CHAPTER IV

DISCUSSION

In the investigation of *Aglaia pirifera* Hance, the leaf powders were exhaustively extracted with methanol by maceration method. The phytochemical screening of this plant suggested the presence of sterols and alkaloids. In the experiment, the concentrated alcoholic extract was partitioned with pentane in a continuous liquid-liquid extractor. This procedure removed most of the chlorophyll pigments and some lipid materials from the aqueous methanolic extract. The presence of sterols in the pentane extract was indicated by a positive Liebermann-Burchard test. When the aqueous alcoholic extract was partitioned with chloroform, alkaloids and some other chloroform soluble materials were removed from the aqueous alcoholic phase.

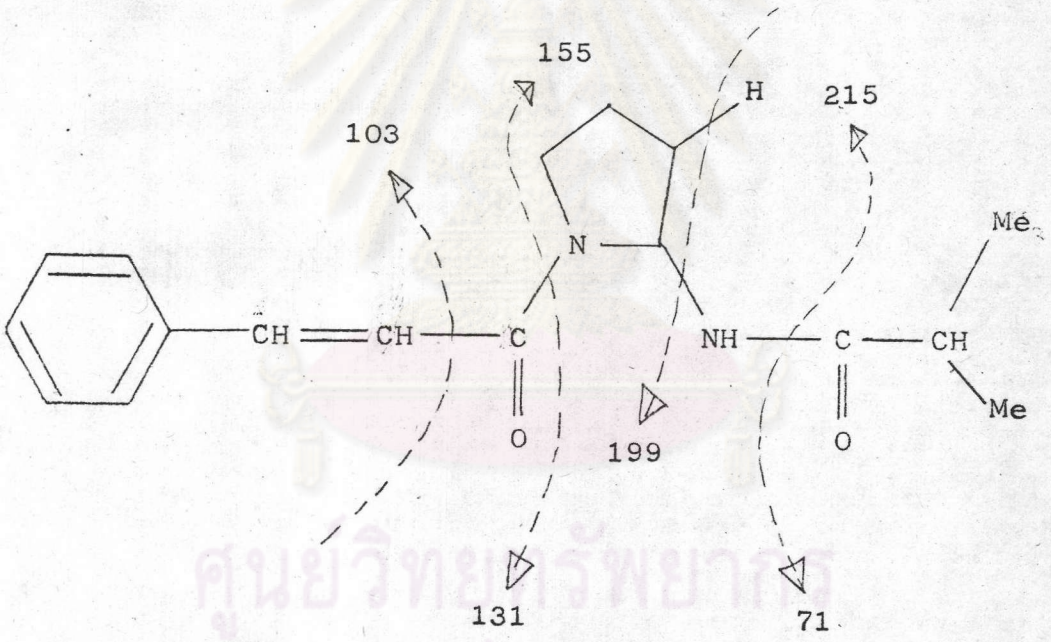
The separation process of individual substances was based on the use of adsorption chromatographic methods. The column chromatographic procedure followed the technique called short column chromatography. This technique was devised and pioneered by Rigby and Hunt (73) in 1967. The separative power of a short column chromatographic method was considerable. This method provides reliable preparative separations of mixtures. Moreover, separations are carried out more rapidly and with less solvent than conventional techniques. It is essential that the appropriate solvent

system be employed, and that the column be packed uniformly. Examination of a mixture to be separated by analytical tlc in several mixed solvent systems enabled selection of an eluting solvent mixture yielding the best separation of the components. Ideally, these components should appear as spots at about hRf values of 30-40 on the plate. Moving these spots around with various solvent combinations of differing polarities were carried out to ensure that each spot was a single compound. Having selected the best solvent system for tlc, the polarity for the column eluent can then be chosen. Since a compound on a column runs somewhat faster than on a plate, the concentration of the more polar component in the column eluent was usually decreased to about 50% of that found to be suitable for analytical tlc. The best system for analytical tlc was found to be chloroform/ethyl acetate (4:6), the eluent mixture used for the column separation was chloroform/ethyl acetate (7:3). The main advantage of using this method of chromatography is that the columns are short resulting in rapid separations, efficient solvent utilization, and excellent material recovery.

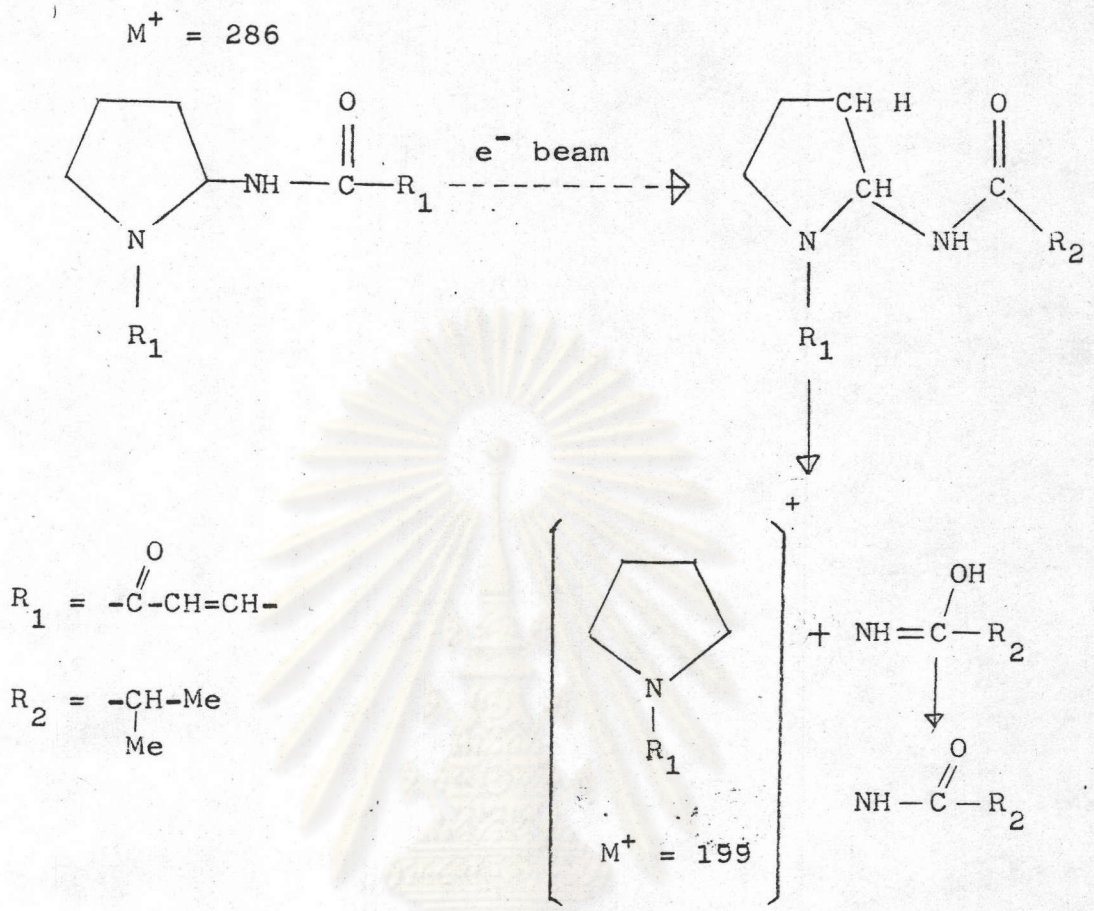
From this study, a colorless crystalline alkaloid (Ag) was isolated from the column. The high resolution mass spectral study of Ag showed molecular ion at *m/e* 286.15813 corresponding to the formula $C_{17}H_{22}N_2O_2$. This result is in agreement with the elemental analysis data which confirms the empirical formula of Ag to be $C_{17}H_{22}N_2O_2$. This compound was identified to be N-cinnamoyl-2(2-methyl-

propanoylamino)pyrrolidine which is apparently a new natural product. Alkaloid Ag is being given the name "piriferine" after the specific name of the plant.

The mass spectrum of piriferine (Fig.12, Page 67) showed the base peak at *m/e* 131.04969 which corresponded to the loss of 2-methylpropanoylamino pyrrolidine moiety. The strong peak at *m/e* 215.11844, 155.11844, and 71.04969 resulting from the loss of isobutanoyl, cinnamoyl and N-cinnamoyl-2-aminopyrrolidine fragments respectively.



In addition, there was a peak at *m/e* 199.09971 corresponding to the loss of 2-methyl propanoic acid amide presumably by a McLafferty rearrangement(74).

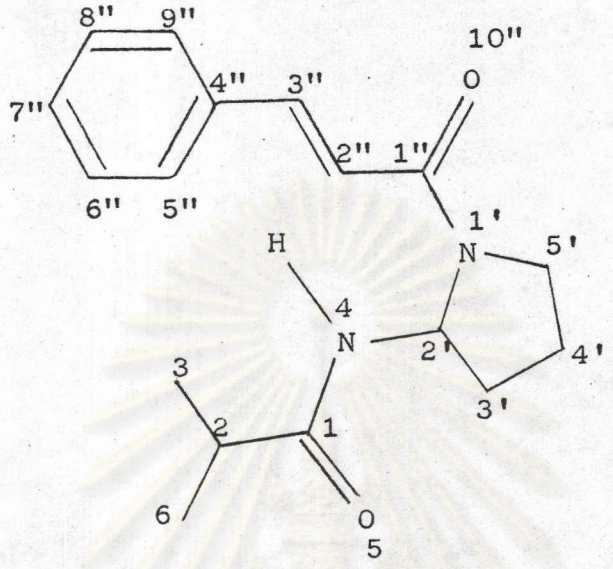


The ir spectrum of piriferine (Fig.9, Page 64) showed NH stretching at ν_{max} 3250 cm^{-1} and the carbonyl stretching at ν_{max} 1640 cm^{-1} and at 1610 cm^{-1}, suggesting the presence of secondary amide and tertiary amide in the nucleus respectively.

The uv spectrum of piriferine (Fig.8, Page 63) revealed the presence of cinnamic acid moiety.

The proton nmr (Fig.10, Page 65) showed that the molecule is a secondary-tertiary bis-amide of a monocyclic unit $C_4H_8N_2$ comprising two methylenes, a methylene bearing nitrogen, and a methine bearing two nitrogens. The corresponding ^1H resonance appeared at δ 1.95 (4H, m), 3.46 (2H, m) and 6.13 (2H, t) respectively. The complete proton

chemical shifts can be assigned as shown in Table III.



Chemical Shift (δ)	Proton	Multiplicity	Coupling Constants
1.10	Gem dimethyl	d	J = 8 Hz
1.18	(6H)		
1.95	3', 4' (4H)	m	
2.50	2(H)	m	J = 8 Hz
3.46	5'(2H)	m	
6.13	2'(H)	t	
6.83	2"(H)	d	J = 14 Hz
7.30-7.50	Ar(5H)	m	
7.76	3"(H)	d	J = 14 Hz

Table III The complete assignment of the 90 MHz proton nmr spectrum ($CDCl_3$) in δ value (ppm) from tetramethylsilane

The ^{13}C nmr spectrum (Fig.11, Page 66) of piriferine was produced to confirm the structure. The result was compared with literature values for N-cinnamoyl-2-(methylpropanoylamino)pyrrolidine compound (75) (shown below in parentheses). The chemical shifts could be assigned to carbons in the molecule as indicated in Fig.2.

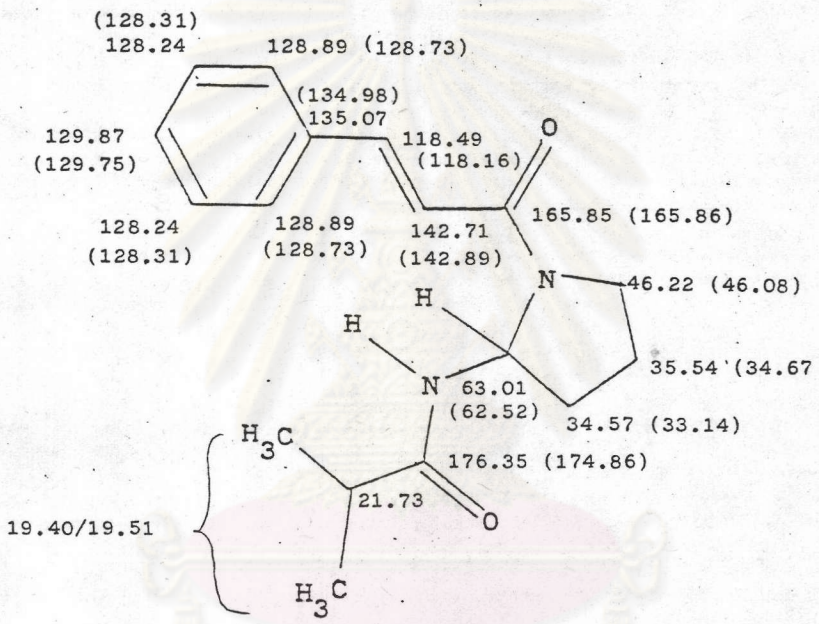


Fig.2 ^{13}C-nmr a signment of piriferine

From the above discussion, it could be concluded that the structure of new alkaloid piriferine is N-cinnamoyl-2-(2-methylpropanoylamino)pyrrolidine (Fig.3).

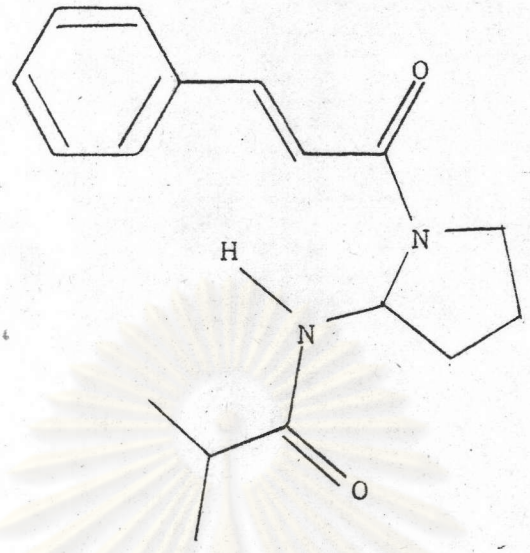


Fig.3 Structure of piriferine

From the literature survey, it was found that the bis-amide of 2-aminopyrrolidine alkaloid was first isolated from *Aglaia roxburghiana* Hiern in 1979 by Purushothaman and co-workers (66). The name roxburghiline (XXXVI) was given to this alkaloid according to its specific name. In the same year, Shiengthong and co-workers (65) worked on the plant called *Aglaia odorata* Lour. and reported the isolation of alkaloid odorine (XXXVI) and odorinol (XXXVII). From the structures of roxburghiline and odorine, it was apparent that these two compounds are identical. Both structures were reported in the same year (1979), and the authors were probably unaware of each other's work. According to Shiengthong's work (76), the picrate salt formation of odorine and odorinol was being attempted but negative result was obtained. This coincide with our re-

sult on piriferine.

In addition, to figure out the stereochemistry of odorine, the synthesis of (-)odorine and (+)dihydroodorine was undertaken (77). The synthetic pathway was started with L-proline and the chemical reaction are summerized in Fig.4.

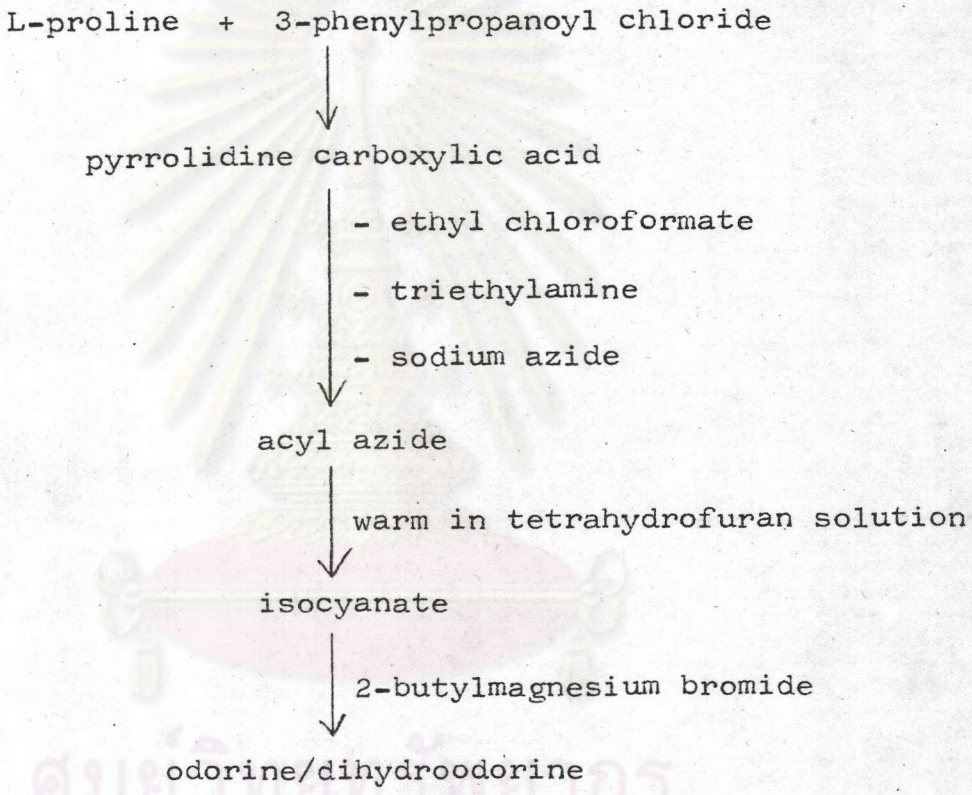


Fig.4 Synthetic pathway of odorine and dihydro-odorine

From this experiment, it was found that the stereochemistry of naturally occurring odorine has been established as (+)-(E,2S,2'R)-2-methyl-N-1'-(1"-oxo-3"-phenyl-prop-2"-enyl)pyrrolidine-2'yl butanamide.

In 1982, Hayashi *et al.* (75) had worked on the

leaves and twigs in Chinese Folklore, Shu-Lan, (*Aglaia odorata* Lour.) it was found that the methanolic extract of the leaves and twigs of the plant showed significant inhibitory activity *in vivo* against P-388 lymphocytic leukemia growth in BDF_1 male mice (T/C = 145%) at 50 mg/kg/day, i.p. From this investigation, the isolation of (-)odorinol was reported as a major active principle of this plant. It was assumed that odorinol was responsible for the antileukemic activity of this plant, however, no firm evidence has been established to prove this matter.

The result of this present investigation exhibited the homogeneity in term of chemical constituents in the genus *Aglaia*. The data obtained are not sufficient to use as evidence in chemotaxonomy until more exhaustive studies of the plants in genus *Aglaia* and some other Meliaceous plants are done.