



CHAPTER IV

DISCUSSION

In the investigation of the leaves of *Aglaia pyramidata* Hance, alkaloid Py and compound X were isolated from the chloroform and methanol residue, respectively.

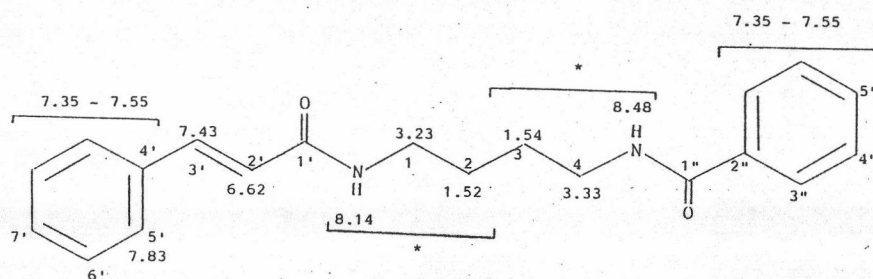
The alkaloid Py was obtained as colorless prisms. Mass spectrum (Fig. 12) showed molecular ion peak at m/z 322, in agreement with the elemental analysis data (Fig. 9) (C 73.85% : H 6.91% : N 8.59%) which indicated the formula of alkaloid Py to be $C_{20}H_{22}N_2O_2$.

The absorption in the UV spectrum (MeOH) (Fig. 10) at λ_{max} 300 (ϵ 3938) and 245 (ϵ 3832) nm suggested the presence of cinnamoyl and benzoyl moiety, respectively. The IR spectrum (Fig. 11) showed NH stretching at ν_{max} 3300 cm^{-1} and CO stretching at ν_{max} 1630 and 1620 cm^{-1} of secondary amide.

The 1H 1-D and 2-D COSY NMR spectra of alkaloid Py (Fig. 13 and 14) indicated the presence of the cinnamoyl part : two aromatic protons at δ 7.83 (d) ppm showing *ortho* coupling (J 6.3 Hz) with the overlapping aromatic protons at δ 7.35-7.55 (m) ppm, an olefinic proton at δ 6.62 (d) ppm showing *trans* coupling (J 15.8 Hz) with the proton at δ 7.43 ppm, the other aromatic protons of cinnamoyl part were in the region of δ 7.35-7.55 (m) ppm.

In addition, the remaining five aromatic protons in the region of δ 7.35-7.55 (m) ppm were assigned as benzoyl protons. The two N-methylene fractions were confirmed by the presence of rather low field quartet signals at δ 3.23 and 3.33 ppm. These signals showed vicinal coupling (J 6.7 Hz) with methylene protons at δ 1.52 and 1.54 ppm and the two amide protons at δ 8.14 and 8.48 ppm, respectively. The 2-D COSY NMR spectrum showed the connectivity of the methylene protons at δ 1.52 and 1.54 ppm. This phenomena indicated the presence of 1,4-butanediamine fragment ($-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}-$).

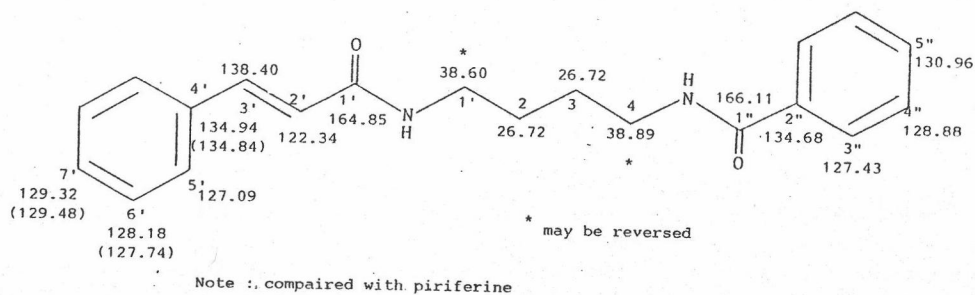
Therefore, the structure of the alkaloid Py was proposed as *N*-cinnamoyl-*N'*-benzoyl-1,4-butanediamine (109).



(109)

The proposed structure was substantiated by the ^{13}C and 2-D HETCOR NMR spectra (Fig. 15 and 16). The fulling decoupling and APT ^{13}C NMR showed the presence of 22 carbons, 2 amide carbonyls, 2 quarternary aromatic

carbons, 10 unsubstituted aromatic carbons, 2 olefinic carbons and 4 methylene carbons. With the reference of complete proton chemical shift assignments and 2-D HETCOR data, the ^{13}C chemical shift assignment (Table 2) were established. Thus, the complete ^{13}C chemical shift assignments was shown as follow (110) :



(110)

The mass spectrum (Fig. 12) showed the base peak at m/z 105 corresponded to the formation of benzoyl ion which subsequently loss of carbonyl (CO) to form benzyl ion at m/z 77. The fragments ion at m/z 131 and 103 corresponded to the formation of cinnamoyl ion which subsequently loss of carbonyl (CO) to form phenyl ethylene ion, respectively. Further loss of acetylene part form phenyl ethylene moiety give ion to the formation of benzyl ion at m/z 77. In addition, the mass spectrum showed ion peaks at m/z 160 and 134 corresponding to the formation of N-methylene cinnamoyl and N-methylene benzoyl ions via the α -cleavage, respectively. The mass fragmentation pattern could be shown in Fig. 2.

Table 2 : ^1H and ^{13}C (HETCOR) NMR assignments of alkaloid
Py

position	^1H (ppm)	^{13}C (ppm)
1	3.23	38.60
2	1.52	26.72
3	1.54	26.72
4	3.33	38.89
1'	-	164.85
2'	6.62	122.34
3'	7.43	138.40
4'	-	134.94
5'-9'	7.83	127.09
6'-8'	7.35-7.55	128.18
7'		129.32
1''	-	166.11
2''	-	134.68
3''-7''		127.43
4''-6''	7.35-7.55	128.88
5''		130.96

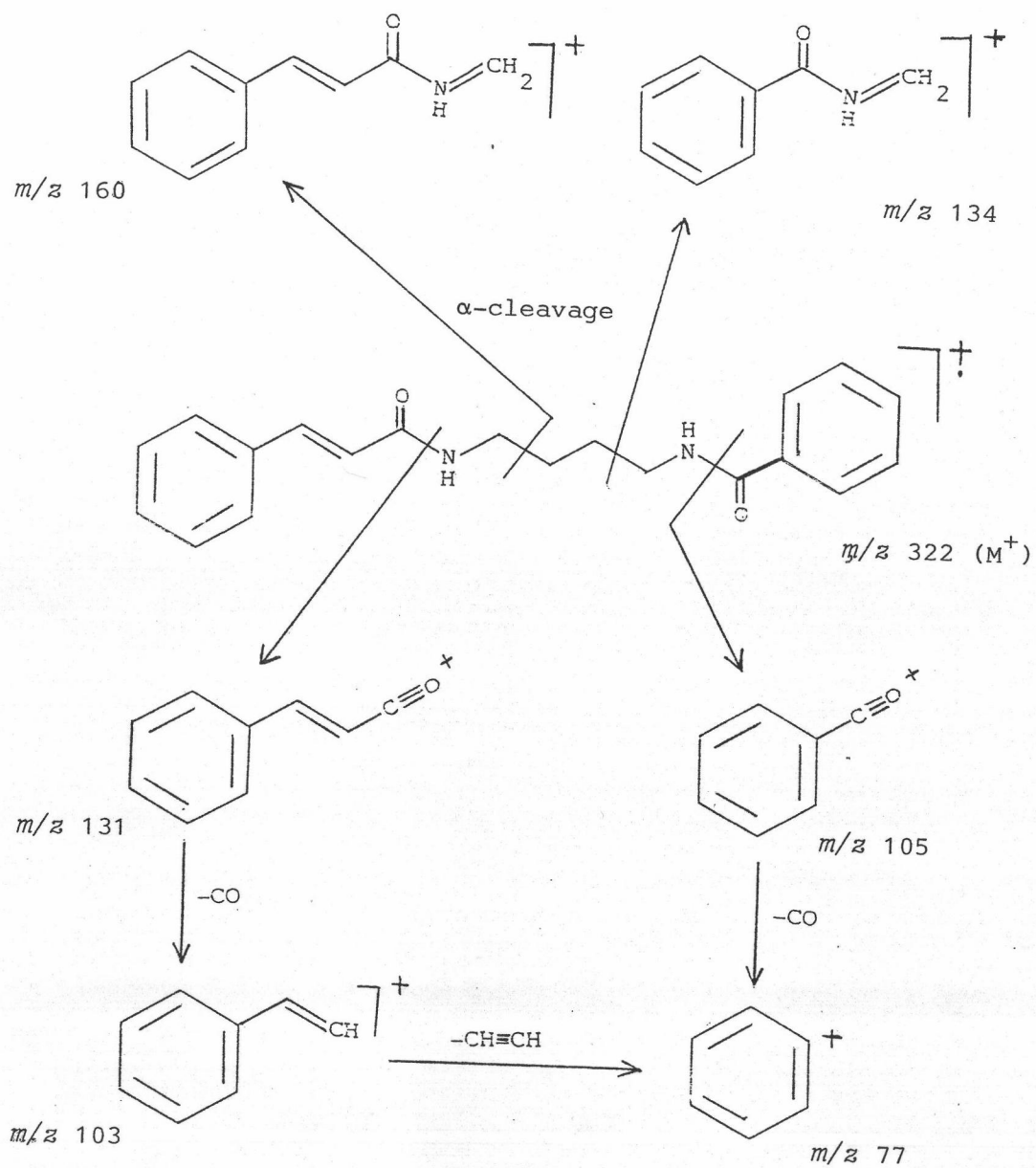


Fig. 2 Massfragmentation pattern in the EI mass spectrum of alkaloid Py

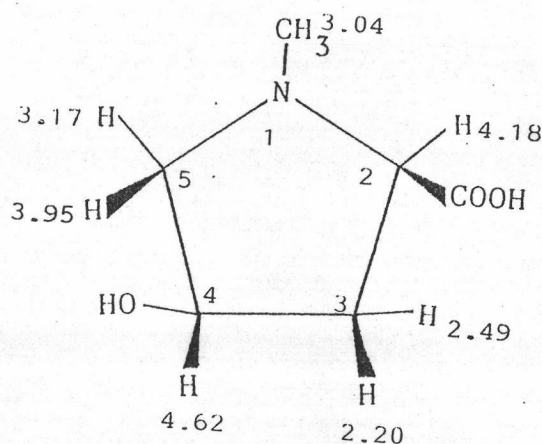
From the spectral evidence, it could be concluded that alkaloid Py is *N*-cinnamoyl-*N'*-benzoyl-1,4-butane-diamine.

The compound X was obtained as colorless needles. Mass spectrum (Fig. 24) showed molecular ion peak at m/z 322, in agreement with the elemental analysis data (Fig. 9) (C 49.66% : H 8.08% : N 9.76%) which indicated the formula of compound X to be $C_6H_{11}NO_3$.

The IR spectrum (Fig. 23) showed OH stretching at ν_{max} 3250 cm^{-1} and CO stretching of carboxylic acid at ν_{max} 1625 cm^{-1} .

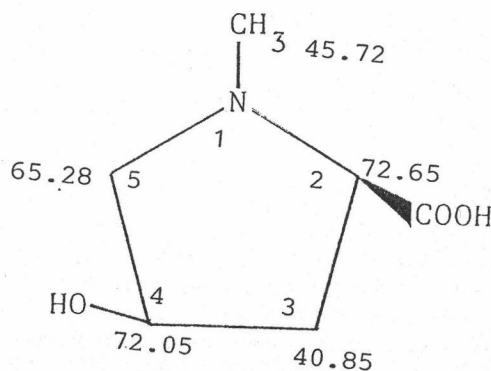
The 1H NMR spectrum of compound X (90 MHz, D_2O) (Fig. 25) contained, in addition to a three protons singlet (N-CH₃) at δ 3.05 ppm, an ABMX system for H-3a, H-3b (AB part), H-2 (M), H-4 (X) whose X multiplet was further split by the A'M'X pattern involving H-4, H-5a and H-5b. The chemical shift and coupling constants, obtained from this analysis with the aid of extensive decoupling and 2-D COSY NMR experiments (Fig. 26) were : a) for the ABMX system δ H-3a 2.49 ppm, δ H-3b 2.21 ppm, δ H-2 4.18 ppm and δ H-4 4.62 ppm ($J_{3a,b}$ 14.1 Hz, $J_{3a,2}$ 7.6 Hz, $J_{3b,2}$ 10.7 Hz, $J_{3a,4}$ 2.1 Hz and $J_{3b,4}$ 4.6 Hz) ; and b) for the A'M'X system δ H-5a 3.07 ppm and δ H-5b 3.95 ppm ($J_{5a,b}$ 12.9 Hz, $J_{5a,4}$ 2.1 Hz and $J_{5b,4}$ 4.6 Hz). It is to be noted that, while in *trans*-4-hydroxyproline, whose 1H NMR spectrum had been analysed in detail by Abraham *et al.* (1964),

protons at C-5 had very similar chemical shift, in compound X the effect of the N-methyl group, deshielding on the *trans* proton and shielding on the *cis* proton (Becconsall and co-workers (1965)), introduces a difference of 0.88 ppm between the chemical shifts of H-5a and H-5b; consequently, the A'B'X pattern in *trans*-4-hydroxyproline is replaced by an A'M'X system in compound X assumes preferentially the conformation in which carboxylate and methyl group bear a *trans* relationship to each other was evidenced by the chemical shifts of the C-5 protons and the relevant vicinal coupling constants. Therefore, the complete ^1H chemical shift assignments was shown as follow (111) :



The proposed structure was substantiated by the ^{13}C and 2-D HETCOR NMR spectra (Fig. 27 and 28). The fulling coupling and decoupling ^{13}C NMR showed the present of 6 carbons, 1 carbonyl, 2 methylene carbons, 2 methine

carbons and 1 methyl carbon. With the reference of complete proton chemical shift assignments and 2-D HETCOR data, the ^{13}C chemical shift assignment (Table 3) were established. Thus, the complete ^{13}C chemical shift assignments was shown as follow (112) :



(112)

Table 3 : ^1H and ^{13}C (HETCOR) NMR assignments of compound X

position	^1H (ppm)	^{13}C (ppm)
N-CH ₃	3.04	45.72
2	4.18	72.65
2-COOH	-	175.42
3	2.20 , 2.49	40.85
4	4.62	72.05
5	3.17 , 3.95	65.28

The mass spectrum (Fig. 24) showed base peak and intense peak at m/z 100 and 82 the coresponded to the loss of carboxylic moiety from the molecular ion and subsequently loss of H_2O , respectively. The mass fragmentation pattern could be shown in Fig. 3.

The melting point, IR, MS, 1H and ^{13}C NMR data obtain were in full agreement with those of reported values for *N*-methyl-*trans*-4hydroxyl-L-proline (111) (Sciuto and co-worker (1983) (Figliuolo *et al.* (1987))). Therefore, the spectral evidence, it could be concluded that compound X is *N*-methyl-*trans*-4-hydorxy-L-proline.

The compound X was previously found in the red algae (*Chondia coerulescens*, family Rhodomelaceae) (Sciuto and co-worker (1983)) and in the leaves and seeds of *Copaifera multijuga* and *C. langsdorfii* (family Caesalpianaceae, collected from Brazil) (Figliuolo *et al.* (1987)). This compound could inhibited the larval developement of seed feeding bruched beetle (*Callosobruchus maculatus*) and had significant feeding deterrence of leaf-feeding lepidoptera (*Spodoptera littoralis*).

From the phytochemical studies of the leaves of *Aglaia pyramidata* Hance, a new natural alkaloid, *N*-cinnamoyl-*N'*-benzoyl-1,4-butanediamine (109) and an amino acid, *N*-methyl-*trans*-4-hydroxy-L-proline (111), were isolated.

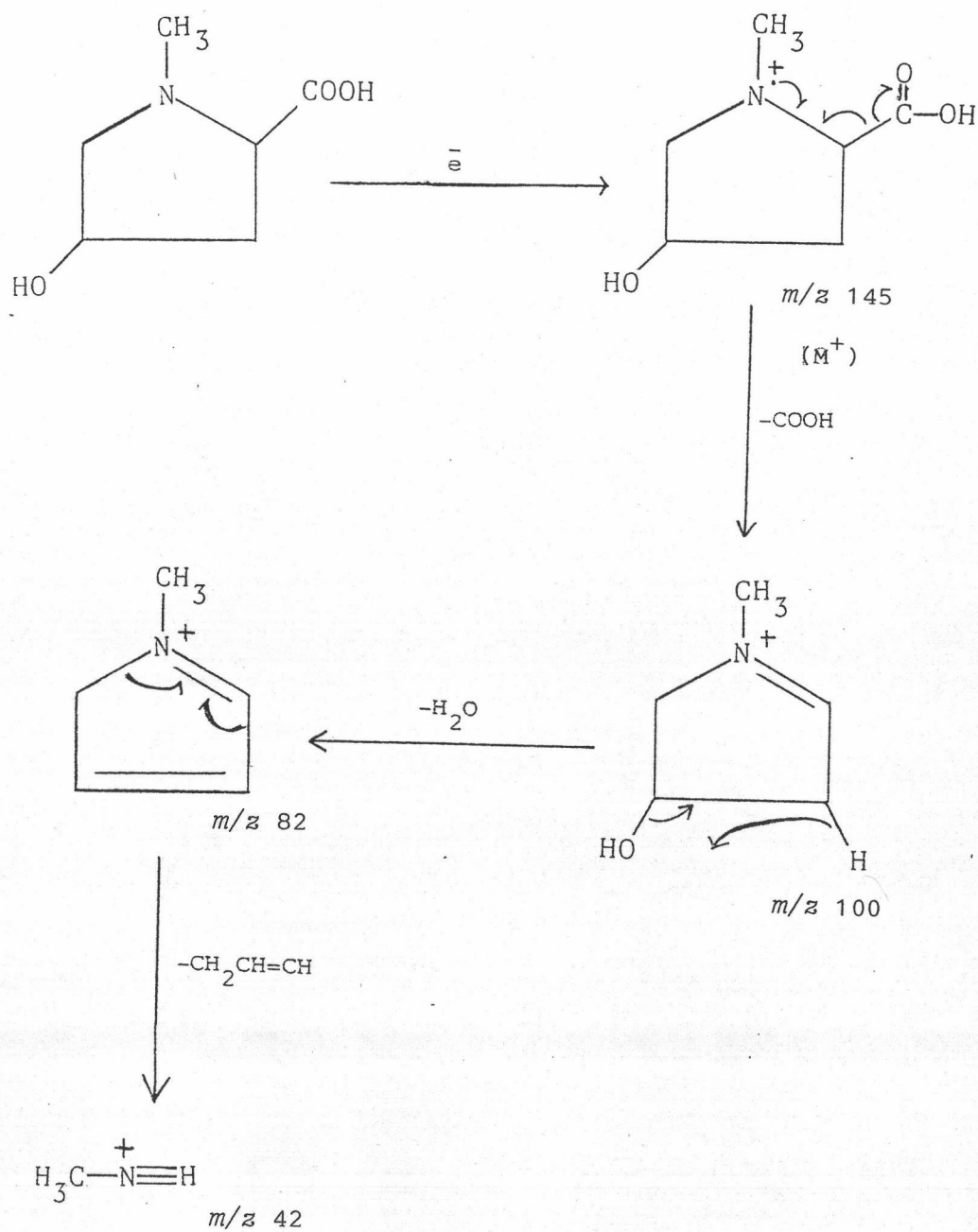


Fig. 3 Massfragmentation pattern in the EI mass spectrum of compound X

The result of this present investigation exhibited the homogeneity in term of chemical constituents in the genus *Aglaia*. However, the data obtained are not sufficient to concluded chemotaxonomy of genus *Aglaia* until more studies of the plants in the genus *Aglaia* are done.