

CHAPTER IV

DISCUSSION

In the investigation of the chemical constituents of the leaves of *Aglaia rubiginosa* (Hiern) Pannell, compound NAT01 was isolated from the chloroform and methanol residue. NAT01 gave positive result with Dragendorff's reagents suggest its alkaloid nature.

The structure elucidation of NAT01

Compound NAT01 was crytalized as colorless needles . The mass spectrum of NAT01 (Fig.3 page 122) showed molecular ion peak at m/z 298, suggesting its molecular formular as containing even number of nitrogen atoms corresponding to the molecular formular $C_{18}H_{22}N_2O_2$.

The IR spectrum (Fig.5 page 124) showed NH stretching band at ν 3285 cm^{-1} and CO stretching of secondary amide at ν 1655 cm^{-1} and 1677 cm^{-1} .

The ^{13}C -NMR and DEPT spectra (Fig. 12 page 131 and Fig. 13 page 132) showed the presence of 15 sets of carbon signals; 2 carbonyls carbons [δ 168.60 ppm (C-1''), δ 172.00 ppm (C-2)], 1 quaternary aromatic carbons [δ 136.2 ppm (C-3)], 1 quaternary olefinic carbon [δ 135.9 ppm (C-4)], 5 unsubstituted aromatic carbons [δ 129.4 ppm (C-5''/C-9''), δ 130.1 ppm (C-6''/C-8''), δ 130.7 ppm (C-7'')], 3 olefinic carbons [δ 121.88 ppm (C-2''), δ 132.5 ppm (C-4), δ 140.8 ppm (C-3'')], 5 methylene carbons [δ 27.8 ppm (C-2'/C-3'), δ 40.2 ppm (C-4'), δ 40.3 ppm (C-1'), δ 59.5 ppm (C-5)], 1 methyl carbon [δ 13.00 ppm (C-6)]

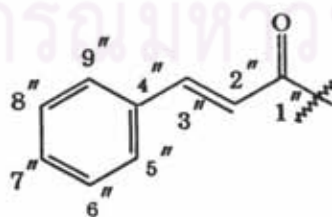
The 1H and 1H - 1H COSY NMR spectra of NAT01 (Fig.6 page 125 and Fig. 14 page 133) indicated the presence of cinnamoyl (311), butylamine (312) and 3-methyl-3-pyrrolin-2-one (313) moieties.

The cinnamoyl moiety exhibited three sets of the aromatic protons signals at δ 7.54 ppm (2H, *dd*, $J=7.63, 1.53\text{Hz}$), δ 7.38 ppm (2H, *m*), δ 7.36 ppm (1H, *m*) and two sets of olefinic protons signals at δ 7.52 ppm (1H, *d*, $J=15\text{Hz}$), δ 6.59 ppm (1H, *d*, $J=15\text{Hz}$) which was assigned as proton at position 5''/9'', 6''/8'', 7'', 3'', 2'' respectively. An olefinic proton signal at δ 7.52 ppm (*d*, H-3'') showed *trans* coupling ($J=15\text{Hz}$) with another olefinic proton at δ 6.59 ppm (*d*, H-2''). The presence of cinnamoyl moiety was confirmed by HMBC spectrum which exhibited the C-H long-range correlation between proton position 9'' and 5'' to carbon position 3'', proton position 2'' to carbon position 4'', proton position 3'' to carbon position 1'' in turn the proton position 3'' to carbon position 5'' and 9'' respectively.

The chemical shift assignment of cinnamoyl part of NAT01 was in agreement with those of Pyramidatine showed in Table 7 (Saifah *et al.* 1993)

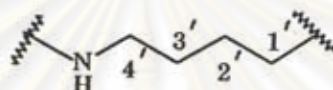
Table 7 The chemical shift assignment of cinnamoyl part of NAT01 and Pyramidatine

Position	NAT01		Pyramidatine	
	¹ H (ppm)	¹³ C (ppm)	¹ H (ppm)	¹³ C (ppm)
1''	-	168.6	-	164.9
2''	6.59	121.8	6.63	122.3
3''	7.52	140.8	7.42	138.5
4''	-	136.2	-	134.9
5''/9''	7.54	129.4	7.54	127.5
6''/8''	7.38	130.1	7.39	128.9
7''	7.36	130.7	7.35	129.4



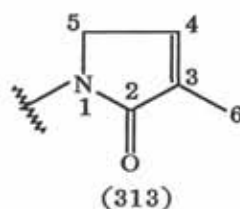
(311)

Butylamine moiety exhibited three sets of methylene protons signals at δ 3.32 ppm, 3.28 ppm, 1.60 ppm which was assigned as H-4', H-1', H2' /3' respectively. The methylene protons at δ 3.32 ppm and 3.28 ppm (*m*, H-4', H-1') showed connectivity with other two methylene protons at δ 1.60 ppm (*m*, H-2' /3') observable as a cross peak in upfield region of the COSY spectrum. The presence of butylamine moiety was confirmed by HMBC spectrum which exhibited the C-H long-range correlation between proton position 2' and 3' to carbons position 4' and 1' respectively. In turn proton at position 1' and 4' to carbons position 2' and 3' respectively.



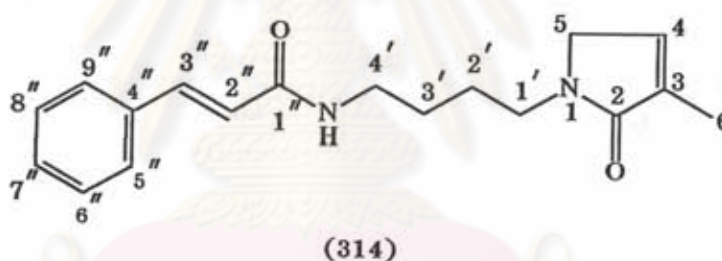
(312)

The 3-methyl-3-pyrrolin-2-one moiety exhibited three sets of proton signals at δ 1.83 (3H, *dt*, $J=1.83\text{Hz}$, 1.22Hz), δ 4.23 ppm (2H, *dq*, $J=6.10, 1.02\text{Hz}$), δ 6.33 ppm (1H, *tq*, $J=6.10, 1.53\text{Hz}$), which was assigned as H-6, H-5, H-4 respectively. An olefinic proton at δ 6.33 ppm (H-4, *tq*) showed connectivity with the methylene protons at δ 4.23 ppm (H-5, *dq*, $J=6.10, 1.02\text{Hz}$). The methyl protons at δ 1.83 ppm (H-6, *dt*, $J=1.83\text{Hz}, 1.22\text{Hz}$) showed long-range correlation with methylene protons at δ 4.23 ppm (H-5, *dq*, $J=6.10, 1.02\text{Hz}$) and an olefinic proton at δ 6.33 ppm (H-4, *tq*) observable in COSY spectrum. Based on HMBC experiment, the olefinic proton at δ 6.33 ppm (H-4) showed three bond correlation to an amide carbonyl carbon at δ 172.0 ppm (C-2) and the methylene proton at δ 4.23 ppm (H-5) showed two bond correlation to a quaternary olefinic carbon at δ 135.9 ppm (C-4) while the methyl protons at δ 1.83 ppm (H-6) showed three bond correlation to an olefinic carbon at δ 6.33 ppm (C-4) in turn methylene proton at δ 4.23 ppm (H-5) showed three bond correlation to methyl carbon at δ 13.0 ppm (C-6)



The connectivity of these three moiety were established through HMBC experiment in which the *N*-methylene protons of position 1' of the butylamine moiety displayed three bond correlation with C-2 carbonyl (δ 172.00 ppm) of 3-methyl-3-pyrrolin-2-one moiety, while *N*-methylene protons of position 4' showed three bond correlation with C-1'' carbonyl carbon (δ 168.60 ppm) of cinnamoyl moiety.

Therefore, the structure of compound NAT01 was proposed as a novel bisamide alkaloid, *N*-[4-(1-oxo-3-phenyl-2-propenyl)aminobutyl]-3-methyl-3-pyrrolin-2-one.



The ^1H and ^{13}C chemical shift assignments of NAT01 were showed in Table 8

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Table 8 ^1H -NMR and ^{13}C -NMR assignments of NAT01

Position	^1H (ppm)	^{13}C (ppm)
1''	-	168.6
2''	6.59	121.8
3''	7.52	140.8
4''	-	136.2
5''/9''	7.54	129.4
6''/8''	7.38	130.1
7''	7.36	130.7
1'	3.28	40.3
2'/3'	1.60	27.8
4'	3.32	40.2
1	-	-
2	-	172.0
3	-	132.5
4	6.33	135.9
5	4.23	59.5
6	1.83	13.0

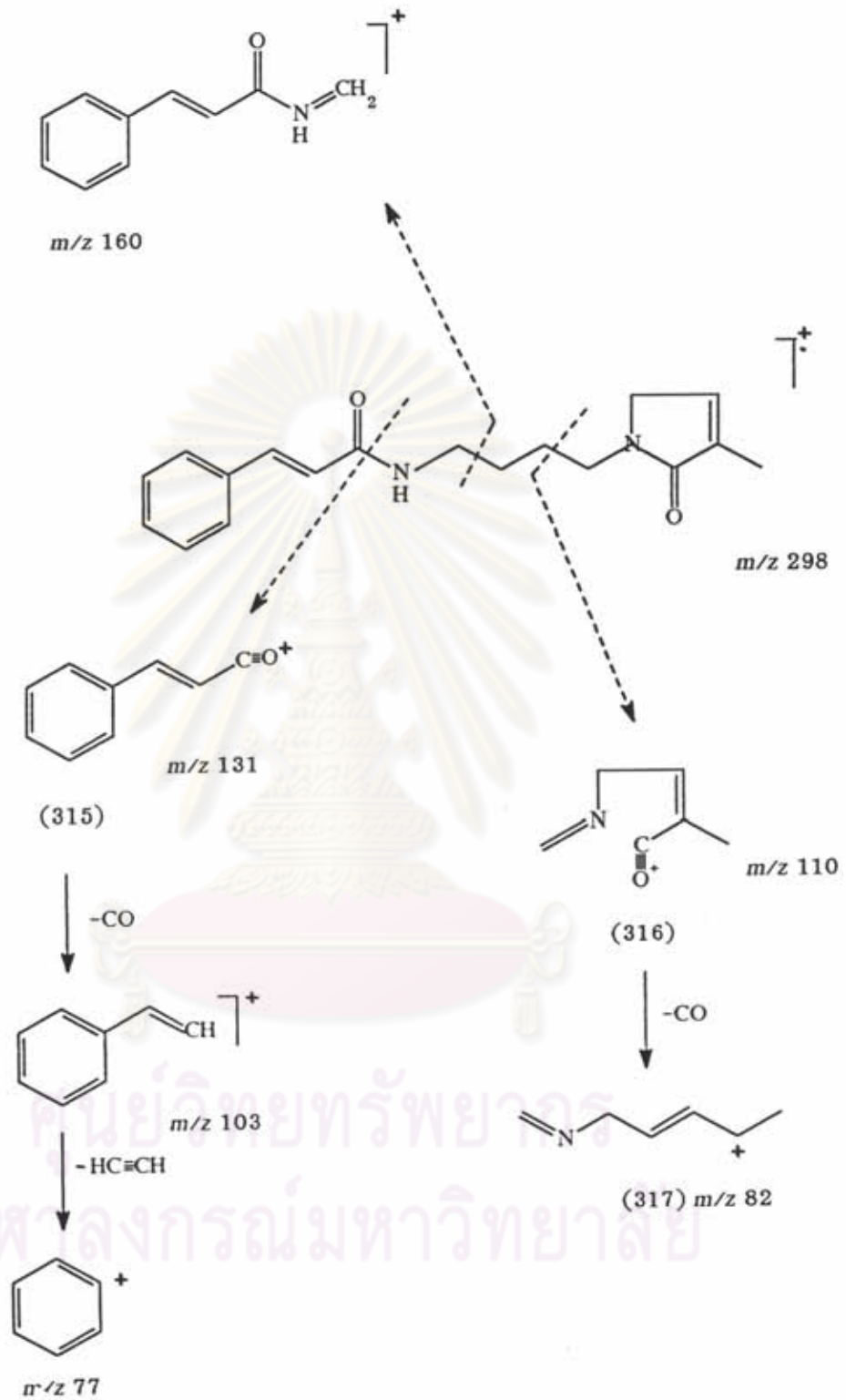
*Chemical shift are reported in ppm from TMS in CD_3OD

The mass spectrum (Fig.3 page 122) showed the base peak at m/z 131. The fragment ion at m/z 131 and 103 corresponded to the formation of cinnamoyl ion which subsequently loss of carbonyl (CO) to form phenyl ethylene moiety give rise to the formation of benzoyl ion at m/z 77. In addition, the mass spectrum showed ion peak at m/z 160 and m/z 110 corresponding to the formation of structure (315) and structure (316) via the α -cleavage which subsequently loss of carbonyl (CO) to form structure (317) (m/z 82) , respectively. The mass fragmentation pattern could be shown in Scheme 1

From the phytochemical studies of the leaves of *Aglaia rubiginosa* (Hiern) Pannell, the novel bisamide alkaloid, *N*-[4-(1-oxo-3-phenyl-2-propynyl)aminobutyl]-3-methyl-3-pyrrolin-2-one was isolated. 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 conclude chemotaxonomy of the genus *Aglaia* until more studies of the plants in the genus *Aglaia* are done.



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Scheme 1 Mass fragmentation of NATO1