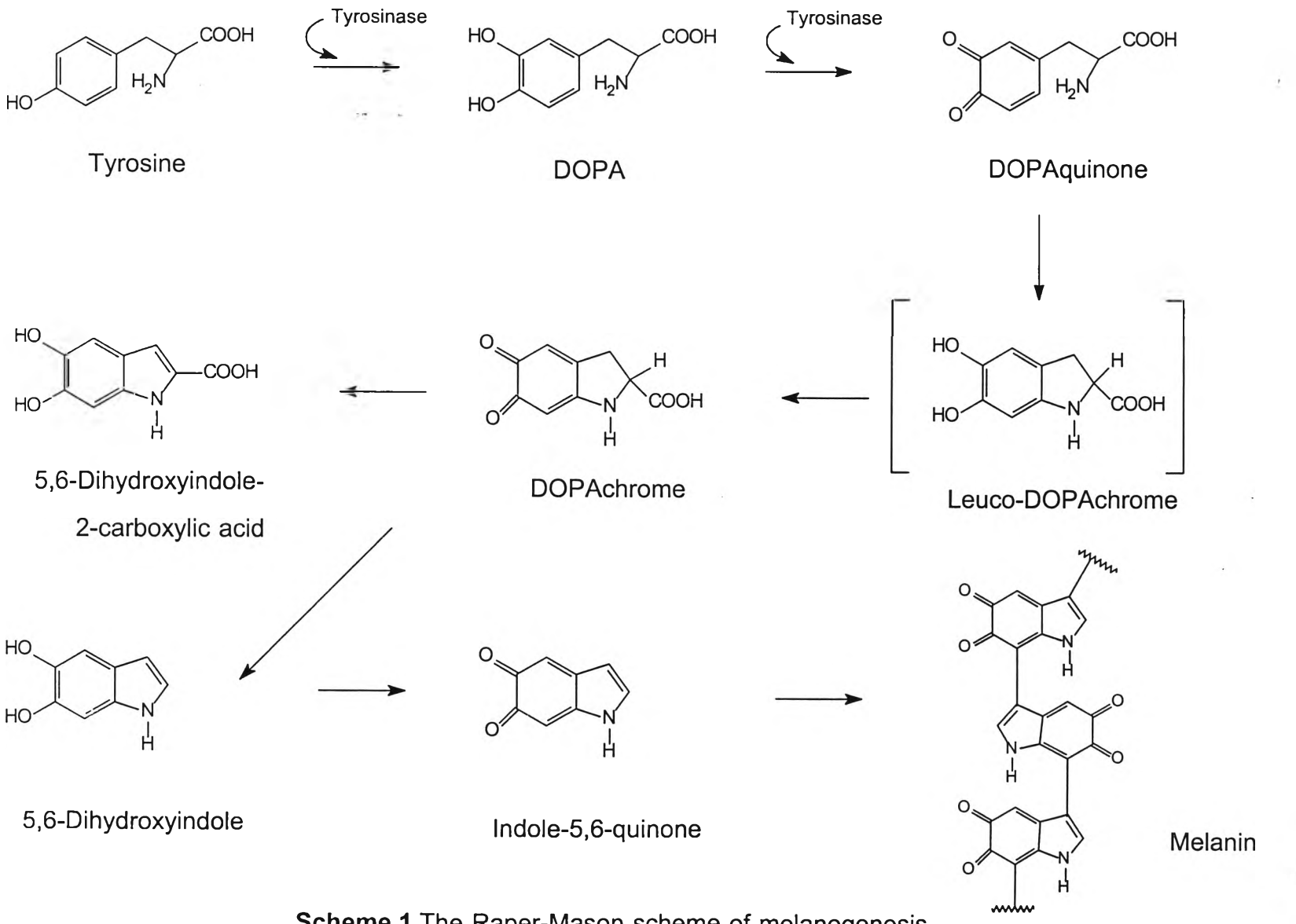
B : The difference of optical density before and after incubation at 492 nm without test sample and enzyme

C : The difference of optical density before and after incubation at 492 nm with test sample

D : The difference of optical density before and after incubation at 492 nm with test sample, but without enzyme

5.4 Calculation of IC_{50}

After the % tyrosinase inhibition of the test sample in each concentration was calculated, the curve of each concentration and its % tyrosinase inhibition was plotted. The IC_{50} of each pure compound was then obtained from the graph.



Scheme 1 The Raper-Mason scheme of melanogenesis