

CHAPTER III

EXPERIMENTAL

1. General Techniques

1.1 Thin Layer Chromatography (TLC)

Analytical

Technique	: one way, ascending
Adsorbent	: a) silica gel G (E. Merck), calcium sulfate binder 13%, 30g/60ml of distilled water b) aluminium oxide G (E. Merck), calcium sulfate binder 10%, 35g/40ml of distilled water
Plate size	: 10 cm X 20 cm
Layer thickness	: 0.25 mm
Activation	: air dried for 15 minutes/heat at 110°C for 1 hour
Solvent systems	: a) diethyl ether : acetone (9:1) b) chloroform : ethyl acetate (4:6) c) chloroform
Distance	: 15 cm
Laboratory temperature	: 25-30°C
Detection	: Dragendorff's spray reagent (72), giving orange color

1.2 Column Chromatography (CC)

Column : flat bottom glass column of 10 cm in diameter

Adsorbent : silica gel 0.040-0.063 mm (E. Merck)

Packing method : wet packing

Solvents : a) chloroform : ethyl acetate (7:3)
b) methanol

1.3 Melting Point

Melting point was determined on Melting Point Apparatus (Gallenkamp) and was uncorrected.

1.4 Ultraviolet Absorption Spectrometry

Ultraviolet absorption spectrum was measured on a Shimadzu Double-Beam Spectrophotometer UV-180.

1.5 Infrared Absorption Spectrometry

Infrared absorption spectrum was obtained on a Perkin Elmer Model 283 Spectrophotometer, absorption bands were reported in wave numbers (cm^{-1}).

1.6 Nuclear Magnetic Resonance (NMR) Spectrometry

^1H and ^{13}C nmr spectra were recorded at 90 MHz on Nuclear Magnetic Resonance Spectrophotometer Model FX 900 (JEOL). Tetramethylsilane was used as an internal standard and chemical shifts were reported on the ppm scale.

1.7 Mass Spectrometry

The low resolution mass spectrum was obtained on a Mass Spectrometer Model DX 300 (JEOL), operating at 70 eV. The high resolution mass spectral study was per-

formed on a CEC 110B Double Focusing Mattauch Herzog spectrometer. The spectrum was recorded on Ionomet plates (deposited AgBr) (Dupont Industries, Monrovia, California).

1.8 Elemental Analysis

Elemental analyses were performed on an Elemental Analyzer Model 240C, Perkin Elmer.

1.9 Optical Rotation

The optical rotation was determined on Polarimeter Model A, Bellingham & Stanley Limited and was uncorrected.

2. Phytochemical Screening

Powdered leaf material (100 g) was macerated with methanol (200 ml) over night. After the mixture was filtered, it was concentrated to a syrupy mass on a steam bath. It was diluted with equal volume of distilled water. The aqueous methanolic extract was partitioned three times with petroleum ether. The aqueous methanolic extract remaining after the petroleum ether shaking out was then partitioned three times with chloroform. The petroleum ether extract and the chloroform extract were saved for further screening procedure.

2.1 Screening for Sterols

The petroleum ether fraction was evaporated to dryness on a steam bath. After the contents were cooled to room temperature, it was used for the Liebermann-Burchard test. The sample, 3 drops of acetic anhydride, and 1 drop

of conc. H_2SO_4 when mixed yielded a progression of colors from blue to green indicating the presence of sterols.

2.2 Screening for Alkaloids

The chloroform fraction was evaporated to dryness on a steam bath. After the contents were cooled to room temperature, a small amount of dil HCl was added to dissolve the residue. After filtration, the filtrate was used for the precipitation test with alkaloidal reagents. The results were shown positive with Dragendorff's and Mayer's reagents.

3. Isolation of Chemical Substances from *Aglaia piriifera* Hance Leaves

3.1 Extraction

Dried powdered leaves (3 kg) were macerated three times for 7 day-periods with methanol (14 l, 13 l, and 13 l). The alcoholic extract was evaporated under reduced pressure to give a syrupy mass and was fractionated according to Fig.1. The syrupy mass was partitioned with n-pentane (2 l) in a continuous liquid-liquid extractor until the pentane layer became colorless. After the two layers were separated, the pentane layer was evaporated to give 155 g of pentane soluble residue. The residue gave negative test for alkaloid, however, it gave positive result with Liebermann-Burchard's test for sterols. This matter was not further investigated. The remain methanolic fraction was suspended in equal volume of distilled water. The aqueous alcoholic solution was exhaustively extracted

with chloroform to give on evaporation 10 g of residue containing crude alkaloids. The crude extract was subjected to silica gel column chromatography, using CHCl_3 : EtOAc (7:3) as eluent. One hundred and fifty fractions (20 ml, each) were collected and the column was washed with methanol. Those of similar fractions when examined on tlc were combined and evaporated. Fractions 1-50 gave negative tests with alkaloidal reagents and was discarded. Fractions 51-150 gave one spot on three tlc systems. They are : 1) silica gel G/diethyl ether : acetone (9:1), 2) silica gel G/chloroform : ethyl acetate (4:6), 3) alumina/chloroform. The combined fractions yielded 2.25 g of pale yellow powder.

3.2 Purification of Isolated Compound

The combined pale yellow powder was crystallized in chloroform/diethyl ether which on recrystallization yielded 1.55 g of colorless prisms. This was designated as Ag and was subsequently identified as N-cinnamoyl-2-(2-methylpropanoylamino)pyrrolidine.

4. Examination of Alkaloid Ag

Ag was obtained as colorless prisms. It was soluble in diethyl ether, chloroform and methanol.

The following data were obtained on sample of alkaloid Ag:

hRf values

- a) 53 (Fig.5, Page 60)
- b) 26 (Fig.6, Page 61)

c) 66 (Fig.7, Page 62)

Melting point

164.5-165.5°C

Ultraviolet absorption spectrum (Fig.8, Page 63)

λ_{max} (EtOH) 283 nm ($\log \epsilon = 4.20$).

Infrared absorption spectrum (Fig.9, Page 64)

ν_{max} (KBr) 3250, 3050, 2960, 2860, 1640, 1610, 1540, 1490, 1390, 1320, 1240, 1190, 1050 cm^{-1} .

Nuclear Magnetic Resonance spectrum

^1H nmr (90 MHz, CDCl_3) δ 1.10 (d, J 8, 3H), 1.18 (d, J 8, 3H), 1.95 (m, 4H), 2.50 (m, J 8, 1H), 3.46 (m, 2H), 6.13 (t, 1H), 6.83 (d, J 14, 1H), 7.30-7.50 (m, 5H), 7.76 (d, J 14, 1H). (Fig.10, Page 65)

^{13}C nmr (CDCl_3) δ 176.35 (s), 165.85 (s), 142.71 (d), 135.07 (s), 129.87 (d), 128.89 (d), 128.24 (d), 118.49 (d), 63.01 (d), 46.22 (t), 35.54 (d), 34.57 (t), 21.73 (t), 19.51 (q), 19.40 (q).

Mass spectrum (Fig.12, Page 67)

m/e 286 (M^+ , 7.15%, $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_2$), 216 (2.19%), 215 (11.50%, $\text{C}_{13}\text{H}_{15}\text{N}_2\text{O}$), 200 (6.41%), 199 (27.75%, $\text{C}_{13}\text{H}_{13}\text{NO}$), 156 (3.84%), 155 (38.61%, $\text{C}_8\text{H}_{15}\text{N}_2\text{O}$), 132 (11.73%), 131 (100%, $\text{C}_9\text{H}_7\text{O}$), 130 (1.57%), 104 (4.37%), 103 (37.07%, C_8H_7), 102 (5.36%), 91.09 (2.09%), 88 (3.40%), 87 (1.10%, $\text{C}_4\text{H}_9\text{NO}$), 86 (5.34%), 85 (59.66%, $\text{C}_4\text{H}_9\text{N}_2$), 77 (18.67%), 71 (3.99%, $\text{C}_4\text{H}_7\text{O}$), 70 (10.31%), 69 (8.17%), 68 (7.32%), 57 (2.88%), 56 (1.79%).

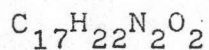
Elemental analysis

Anal. Calcd. : C, 71.30; H, 7.74; N, 9.78

Found : C, 71.31; H, 7.82; N, 9.73

Molecular weight

286

Empirical formulaSpecific rotation

$$\left[\alpha \right]_D^{28} + 30^\circ \quad (c = 0.01 \text{ in abs. alc.})$$

5. The Picrate Salt of Ag

A solution was prepared by dissolving 57 mg (0.2 mmol) of Ag in a small amount of absolute ethanol. Forty five milligrams (0.2 mmol) of picric acid was dissolved in a small amount of absolute ethanol. The acid solution was poured into the alkaloid solution. The mixture was evaporated to a small volume under the stream of nitrogen gas. After it was left overnight at room temperature and the resinous mass was produced. Attempt to crystallize the salt by mean of absolute ethanol was unsuccessful.