

CHAPTER IV

DISCUSSION

During the investigation of the chemical constituents of the leaves of *Aglaia edulis* A. Gray, compound ED01, ED02 and ED03 were isolated from the chloroform extract. Those compounds gave positive results with Dragendorff's and Mayer's reagents, suggesting their alkaloid nature. Another compound, ED04, was isolated from the methanol extract and responded positively with Shinoda's test suggesting it to be a flavonoid.

The Structure Elucidation of The Isolated Compounds

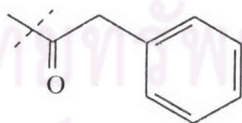
Compound ED01

Compound ED01 was crystallized as colorless needles from methanol. The EIMS spectrum (Figure 3) ED01 showed a molecular ion peak at m/z 324, suggesting its molecular formula as containing even number of nitrogen atoms. The base peak at m/z 91 suggested the presence of benzyl moiety in the structure. The high resolution mass spectrum revealed molecular ion peak at 324.4081 established its formula as $C_{20}H_{24}N_2O_2$.

The IR spectrum (Figure 5) showed NH stretching band at ν 3250 cm^{-1} and CO stretching of secondary amide at ν 1630 cm^{-1} .

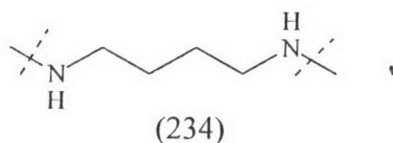
The ^{13}C -NMR and DEPT spectra (Figure 8 and 9) showed eight sets of carbon signals ; 2 amide carbonyl carbons (δ 173.9 ppm), 2 quaternary aromatic carbons (δ 136.9 ppm), 10 unsubstituted aromatic carbons (δ 127.8 ppm, C-6', δ 129.5 ppm, C-5'/C-7', δ 129.9 ppm, C-4'/C-8') and 6 methylene carbons (δ 27.6 ppm, C-2/3, δ 40.1 ppm, C-1/4, δ 43.8 ppm, C-2').

The ^1H and ^1H - ^1H COSY NMR spectra of ED01 (Figure 6 and 11) indicated the presence of two phenylacetyl moiety (233), each displaying four sets of equivalent signals, The singlet at δ 3.46 ppm could be assigned as the signal of methylene protons, while the other three signals in the aromatic region at δ 7.21 (*m*) ppm, 7.26 (*m*) ppm and 7.28 (*m*) ppm were assigned to the aromatic proton positions H-6' and H-4'/H-8' and H-5'/H-7', respectively. The connectivity of methylene and aromatic protons were established by the presence of a cross-peak from long-range coupling between methylene protons and the aromatic protons at position 4'/8' observable in the ^1H - ^1H COSY spectrum.

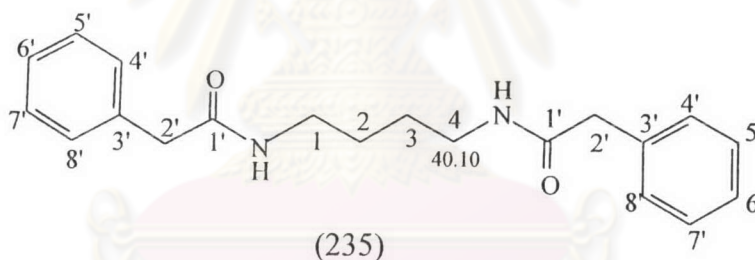


(233)

Another major cross-peak was observed between the downfield, N-methylene protons at δ 3.15 ppm (*m*, H-1) and the signal at δ 1.47 ppm (*m*, H-2). The equivalent signals of these positions indicated the existenc of a 1,4-butanediamine moiety (234) in the molecule.



From HMBC experiment, the N-methylene proton signal of H-1 and H-4 of 1,4-butanediamine moiety displayed long-range coupling with C-1' of phenylacetyl moiety, indicated that 1,4-butanediamine moiety was connected to phenylacetyl moiety through the nitrogen atom. This was supported by the two-bond correlation observable between methylene protons at position 2 with amide carbonyl carbon at δ 174.0 ppm (C-1'). Therefore, the structure of ED01 was proposed as a novel bisamide alkaloid, *N*-[4-(1-oxo-2-phenylethyl) aminobutyl] phenylacetamide (235).



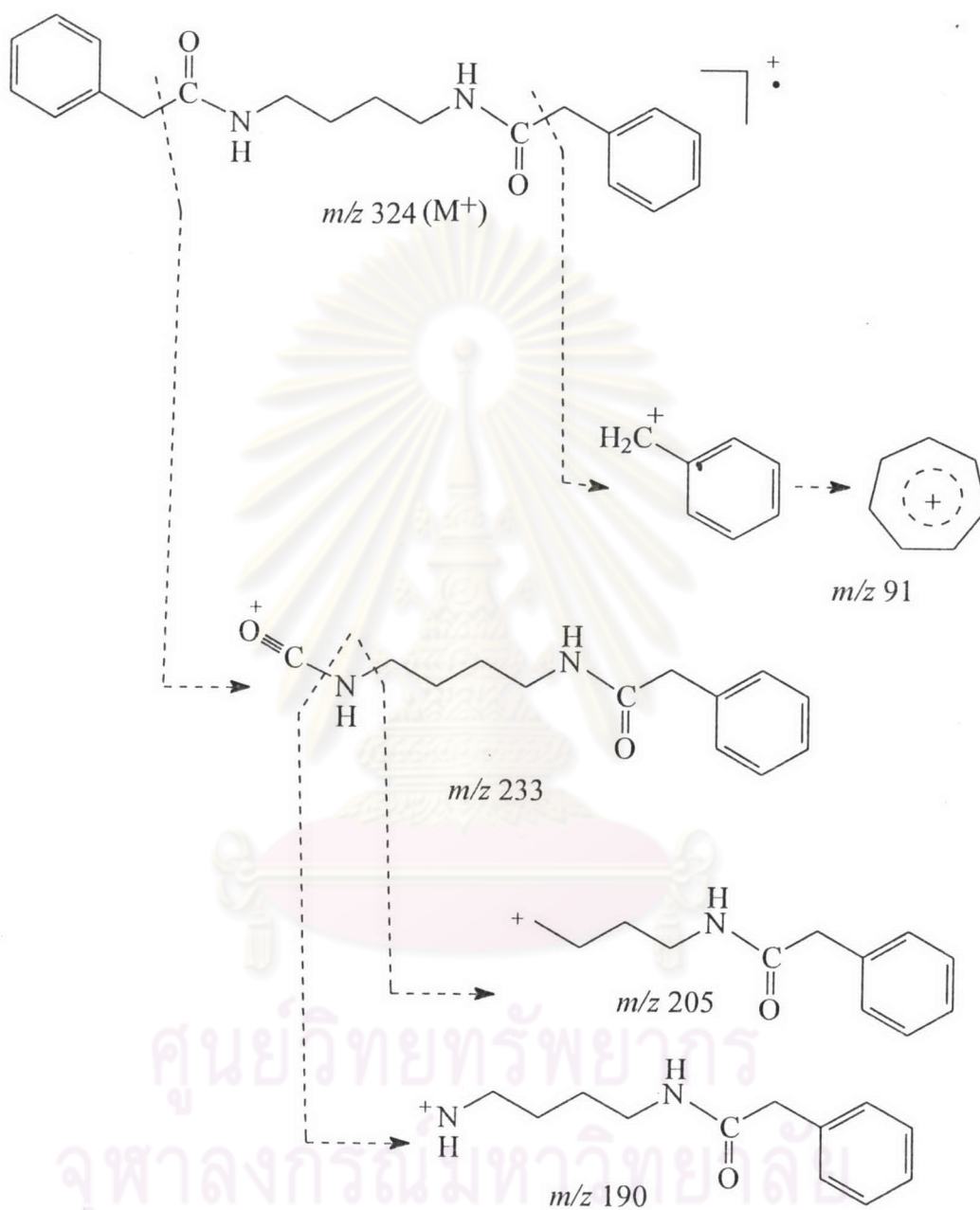
The ^1H and ^{13}C chemical shift assignments of ED01 were shown in Table 6 and the proposed fragmentation pattern of ED01 was shown in Scheme 1.

Table 6 ^1H -NMR and ^{13}C -NMR assignments of ED01*

Position	^1H	^{13}C
1	3.15(<i>m</i>)	40.1
2	1.47(<i>m</i>)	27.6
3	1.47(<i>m</i>)	27.6
4	3.15(<i>m</i>)	40.1
1'	-	173.9
2'	3.46(<i>s</i>)	43.8
3'	-	136.9
4',8'	7.26(<i>m</i>)	129.9
5',7'	7.28(<i>m</i>)	129.5
6'	7.21(<i>m</i>)	127.8

* Chemical shifts are reported in ppm from TMS in CD_3OD ; multiplicity and coupling constants in Hz are in parentheses.

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Scheme 1 Mass fragmentation of ED01

Compound ED02

Compound ED02 was crystallized as colorless needles from methanol. The high resolution mass spectrum of ED02 exhibited a molecular ion at m/z 306.3213 corresponded to the molecular formula of $C_{16}H_{22}N_2O_2S$.

The IR spectrum (Figure 17) showed NH stretching at ν 3260 cm^{-1} and CO stretching of secondary amide at ν 1640 cm^{-1} and 1600 cm^{-1} .

The ^{13}C -NMR and DEPT spectra (Figure 20 and 21) showed the presence of 16 carbons ; 2 amide carbonyl carbons (δ 167.3, 174.0 ppm), 1 quaternary aromatic carbons (δ 137.0 ppm), 5 unsubstituted aromatic carbons (δ 127.8 ppm, C-6" , δ 129.5 ppm, C-5"/7", δ 130.0 ppm, C-4"/8"), 2 olefinic carbons (δ 116.7, 143.4 ppm), 1 rather upfield carbon (δ 14.3 ppm) and 5 methylene carbons (δ 27.7 ppm, C-2/3, δ 39.9 ppm, C-1, δ 40.1 ppm, C-4).

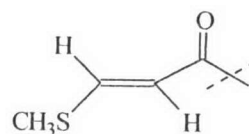
The 1H and 1H - 1H COSY NMR spectra of compound ED02 (Figure 18 and 22) indicated the presence of phenylacetyl (233), 1,4-butanediamine (234) and *trans*-3-methylthio-2-propenoyl (236) moieties.

The phenylacetyl moiety exhibited four sets of equivalent signals. The two proton singlet at δ 3.47 ppm was that of the methylene protons, while the other three signals in the aromatic region at δ 7.22 (1H, *m*) ppm, 7.27 (2H, *m*) ppm and 7.28 (2H, *m*) ppm were assigned to the aromatic proton positions 6" and 4"/8" and 5"/7",

respectively. Long-range correlation between methylene protons at position 2" to the aromatic proton at 4"/8" could be detected in the ^1H - ^1H COSY spectrum. The connectivity between the 2-methylene protons and the carbonyl carbon (δ 174.0 ppm) was also confirmed by HMBC experiment.

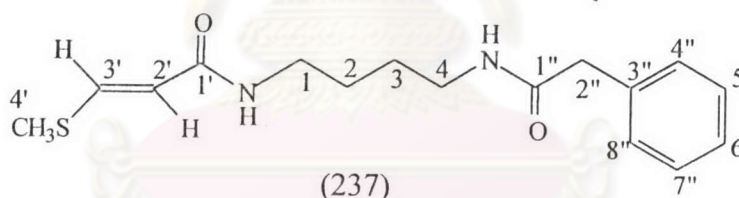
Two sets of N-methylene protons at δ 3.18 and 3.21 (*m*, H-1,H-4) ppm showed connectivity with other two sets of methylene protons at δ 1.50 ppm (H-2,H-3), observable as a cross-peak in the upfield region of the COSY spectrum. The presence of 1,4-butanediamine moiety was confirmed by HMBC spectrum which exhibited the C-H long-range correlations between protons position 1 and 4 to carbon position 3 and 2, respectively, and in turn, proton position 2 and 3 to carbon position 3 and 2, respectively.

An olefinic proton signal at δ 5.79 ppm (*d*, H-2') belonging to the *trans*-3-methylthio-2-propenoyl moiety (236) showed *trans* coupling ($J = 14.7$ Hz) with another olefinic proton at δ 7.53 ppm (*d*, H-3'). The thiomethyl protons appeared as a singlet at δ 2.33 ppm. Based on HMBC experiment, the thiomethyl proton H-4' showed three-bond correlation to an olefinic carbon C-3' at δ 143.4 ppm and the olefinic proton H-3' in turn, showed correlation to an amide carbonyl carbon (C-1') at δ 167.3 ppm. The base peak in the mass spectrum of compound ED02 at m/z 101 also corresponded to the existence of this *trans*-3-methylthio-2-propenoyl moiety in the structure.



(236)

The connectivity of these three moieties were established through HMBC experiment in which the N-methylene protons of position 1 (δ 3.21 ppm) of the 1,4-butanediamine moiety displayed long-range correlation with C-1' carbonyl (δ 167.3 ppm) of *trans*-3-methylthio-2-propenoyl moiety, while the N-methylene protons of position 4 correlated with C-1'' carbonyl (δ 174.0 ppm) of phenylacetyl moiety. Therefore, the structure of compound ED02 was proposed as a novel bisamide alkaloid, *N*-[4-(1-oxo-*trans*-3-methylthio-2-propenyl) aminobutyl] phenylacetamide (237).



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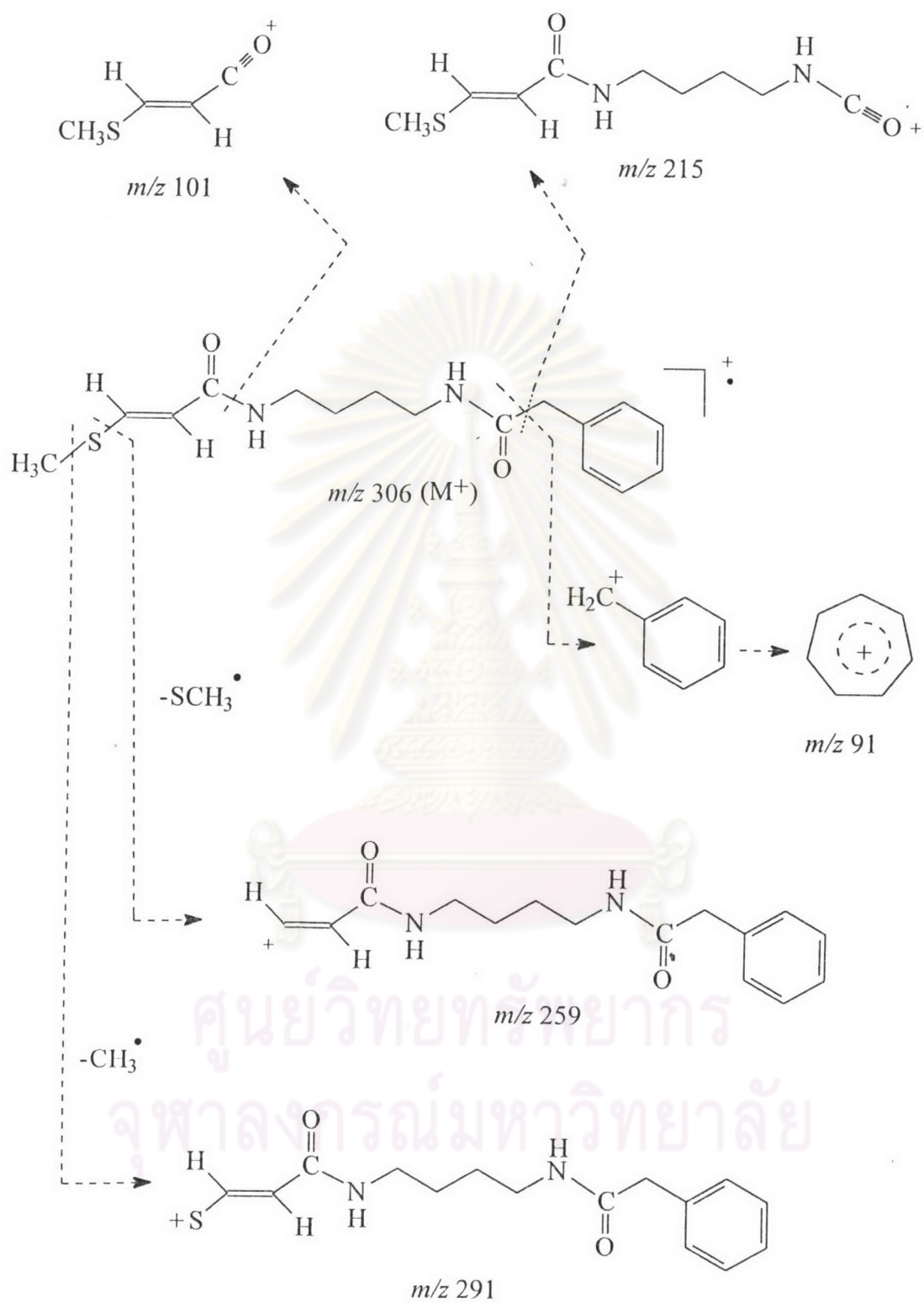
The structure of ED02 was confirmed by the mass spectral data (Figure15) the fragment peak at m/z 291 and 259 corresponded to loss of methyl and thiomethyl fragments from the structure, respectively. In addition, the mass spectrum also showed corresponding peak at m/z 215 and 91, the former successively loss a C-O fragment, indicating the presence of phenylacetyl moiety in the molecule.

The ^1H and ^{13}C chemical shift assignments of ED02 were shown in Table 7 and the proposed fragmentation pattern of ED02 was shown in Scheme 2.

Table 7 ^1H -NMR and ^{13}C -NMR assignments of ED02*

Position	^1H	^{13}C
1	3.21(<i>m</i>)	39.9
2	1.5(<i>m</i>)	27.7
3	1.5(<i>m</i>)	27.8
4	3.18(<i>m</i>)	40.1
1'	-	167.3
2'	5.79(<i>d</i> ,14.7)	116.7
3'	7.53(<i>d</i> ,14.7)	143.4
4'	2.33(<i>s</i>)	14.3
1"	-	174.0
2"	3.47(<i>s</i>)	43.9
3"	-	137.0
4",8"	7.27(<i>m</i>)	130.0
5",7"	7.29(<i>m</i>)	129.5
6"	7.22(<i>m</i>)	127.8

* Chemical shifts are reported in ppm from TMS in CD_3OD ; multiplicity and coupling constants in Hz are in parentheses.



Scheme 2 Mass fragmentation of ED02

Compound ED03

Compound ED03 was crystallized as pale orange needles from methanol. The high resolution mass spectrum of ED03 exhibited a molecular ion at m/z 288.2670 corresponding to the molecular formula of $C_{12}H_{20}N_2O_2S_2$. The fragment at m/z 273 and 241 corresponded to loss of methyl and thiomethyl radical, respectively.

The IR spectrum (Figure 28) showed NH stretching at ν 3310 cm^{-1} and CO stretching of secondary amide at ν 1600 cm^{-1} .

The ^{13}C -NMR and DEPT spectra (Figure 31 and 32) displayed six carbon signals, representing 2 sets of equivalent carbon atoms. These 12 carbons were 2 amide carbonyl carbons (δ 167.4 ppm), 4 olefinic carbons (δ 116.8, 143.4 ppm), 2 methyl carbons (δ 14.3 ppm) and 4 methylene carbons (δ 27.9, δ 40.0 ppm). The 1H and ^{13}C chemical shift assignments of ED03 were shown in Table 8 and the proposed fragmentation pattern of ED03 was shown in Scheme 3.

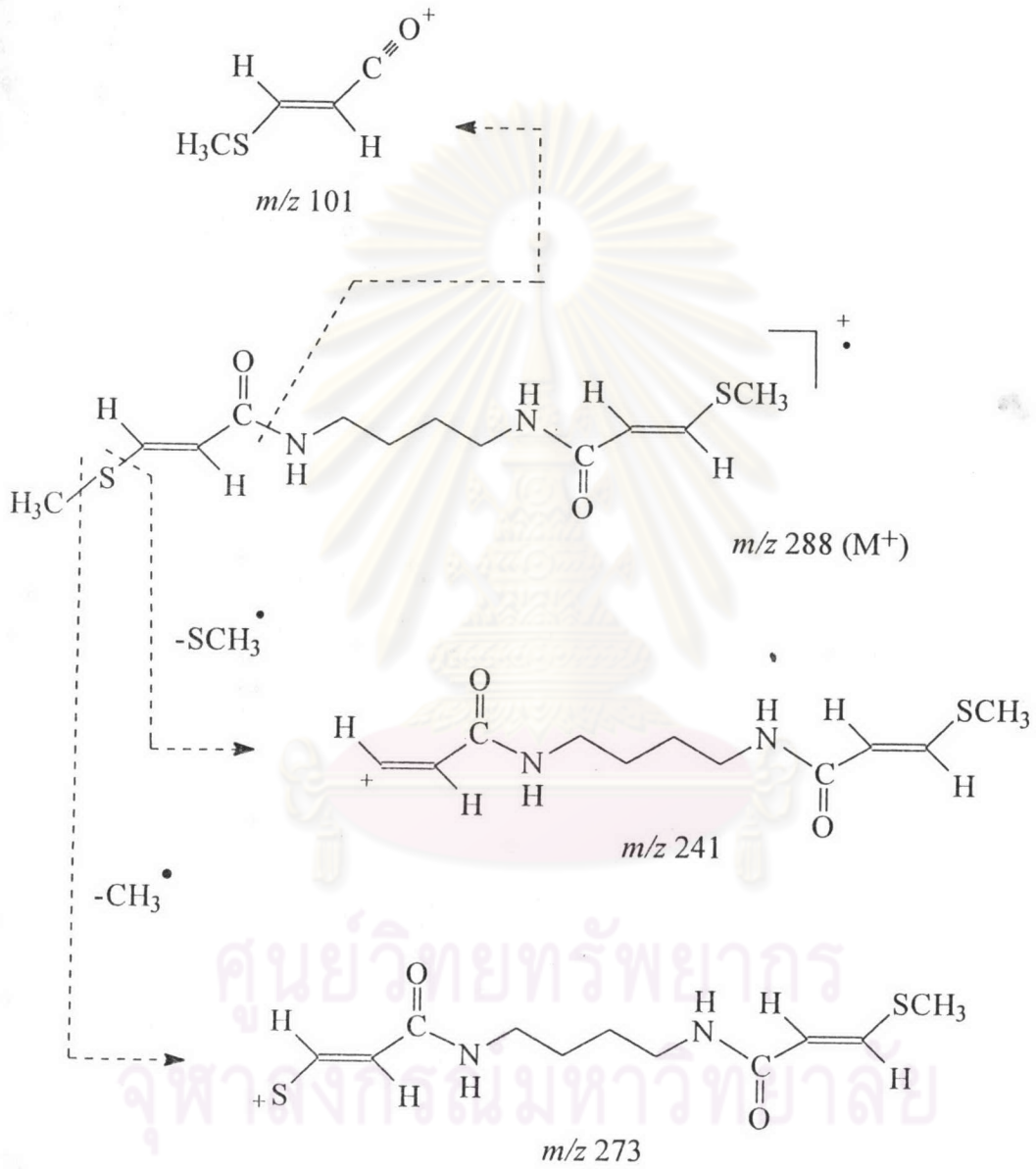
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Table 8 ^1H -NMR and ^{13}C -NMR assignments of ED03*

Position	^1H	^{13}C
1	2.33(<i>m</i>)	40.0
2	1.55(<i>m</i>)	27.9
3	1.55(<i>m</i>)	27.9
4	2.33(<i>m</i>)	40.0
1'	-	167.4
2'	5.80(<i>d</i> ,14.7)	116.8
3'	7.53(<i>d</i> ,14.7)	143.4
4'	2.52(<i>s</i>)	14.3

* Chemical shifts are reported in ppm from TMS in CD_3OD ; multiplicity and coupling constants in Hz are in parentheses.

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Scheme 3 Mass fragmentation of ED03

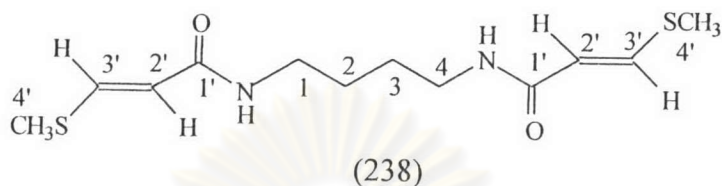
The ^1H and ^1H - ^1H COSY NMR spectra of ED03 (Figure 29 and 33) indicated the presence of 1,4-butanediamine (234) and two *trans*-3-methylthio-2-propenoyl (236) moieties.

Two N-methylene protons at δ 2.33 ppm (*m*, H-1 and H-4) exhibited connectivity with the methylene protons at δ 1.55 ppm (H-2 and H-3) in the COSY spectrum. The presence of 1,4-butanediamine moiety was confirmed by HMBC experiment which exhibited C-H long-range correlations between proton position 1 and 4 to carbon position 3 and 2, respectively, and in turn, proton position 2 and 3 to carbon position 3 and 2, respectively.

An olefinic proton at δ 5.80 (*d*, H-2') ppm in *trans*-3-methylthio-2-propenoyl moiety showing *trans* coupling ($J = 14.7$ Hz) with H-3' olefinic proton at δ 7.53 ppm. The thiomethyl protons appeared as a singlet at δ 2.52 ppm which on HMBC spectrum showed three-bond correlation to C-3' at δ 143.4 ppm while the olefinic proton H-3' could be connected to the amide carbonyl carbon (C-1') at δ 167.4 ppm. The base peak at m/z 101 in the EIMS spectrum also corresponded to the presence of *trans*-3-methylthio-2-propenoyl moiety in the structure.

From HMBC experiment, both N-methylene protons of the position 1 and 4 of 1,4-butanediamine moiety displayed long-range coupling with C-1' of each *trans*-3-methylthio-2-propenoyl moiety, indicating the connectivity of 1,4-butanediamine moiety to two *trans*-3-methylthio-2-propenoyl moieties at both ends. Therefore, the

structure of compound ED03 was proposed as a novel bisamide alkaloid, *Trans*-3-methylthio-*N*-[4-(1-oxo-*trans*-3-methylthio-2-propenyl) aminobutyl] propenylamide (238).



Compound ED04

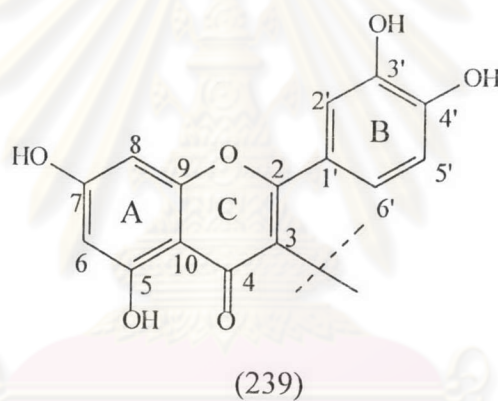
Compound ED04 was crystallized as yellow needles from methanol, It gave bright pink color with Shinoda's test indicated that it was a flavonoid.

In the IR spectrum (Figure 41) revealed absorption bands at 3381 and 1647 cm^{-1} , suggested the presence of the hydroxyl groups and the chelated carbonyl, respectively. Its UV spectra (Figure 38, 39 and 40) showed the characteristic of a flavonol chromophore at λ_{max} 256 nm (ϵ 45965 , band II) and 349 nm (ϵ 34765 , band I), respectively. The bathochromic shift (82 nm) of band I in the presence of AlCl_3 indicated 5-OH substitution on the flavonol nucleus whilst, the bathochromic shift (17 nm) of band II in the presence of NaOAc and the bathochromic shift (20 nm) of band I in the presence of $\text{NaOAc}/\text{H}_3\text{BO}_3$ indicated 7-OH substitution and B-ring *o*-diOH substitution on the flavonol nucleus, respectively.

The substitution positions of aromatic A-ring was substantiated by the *meta* coupling of aromatic protons at δ 6.20 ppm (*d*, 2.14 Hz) and at δ 6.37 ppm

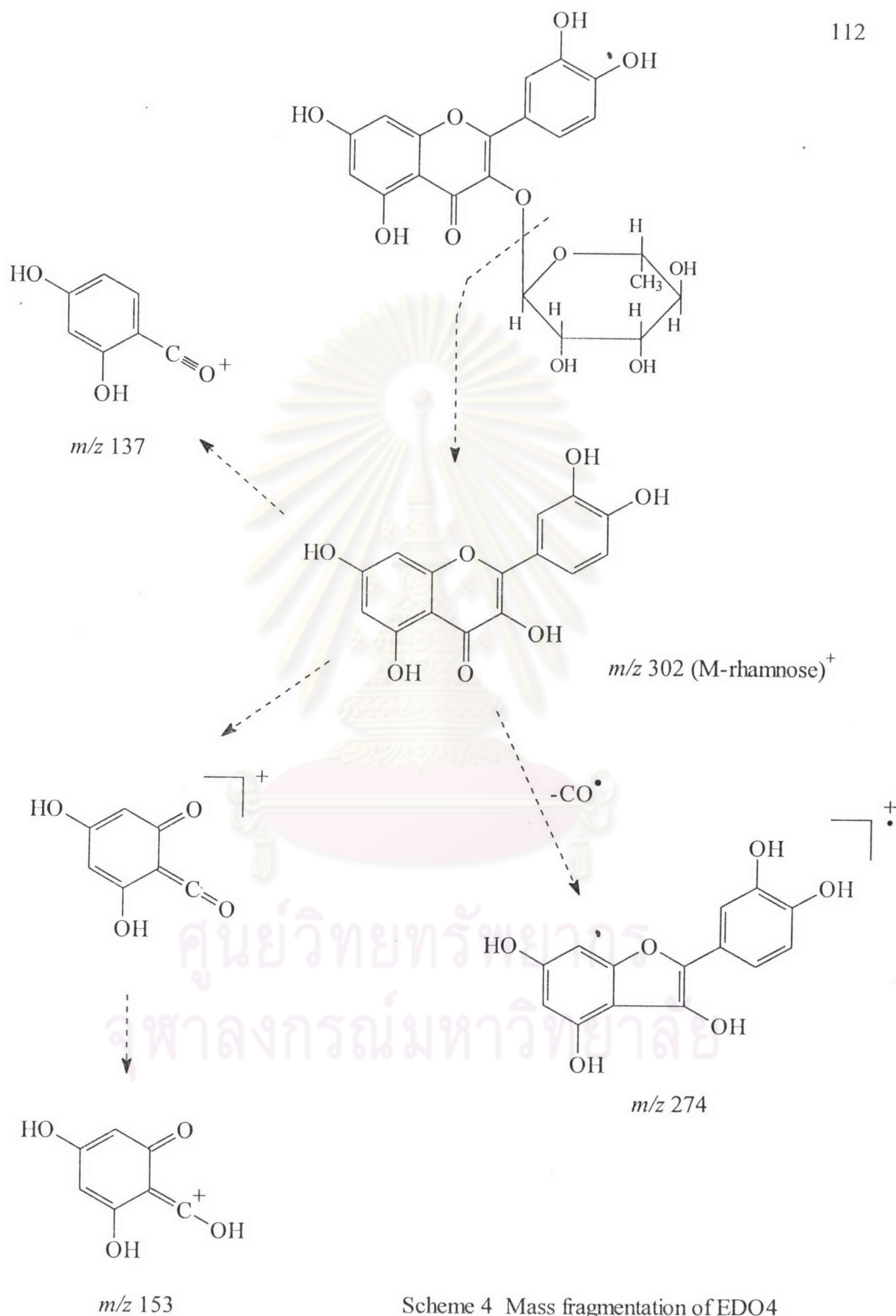
(*d*, 2.14 Hz) , observable in ^1H - ^1H COSY NMR spectrum which were assigned to the aromatic proton positions H-6 and H-8, respectively.

The substitution pattern of aromatic B-ring was deduced by the *ortho* coupling of aromatic proton at δ 7.31 ppm to the signal at 6.91 ppm (*d*, 8.2 Hz) and also *meta* coupling to another signal at δ 7.34 ppm (*d*, 2.1 Hz). Therefore the *o*-diOH groups could be assigned to the 3' and 4' position of ring B and the proposed structure of the aglycone part of ED04 is shown below (239).

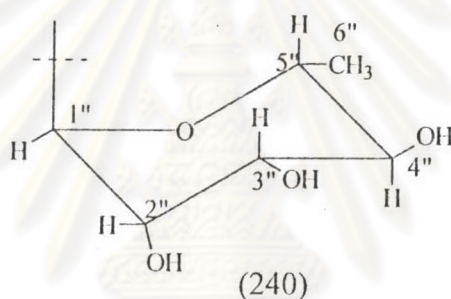


The structure of the aglycone (239) was confirmed by the mass spectral data and the mass fragmentation pattern of was shown in Scheme 4.

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Six proton-signals were possessed to a sugar molecule (240). The coupling constants between each vicinal proton; an anomeric proton (δ 5.35 ppm, *d*, H-1''), H-2'' (δ 4.23 ppm, *dd*), H-3'' (δ 3.76, *dd*), H-4'' (δ 3.35, *t*), H-5'' (δ 3.42, *dq*) and H-6'' (3H, δ 17.61, *d*); were 1.68, 1.68 and 3.36, 3.36 and 9.46, 9.46, 9.46 and 6.1, 6.10 Hz, respectively. These showed that the stereochemistry of each proton on the pyran sugar ring; H-1'', H-2'', H-3'', H-4'' and H-5'' ; must be equatorial-equatorial-axial-axial-axial relation. And the methyl group, which showed H-6'' signal was substituted on C-5'' in the equatorial position



From HMBC experiment, the anomeric proton of sugar moiety (H-1'') displayed three-bond coupling with C-3 of flavonol aglycone indicated that the sugar moiety was attached at the position C-3 of the aglycone. Therefore it could be concluded that ED04 is the known quercetin-3-*O*-rhamnoside (241). The proton and carbon chemical shift assignments were shown in table 9. These data were in agreement with reported value (Markham and Chari, 1982).

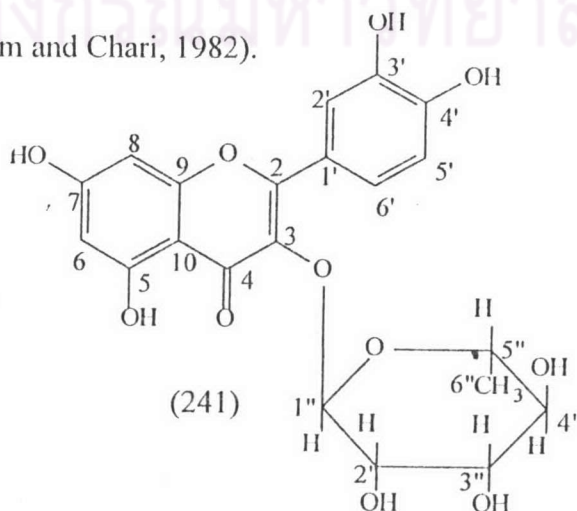


Table 9 ^1H -NMR and ^{13}C -NMR assignments of ED04*

Position	^1H	^{13}C
1	-	-
2	-	159.2
3	-	136.1
4	-	179.5
5	-	163.1
6	6.20(<i>d</i> ,2.1)	99.7
7	-	165.8
8	6.37(<i>d</i> ,2.1)	94.7
9	-	158.4
10	-	105.8
1'	-	122.9
2'	7.34(<i>d</i> ,2.1)	116.9
3'	-	146.3
4'	-	149.7
5'	6.91(<i>d</i> ,8.2)	116.3
6'	7.31(<i>dd</i> ,8.2,2.1)	116.3
1''	5.35(<i>d</i> ,1.6)	103.4
2''	4.23(<i>dd</i> ,1.6,3.3)	71.8
3''	3.76(<i>dd</i> ,3.3,9.4)	72.0
4''	3.35(<i>t</i> ,9.4)	73.2
5''	3.42(<i>dq</i> ,6.9,6.1)	71.9

6"

0.95(d,6.1)

17.61

* Chemical shifts are reported in ppm from TMS in CD₃OD ; multiplicity and coupling constants in Hz are in parentheses.

It could be concluded from the results of this investigation that the chemical characterizations of *Aglaia edulis* A. Gray shows the presence of three alkaloids and one flavonoid. The isolation of alkaloids ED01, ED02 and ED03 from this plant represents the first occurrence of sulfur containing alkaloid in the family Meliaceae, and ED01, ED02, ED03 are apparently new natural products. These three alkaloids are bisamide in nature similar to those previously found in other *Aglaia spp.* (Shiengthong *et al.*, 1979, Hayashi *et al.*, 1982, Joshi *et al.*, 1986, Saifah *et al.* 1988 ; 1993) and therefore, might be considered as significant chemotaxonomic informations of Meliaceous plant.

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