CHAPTER III

EXPERIMENTAL

General technique

Chromatographic tecniques

Thin-layer chromatography (TLC)

Technique	:	One way ascending
Adsorbent	:	Silica gel G. (E. Merck)
		(30 g in 60 ml distilled water)
Plate size	:	5 X 20 cm
		10 X 20 cm
		20 X 20 cm
Layer thickness	:	0.25 mm
Activation	:	Air-dried for 15 minutes / heat at 110 ^{o}C for 1 hour
Solvent system	:	a) 4% methanol in chloroform
		b) 30% methanol in chloroform
Distance	:	15 cm
Temperature	:	28 - 33 ^{o}C
Detection	:	a) Dragendorff's reagent.
		b) Anisaldehyde-sulfuric acid spraying reagent

Column chromatography (CC)

Column	:	Flat bottom glass column (various diameter)
Adsorbent	:	Silica gel 60 (E. Merck)
Packing method	:	Wet packing
Solvent system	:	a) 4% methanol in chloroform b) 30% methanol in chloroform

Melting point

Melting points were determined on a Gallenkamp, Melting point, Apparatus Model MFB 595 and were uncorrected.

Ultra-violet spectroscopy (UV)

Ultra-violet absorption spectra were determined on a double beam Hitachi SP-150-20.

Infrared spectroscopy (IR)

Infrared absorption spectra were recorded as KBr disc on a Perkin Elmer Model 283 spectrophotometer. The absorption bands were reported in wave number (cm^{-1}).

Nuclear magnetic resonance (NMR)

^{1}H NMR and ^{13}C NMR spectra were obtained on two difference instuments as follows :

a) JEOL. nuclear magnetic resonance spectrometer model FX. 90.

b) Varian nuclear magnetic resonance spectrometer model XL-300.

Tetramethylsilane (TMS) was used as an internal standard (chemical shift (δ) = 0.00 ppm) and chemical shifts were reported on ppm scale.

Mass spectroscopy (MS)

The low resolution mass spectra were obtained on a JEOL. mass pectrometer model DX. 300, operating at 70 eV, temperature range between 150 ^{o}C to 400 ^{o}C.

Elemental analysis

Elemental analysis were performed on a Perkin-Elmer elemetal analyzer model 2400.

Optical rotation

The optical rotation was recorded on the Polax no. 45014.

Solvent

The solvents were used as commercial and analytical grades.

Phytochemical screening

Powder of leaf material (115 g) was macerated with methanol (150 ml). After 3 days the mixture was filtered, then concentrated to the syrupy mass on a waterbath. It was mixed with kieselguhr and the mixture was eluated by hexane, chloroform and methanol, respectively. The hexane, chloroform and methanol extracts were evaporated to dryness on waterbath for further screening procedure.

Screening for sterols and triterpenes

Dissloved the small amount of the hexane extract in 3 drops of acetic anhydride, then one drop of conc sulfuric acid was add. The developing of blue to green colors, indicated the present of sterols.

Screening for alkaloids

Dissolved small amount of chloroform extract with 5 ml of dil HCl and filtered. The filtrate gave precipitates with modified Dragendorff's and Mayer's reagents, indicated the present of alkaloids.

Screening for flavonoids.

Dissolved small amount of the extracts in 2

ml of ethanol, the magnesium ribbons and 2 ml of conc HCl were added. The results showed negative test for flavonoids.

Isolation of chemical substances from the leaves of *Aglaia pyramidata* Hance

Extraction

Dried powdered leaves (6.2 Kg) were macerated three times for 10 day periods with methanol (25, 20 and 20 l). The methanolic extract was concentrated under reduced pressure to give a residue (1.27 Kg) which was fractionated, according to Fig. 1.

The residue was preadsorbed on kieselguhr. It was then eluted with n-hexane in a large cone percolator until the hexane extract gave negative test to Liebermann-Burchard test. The hexane extract was evaporated to dryness to give 650 g of hexane residue. The residue gave negative test for alkaloids. This residue was not further investegated. The remaining air dried kieselguhr residue was then exhaustively eluted with chloroform to give on evaporation 252 g of chloroform residue containing crude alkaloid. The remaining air dried kieselguhr residue was exhaustively extract with methanol to give on evaporation, 206 g of the methanol residue. The chloroform and methanol residues were subjected to column chromatography for further purification.

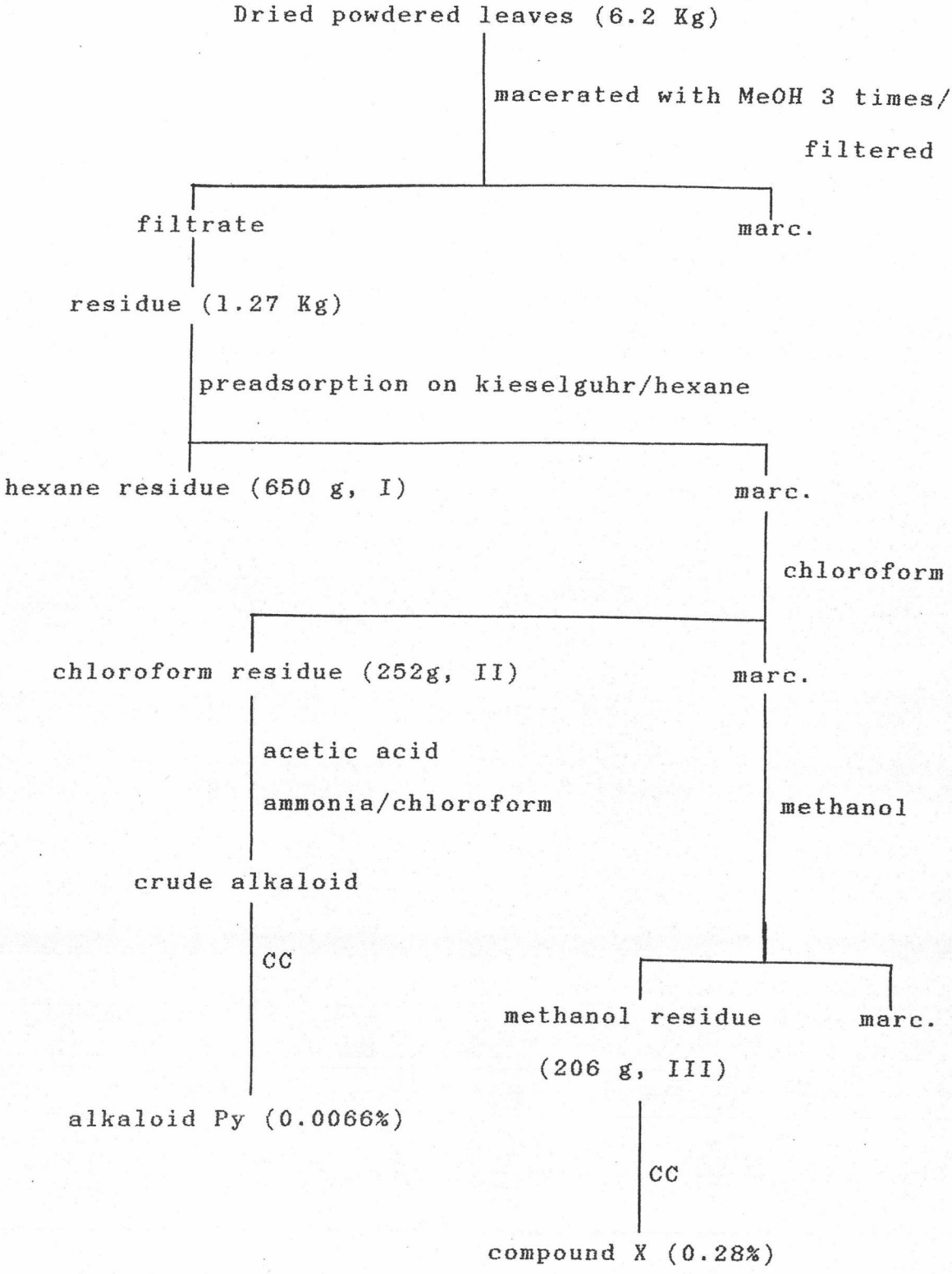

Fig. 1 : The extraction and fractionation of *Aglaia pyramidata* Hance Leaves

Fractionation of chloroform residue

The chloroform residue (252 g) was divided to five portions, each portion (50 g) was subjected to silica gel column in the same manner. It was dissolved in glacial acetic acid (200 ml). The acid solution was then diluted with distilled water (300 ml) until complete precipitation of chlorophyll and some other chloroform soluble impurities had occurred. The precipitate was separated by vacuum filtration. The clear acid filtrate was basified with 25% ammonium hydroxide solution to approximately pH 10, using universal pH paper as an indicator.

The alkaline extract was exhaustively extracted with chloroform to give 4.53 g of the crude alkaloid residue. It was then subjected to silica gel column chromatography, using 4% methanol in chloroform as an eluent. Sixty five fractions (40 ml, each) were collected and the column was finally washed with methanol. Those of similar fraction, on TLC plates were combined and evaporated. Fractions 1 - 18 gave negative tested with modified Dragendorff's reagent and were discarded. Fractions 19 - 32 gave an identical alkaloid positive spot on TLC plate. They were combined and crystallized/ recrystalized from actone to give 0.081 g (0.0066%) of colorless prisms, which was designated as alkaloid Py. The alkaloid Py was identified to be *N*-cinnamoyl-*N'*-benzoyl-1,4-butanediamine.

Fractionation of methanol residue

The methanol residue (206g, III) was divided to four portions, each portion (50 g) was subjected to silica gel column chromatography, using 30 % methanol in chloroform as an eluent. Two hundred and sixteen fractions (40 ml, each) were collected and the column was eluted with methanol. Those similar fractions, on TLC plates, were combined and evaporated to dryness. Fractions 1 - 109 gave negative test with anisaldehyde sulfuric acid spraying reagent and were discarded. Fractions 110-216 gave one opaque spot on TLC plate when sprayed with the same reagent. They were combined and evaporated to give residue. The residue was crystallized in methanol/acetone and subsequently re-crystallized in methanol to yield 4.2 g (0.28 %) of colorless needle. This was designated as compund X. The compound X was identified as *N*-methyl-*trans*-4-hydroxy-L-proline.

Characterization of alkaloid Py

Alkaloid Py was obtained as colorless prism re-crystallized from acetone. It gave positive test with modified Dragendorff's reagent. It is soluble in chloroform, ethanol and methanol.

hRf values

a) 35.6 (4% methanol in chloroform)
(Fig. 4)

b) 2.6 (chloroform)
(Fig. 5)

c) 6.5 (10% acetone in chloroform)
(Fig. 6)

d) 1.5 (30% benzene in chloroform)
(Fig. 7)

e) 15.8 (10% ethyl acetate in chloroform)
(Fig. 8)

Melting point

173 - 174 °C

Elemental analysis (%) (Fig. 9)

Anal. Calcd. : C, 74.53 ; H, 6.83 ; N, 8.70

Found : C, 73.85 ; H, 6.91 ; N, 8.59

Molecular weight

322

Molecular formular

$C_{20}H_{22}N_2O_2$

Ultra-violet absorption spectum (Fig. 10)

λ max (ε) (MeOH) 230 (4280), 245 (3832), 250 (3706), 300 (3938) nm.

Infrared absorption spectum (Fig. 11)

ν max (KBr) (cm^{-1}) 3300, 2950, 1630, 1620, 1530, 1480, 1320, 1210, 980, 960, 760.

Mass spectrum (Fig. 12)

(EIMS)

m/z (% rel int.) 322 (15.29), 217 (1.08), 202 (6.41), 191 (7.64), 188 (9.11), 174 (44.73), 160 (5.30), 148 (11.43), 146 (7.33), 131 (88.19), 105 (100.00), 103 (42.07), 77 (50.67), 70 (40.54).

Proton nuclear magnetic resonance (^1H NMR)

(300 MHz, DMSO-d_6) (Fig. 13)

Chmical shift (ppm)	Proton	multiplicity
1.520	2H	board
1.538	2H	board
3.228	2H	q (*J* 6.7 Hz)
3.325	2H	q (*J* 6.4 Hz)
6.622	1H	d (*J* 15.8 Hz)
7.346 - 7.551	9H	m
7.826	2H	d (*J* 6.3 Hz)
8.136	1H	t (*J* 6.7 Hz)
8.481	1H	t (*J* 6.4 Hz)

Carbon-13 nuclear magnetic resonance (^{13}C NMR) (75 MHz, DMSO-d_6) (Fig. 15)

Chemical shift (ppm)	
26.724	2 CH_2
38.604	CH_2
38.891	CH_2
122.338	CH
127.089	2 CH
127.428	2 CH
128.184	2 CH
128.881	2 CH
129.317	CH
130.961	CH
134.677	C
134.941	C
138.398	CH
164.846	C
166.113	C

Characterization of compound X

Compound X was obtained as colorless needles recrystallized from methanol. It was soluble in water, hot methanol, slightly soluble in methanol and insoluble in other non polar organic solvents.

hRf values

a) 30.1 (50% methanol in chloroform)
(Fig. 17)

b) 4.2 (30% ethanol in chloroform)
(Fig. 18)

c) 14.7 (50% acetone in methanol)
(Fig. 19)

d) 11.6 (50% ethyl acetate in methanol)
(Fig. 20)

e) 40.5 (30% amonia in n-propyl alcohol)
(Fig. 21)

Melting point

237 - 239 ^{o}C (decomp.)

Elemental analysis (%) (Fig. 9)

Anal. Calcd. : C, 49.66 ; H, 7.59 ; N, 9.66
Found : C, 49.71 ; H, 8.08 ; N, 9.76

Molecular weight

145

Molecular formular

$C_6H_{11}NO_3$

Specific rotation

$[\alpha]_D^{25}$ = -83.3 (c 0.1, water)

Ultra-violet absorption spectrum (Fig. 22)

λ max (ε) (H_2O) 217 (2610), 240 (410) nm.

Infrared absorption spectrum (Fig. 23)

ν max (KBr) (cm^{-1}) 3250, 2920, 2500, 1625, 1400, 1300, 1200, 1070, 920, 700.

Mass spectrum (Fig. 24)

(EIMS)

m/z (% rel int.) 145 (4.02), 100 (100.00), 82 (37.65), 42 (23.9).

Proton nuclear magnetic resonance (^{1}H NMR)

(90 MHz, D_2O) (Fig. 25)

Chmical shift (ppm)	Proton	multiplicity
2.21	1H	ddd (J 4.6, 10.7, 14.1 Hz)
2.49	1H	dddd (J 2.1, 7.6, 14.1 Hz)
3.05	3H	s
3.07	1H	ddd (J 2.1, 12.9 HZ)
3.95	1H	dd (J 4.6, 12.9 Hz)
4.18	1H	dd (J 7.6, 10.7 Hz)
4.62	1H	m

Carbon nuclear magnetic resonance (^{13}C NMR)

(22.5 MHz, D_2O) (Fig. 27)

Chemical shift (ppm)	multiplicity
40.847	t (CH_2)
45.723	q (CH_3)
65.280	t (CH_2)
72.052	d (CH)
72.648	d (CH)
175.417	s (C)