CHAPTER III

RESULTS AND DISCUSSION

The root bark of *Harrisonia perforata* Merr. studied in this investigation was collected from Kladkaw district, Chonburi province, Thailand during November 1992. Its root bark was sun-dried, milled, and extracted with methanol. The methanol extract was evaporated to give dark-red residue. It was then reextracted with diethyl ether. The remaining solid was redissolved in methanol. The methanol solution was then evaporated. These methods explained above were shown in Scheme I. All of the two extract parts were separated by the column chromatography as discussed in Chapter II.

### 3.1 Structural Elucidation of PA-1

PA-1 was isolated from the diethyl ether parts which was separated on the silica gel column chromatography of fraction 3-4 by 100 % n-hexane. Its IR spectrum shown in Fig. 2 and Table 7, indicated the presence of unsaturated hydrocarbon of olefin (=CH streching vibration peak at 3078 cm^{-1} and C=C stretching vibration peak at 1672 cm^{-1}). The C-H stretching vibration peaks of an aliphatic compound were observed at 2960, 2929 and 2863 cm^{-1} and the peak at 1759 cm^{-1} due to C=O stretching vibration of α,β-unsaturated of lactone. The absorption peak at 1377 cm^{-1} corresponded to C-H symmetric bending vibration mode of CH$_3$ group.

Table 7 The IR absorption band assignments of PA-1

Frequency (cm^{-1})	Band type	Tentative assignments
3078	w	=C-H stretching vibration
2960-2863	s	C-H stretching vibration of -CH$_3$, -CH$_2$
1759	s	C=O stretching vibration of α,β-unsaturated of lactone
1672	s	C=C stretching vibration
1547, 1453	s	C-H bending vibration of -CH$_2$, -CH$_3$
1377	s	C-H symmetric bending vibration of -CH$_3$
893	m	C-H out of plane bending vibration of gem-disubstituted olefin

From studing the chemical constituents of this mixture, PA-1 was analyzed by GC-MS spectrometer and gas chromatogram (Fig. 3) was obtained.   The gas chromatogram displayed peaks at retention times (R_t) 12.72, 16.09, 18.11, 18.42, 20.22, 21.48, 21.77, 22.21, 22.58, 22.71, 23.88, 25.26, 25.63, 25.88, 26.49, 27.00, 29.20, 33.36, 34.13, 35.40, 37.38, 41.06, 45.83, 46.16, 46.93, 47.08 and 50.91 min., respectively.   Each peak was analyzed by mass spectrometer and comparing the fragmentation ions pattern of mass spectra with those substances through library search of GC-MS spectrometer (NIST database).   The PA-1 was appeared to be composed of saturated hydrocarbon compounds, unsaturated hydrocarbon compounds and the other components which were unidentified. The discussion of the identified compounds was given as follow.
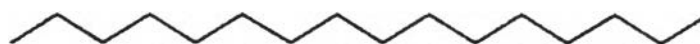
Table 8 contained saturated hydrocarbon observed at R_t 12.72, 16.09 and 25.62 min.. They were 3,6-dimethyldecane ($C_{12}H_{26}$), tridecane ($C_{13}H_{28}$) and hexadecane ($C_{16}H_{34}$), respectively. However, the identity of this compound ought to be comfirmed by comparison with an authentic sample.
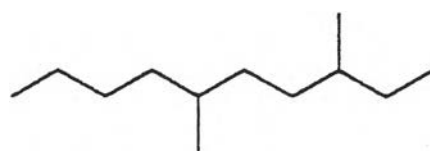
3,6-dimethyldecane

tridecane

hexadecane

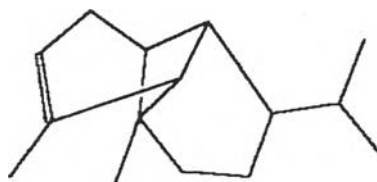**Table 8** **The component of the saturated hydrocarbon compounds**

contained in PA-1

| R_t (min.)	Formula	Name
12.72	$C_{12}H_{26}$	3,6-dimethyldecane
16.09	$C_{13}H_{28}$	tridecane
25.62	$C_{16}H_{34}$	hexadecane

Table 9 contained saturated hydrocarbon observed at R_t 18.11, 18.42, 20.22, 21.48, 21.77, 22.21, 22.71, 23.88, 27.00, 29.20 and 35.40 min.. They were 1,2,3, 5,6,7,8,8A-octahydro-1,8-dimethyl-7-(1-methylethyl)-naphthalene ($C_{15}H_{24}$), copaene ($C_{15}H_{24}$), 1H-cyclopropa A ($C_{15}H_{24}$), 1,2,3,4,4A,5,6,8A-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene ($C_{15}H_{24}$), decahydro-4A-methyl-1-methylene-7-(1-methylethyl)-naphthalene ($C_{15}H_{24}$), 1,2,4A,5,6,8A-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene ($C_{15}H_{24}$), 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene ($C_{15}H_{22}$), 4A,5,6,7,8,8A-hexahydro-7-isopropyl-4,8A-dimethyl-2(1H)-naphthalenenone ($C_{15}H_{22}O$), 1,6-dimethyl-4-(1-methylethyl)-naphthalene ($C_{15}H_{18}$), 2H-cyclopropa A ($C_{15}H_{22}O$), squalene ($C_{30}H_{50}$), respectively. However, the identity of this compound ought to be comfirmed by comparison with an authentic sample.
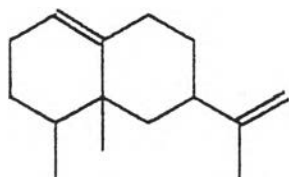
1,2,3,5,6,7,8,8A-octahydro-1,8-
dimethyl-7-(1-methylethyl)-naphthalene

copaene
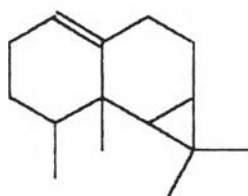
1H-cyclopropa A
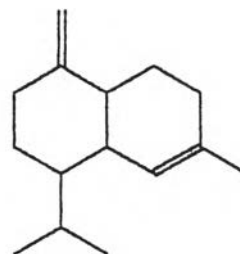
1,2,3,4,4A,5,6,8A-octahydro-7-methyl-
4-methylene-1-(1-methylethyl)-naphthalene
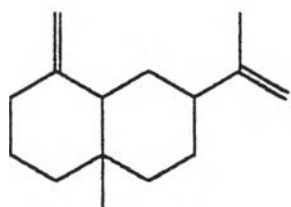
decahydro-4A-methyl-1-methylene-
7-(1-methylethyl)-naphthalene
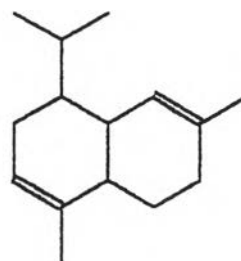
1,2,4A,5,6,8A-hexahydro-4,7-
dimethyl-1-(1-methylethyl)-naphthalene

1,2,3,4-tetrahydro-1,6-dimethyl-
4-(1-methylethyl)-naphthalene

4A,5,6,7,8,8A-hexahydro-7-isopropyl-
4,8A-dimethyl-2(1H)-naphthalenenone

1,6-dimethyl-4-(1-methylethyl)-naphthalene
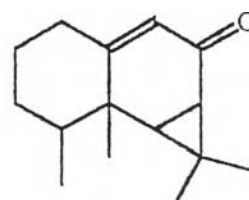
2H-cyclopropa A
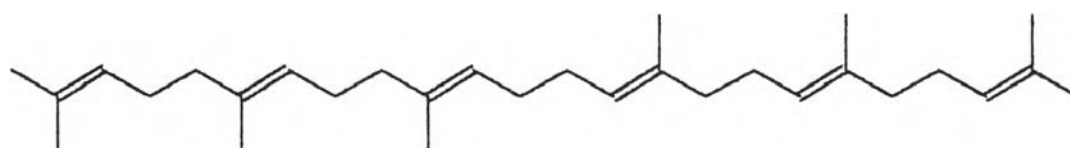
squalene

Table 9 The component of the unsaturated hydrocarbon compounds

contained in PA-1

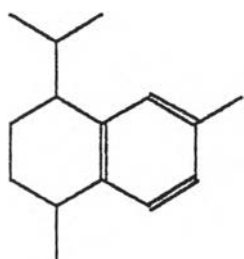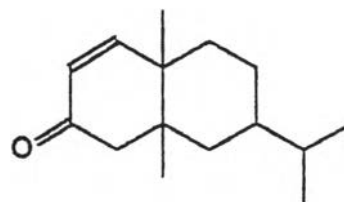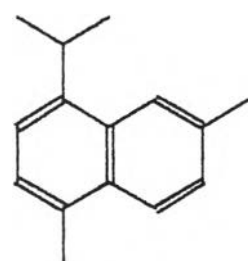| R_t (min.)	Formula	Name
18.11	$C_{15}H_{24}$	1,2,3,5,6,7,8,8A-octahydro-1,8-dimethyl-7-(1-methylethyl)-naphthalene
18.42	$C_{15}H_{24}$	copaene
20.22	$C_{15}H_{24}$	1H-cyclopropa A
21.48	$C_{15}H_{24}$	1,2,3,4,4A,5,6,8A-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene
21.77	$C_{15}H_{24}$	decahydro-4A-methyl-1-methylene-7-(1-methylethyl)-naphthalene
22.21	$C_{15}H_{24}$	1,2,4A,5,6,8A-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene
22.71	$C_{15}H_{22}$	1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene
23.88	$C_{15}H_{24}O$	4A,5,6,7,8,8A-hexahydro-7-isopropyl-4,8A-dimethyl-2(1H)-naphthalenenone
27.00	$C_{15}H_{18}$	1,6-dimethyl-4-(1-methylethyl)-naphthalene
29.20	$C_{15}H_{22}O$	2H-cyclopropa A
35.40	$C_{30}H_{50}$	squalene

The components which were located at R_t 22.58, 23.88, 25.26, 25.88, 26.49, 33.36, 34.13, 37.38, 41.06, 45.83, 46.16, 46.93, 47.08 and 50.91 min. (Fig. 12, 14, 15, 18, 21, 22, 24, 25, 26, 27, 28, 29, and 30, respectively) could not identified because the fragmentation ion pattern of these components were not similar to these spectral contained in the NIST library. The composition of compound were contained in PA-1 was presented in Table 10.

Table 10 The composition of compounds contained in PA-1

R_t (min.)	Formula	Name	%
12.72	$C_{12}H_{26}$	3,6-dimethyldacane	0.62
16.09	$C_{13}H_{28}$	tridecane	0.38
18.11	$C_{15}H_{24}$	1,2,3,5,6,7,8,8A-octahydro-1,8-dimethyl-7-(1-methylethyl)-naphthalene	2.20
18.42	$C_{15}H_{24}$	copaene	2.96
20.22	$C_{15}H_{24}$	1H-cyclopropa A	13.92
21.48	$C_{15}H_{24}$	1,2,3,4,4A,5,6,8A-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-naphthalene	1.90
21.77	$C_{15}H_{24}$	decahydro-4A-methyl-1-methylene-7-(1-methylethyl)-naphthalene	4.20
22.21	$C_{15}H_{24}$	1,2,4A,5,6,8A-hexahydro-4,7-dimethyl-1-(1-methylethyl)-naphthalene	1.83
22.58	-	unidentify	1.80
22.71	$C_{15}H_{22}$	1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-naphthalene	0.95
23.88	$C_{15}H_{24}O$	4A,5,6,7,8,8A-hexahydro-7-isopropyl-4,8A-dimethyl-2(1H)-naphthalenenone	1.18
25.26	-	unidentify	2.92
25.63	$C_{16}H_{34}$	hexadexane	0.46
25.88	-	unidentify	1.82
26.49	-	unidentify	1.21
27.00	$C_{15}H_{18}$	1,6-dimethyl-4-(1-methylethyl)-naphthalene	2.33
29.20	$C_{15}H_{22}O$	2H-cyclopropa A	4.99
33.36	-	unidentify	0.92
34.13	-	unidentify	3.31
35.40	$C_{30}H_{50}$	squalene	10.37
37.38	-	unidentify	1.54
41.06	-	unidentify	12.78
45.83	-	unidentify	1.11
46.16	-	unidentify	4.98
46.93	-	unidentify	1.47
47.08	-	unidentify	7.30
50.91	-	unidentify	1.82

3.2 Structural Elucidation of PA-2

PA-2 was obtained from the diethyl ether parts which was separated on the silica gel column chromatography of fraction 31-35. The oil was removed from solid by washing with methanol and then the solid residue was recrystallized from hot methanol to give a white needle crystals, 0.20 % wt. by wt of diethyl ether crude and the R_f value was 0.45 using 50 % chloroform in n-hexane as a developing solvent and using silica gel TLC plate.

This compound was soluble in chloroform, acetone, ethyl acetate, diethyl ether, hot methanol and slightly soluble in n-hexane. The color tests of this compound indicated that this compound had a steroidal skeleton and unsaturation part in molecule.

The IR spectrum of PA-2 was shown in Fig. 31 and the absorption peaks are assigned in Table 11. Its IR spectrum showed important absorption bands at 3430(b) and 1050 cm^{-1} (O-H and C-O stretching vibration of secondary alcohol), 1670-1630 cm^{-1} (C=C stretching vibration of olifinic), 840 and 800 cm^{-1} (C-H out of plane bending vibration coincided with the characteristic absorption peaks of trisubstituted olefin). The absorption peak at 1380 cm^{-1} corresponded to C-H symmetric bending vibration mode of CH$_3$ group.

Table 11   The IR absorption band assignments of PA-2

Frequency (cm-1)	Band type	Tentative assignments
3430	b	O-H stretching vibration
2960-2840	s	C-H stretching vibration of -CH_3, -CH_2
1650	w	C=C stretching vibration
1460	m	C-H bending vibration of -CH_2, -CH_3
1380	m	C-H in plane bending vibration of trisubstituted of olefin
1050	m	C-O stretching vibration of secondary alcohol
970, 950	m	C-H out of plane bending vibration of trans configuration
840, 800	w	C-H out of plane bending vibration of trisubstituted olefin

The ^{1}H NMR spectrum ($CDCl_3$) of PA-2 (Fig. 32) showed the signals at 0.68-2.30 ppm., which were the signals of angular methyl, methylene and methine groups (CH_3, -CH_2-, -CH-, respectively) of steroids.   The proton of hydroxyl group (-OH) was shown the multiplet signal at 3.50 ppm. while the double of doublet at 5.09 ppm. was the signal of disubstituted vinyl protons (-CH=CH-).   The last signal at 5.32 ppm. was the signal of trisubstituted vinyl proton (-CH=C).

The ^{13}C NMR spectrum ($CDCl_3$) (Fig. 33) exhibited the olefinic carbon signals at 121.63, 129.20, 138.23 and 140.68 ppm.   The ^{13}C NMR spectrum showed the signals that corresponded to those of a mixture of campesterol, stigmasterol and β-sitosterol showed in Table 12.

Table 12   The ^{13}C NMR chemical shifts of β-sitosterol, stigmasterol

and PA-2

Carbon position	Chemical shift (ppm.)		
	β-sitosterol	stigmasterol	PA-2
1	37.1	37.4	37.3
2	31.8	31.7	31.6
3	71.9	71.8	71.7
4	42.4	42.4	42.3
5	140.9	140.0	140.8
6	121.8	121.7	121.6
7	32.0	31.9	31.9
8	32.0	31.9	31.9
9	50.3	50.3	50.2
10	36.6	36.6	36.5
11	21.1	21.1	21.1
12	39.9	39.8	39.7
13	42.4	42.4	42.3
14	56.8	57.0	56.8
15	24.3	24.4	24.3
16	28.2	28.9	28.2 , 28.9
17	56.2	56.0	56.0
18	11.9	12.2	11.8 , 12.2
19	19.4	19.4	19.4
20	36.2	40.5	36.2 , 40.4
21	19.1	21.1	19.0 , 21.1
22	34.0	138.4	34.0 , 138.3
23	29.3	129.4	29.2 , 129.3
24	50.3	51.3	50.2
25	26.2	31.9	26.1 , 31.9
26	18.8	19.0	19.0
27	19.8	21.1	19.8 , 21.2
28	23.1	25.4	23.1 , 25.4
29	11.9	12.0	11.8 , 12.0

The mass spectrum (Fig. 34) exhibited the expected molecular ion peak of β-sitosterol at m/e 414.0 and also revealed the fragmentation ions pattern of C-29 steroids. However, two significant peaks at m/e 412.0 and m/e 400.0 could not be accounted for the β-sitosterol structure. The fragmentation ions mass spectrum pattern of this compound indicated that this compound was a mixture compound of steroids generally found in most plants. However, this mixture could not be separated even though the adsorbent of the column and the developing solvent system were varied. The possible mass fragmentation ions pattern of PA-2 was presented in Scheme 2.

The presence of β-sitosterol in plants was frequently accompanied by one or more steroids such as stigmasterol and campesterol. When compared the GLC analysis data of PA-2 (Fig. 35) with those of the authentic choresterol, campesterol, stigmasterol, and β-sitosterol, it indicated that PA-2 corresponded to campesterol, stigmasterol and β-sitosterol as in Table 13.

Table 13   The comparison of GLC retention times between PA-2

and authentic steroids

standard steroid	retention time (min)		%
	standard steroid	PA-2	
cholesterol	5.73		-
campesterol	6.79	6.99	10.1
stigmasterol	7.27	7.38	46.6
β-sitosterol	7.94	8.14	43.3

DB-1 capillary column, col. temp. Isothermal 290 °C, injection temp. 250 °C

Mass detector , He gas 50 cm/sec,

The results above indicated that PA-2 was a mixture of campesterol, stigmasterol and β-sitosterol.   The structure of these three steroids were given below :
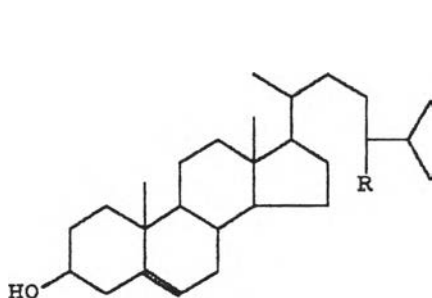
R = Et   , β-sitosterol   stigmasterol

R = Me , campesterol

PA-2 : mixture of steroid

Scheme 2 The possible mass fragmentation pattern of PA-2

3.3 Structural Elucidation of PA-3

PA-3 was obtained from the silica gel column chromatography of fraction 40-45 by 20 % diethyl ether in n-hexane. The compound was recrystallized from a mixture of n-hexane and chloroform to give a white amorphous solid, 0.26 % wt. by wt. of die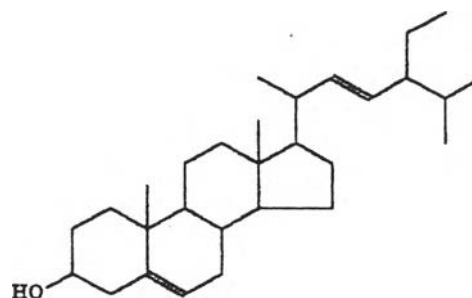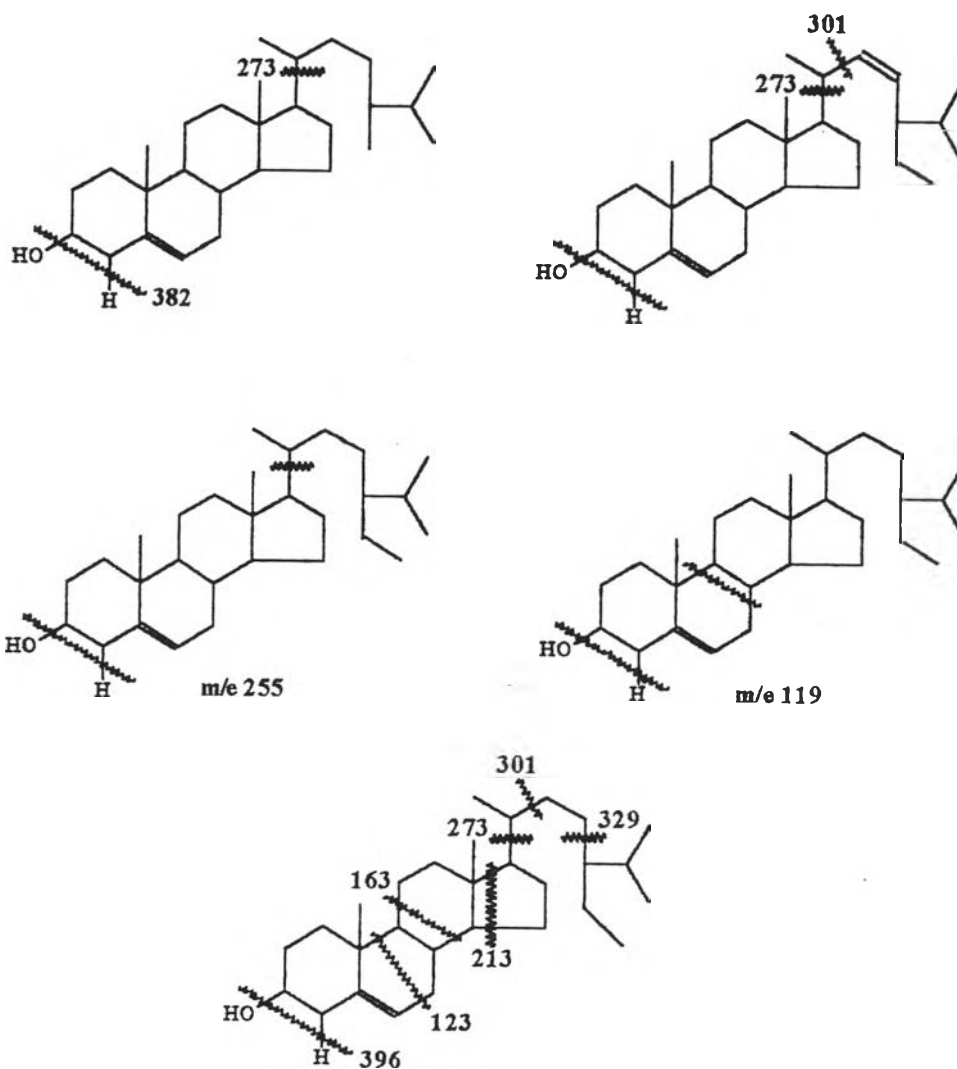thyl ether crude. It melted at 154-155 ^{o}C and R_f value of this compound was 0.40 using chloroform as a developing solvent. This compound was soluble in chloroform, ethyl acetate, diethyl ether, hot ethanol.

The IR spectrum of PA-3 was shown in Fig. 36 and the absorption peaks were tabulated in Table 14. Its IR spectrum showed important absorption bands at 3446(b) cm^{-1} (O-H stretching vibration), 3152 and 3076 cm^{-1} (C-H stretching vibration of olefinic), 2986, 2953 and 2903 cm^{-1} (C-H stretching vibration), 1742 cm^{-1} (C=O stretching vibration of lactone) , 1718 cm^{-1} (C=O stretching vibration of ketone), 1631 cm^{-1} (C=C stretching vibration of olefinic), 1267, 1211 and 1185 cm^{-1} (C-O-C stretching vibration), and 876 cm^{-1} (out of plane bending vibration coincided with the characteristic absorption peak of furan). The absorption peak at 1380 cm^{-1} corresponed to C-H symmetric bending vibration mode of CH_3 group.

Table 14 The IR absorption band assignments of PA-3

Frequency	Band type	Tentative assignment
3446	s, br	O-H stretching vibration
3040	w	CH stretching vibration of =CH
2986, 2953, 2903	m	CH stretching vibration of $-CH_2$, $-CH_3$
1742	s	C=O stretching vibration of lactone
1718	s	C=O stretching vibration of ketone
1631	w	C=C stretching vibration
1552, 1504, 1440, 1405	m	C-H bending vibration of $-CH_2$, $-CH_3$
1386	s	C-H symmetric bending vibration of $-CH_3$
1267, 1211, 1185	s	C-O stretching vibration of -CO-O-C=C
876	m	C-H bending vibration of furan

The ^1H NMR spectrum ($CDCl_3$) of PA-3, Fig. 37, exhibited the signals at 1.16-1.50 ppm., which were the signals of angular methyl groups at side chain of C-18, C-19, C-24, C-25 and C-26. The signals at 1.56-1.95 ppm. exhibited methylene group ($-CH_2-$) of C-11 and C-12.   The signals at 3.0 ppm. was shown the methine group ($-CH-$) of C-9 and the proton of hydroxyl group showed the singlet signals at 3.61 an 5.06 ppm. while the methoxy protons ($-OCH_3$) appeared at 3.79 ppm.   The signal of a methine protons attacted to a carbon atom bearing a oxygen atom at 4.29 and 5.67 ppm. (C-15 and C-17, respectively) and doublet signals in olefinic region which should be a methine protons at 5.76 and 6.00 ppm. (2H, J=12 Hz.). The broad singlet signals of furan ring appeared at chemical shift 6.33, 7.40 and 7.42 ppm. (3H).

The ^{13}C NMR, DEPT-135 and DEPT-90 ^{13}C NMR spectra, Fig 38, 39, exhibited the carboxyl carbon and carbonyl carbon at 166.6, 167.74 and 216.6 ppm., respectively. The singlet signals at 123.0 and 153.8 ppm. were assigned to carbon-carbon double bond of olefin (-C=C-) that the signals were assigned to β-substituted furanoid carbon at 109.8, 120.9, 141.0 and 142.9 ppm. The signal at 108.2 ppm. could be assigned to the carbon atom with two substituents group of oxygen atom and the signals at 57.2, 68.5, 78.3, 80.8 and 88.5 ppm. should be determined to the carbon atom with attacted to one oxygen atom. The signal of methoxy carrbon appeared at 51.9 ppm. and the signals at 14.6-49.8 ppm. were attributed to methyl, methylene and methine carbon.

The ^{13}C NMR, DEPT-135 and DEPT-90 ^{13}C NMR spectra, Fig 37-42, gave important information which corresponded to the information from ^{13}C NMR spectrum and could be interpreted as follows in Table 15.

Table 15 Comparison of spectroscopic data of PA-3

chemical shift (ppm.)				wave number (CM^{-1})	Tentative assignment
^{1}H	^{13}C	DEPT-135	DEPT-90	IR	
	14.6	14.6 ^{u}			CH3
	15.1	15.1 ^{d}			CH2
	17.2	17.2 ^{u}			CH3
	18.2	18.2 ^{u}			CH3
	24.0	24.0 ^{u}			CH3
	26.2	26.2 ^{d}			CH2
	27.3	27.3 ^{u}			CH3
	39.4				CR3
	46.7	46.7 ^{u}	46.7		CHR2
	49.6				CR3
	49.8				CR3
3.80	51.9	51.9 ^{u}			-O-CH3
4.30	57.2	57.2 ^{u}	57.2		CHR2
	68.5				-O-CR3
5.70	78.3	78.3 ^{u}	78.3		-O-CHR2
	80.8				-O-CR3
	88.5				-O-CR3
	108.2				-O2-CR2
6.54	109.8	109.8 ^{u}	109.8		=CHR
	120.9				=CR2
5.76	123.0	123.0 ^{u}	123.0		=CHR
7.42	141.0	141.0 ^{u}	141.0	1631	=CHO
7.40	142.9	142.9 ^{u}	142.9		=CHO
6.00	153.8	153.8 ^{u}	153.8		=CHR
	166.6				-COOR
	167.7			1718	-COOR
	216.3			1742	-COR

It could be concluded that PA-3 exhibited the ^{13}C NMR chemical shifts similar to harrisonin [6]. The ^{13}C NMR chemical shifts of PA-3 and harrisonin could be compared as in Table 16.

Table 16   The ^{13}C NMR chemical shifts of PA-3 and Harrisonin.

Carbon	Chemical shift (ppm.)	
	PA-3	Harrisonin
1	153.8	153.9
2	123.0	123.1
3	166.6	166.7
4	88.5	88.6
5	80.8	80.9
6	213.6	216.8
7	108.2	108.2
8	49.8	50.0
9	46.9	46.8
10	-	-
11	-	-
12	-	-
13	-	-
14	68.5	68.6
15	57.2	57.3
16	167.7	167.9
17	78.3	78.5
18	-	-
19	-	-
20	120.9	121.0
21	141.0	141.2
22	109.8	109.9
23	142.9	143.0
24	-	-
25	-	-
26	-	-
OCH$_3$	52.0	51.9

The mass spectrum which was obtained by chemical ionization technique (Fig. 43) did give the molecular ion peak at 517 (MH^+) which agrees with the molecular formular $C_{27}H_{32}O_{10}$ and the signal at 499 (MH^+-18) could be account for the loss of one hydroxyl group and the signal at 471 (MH^+-28) ought to account for the loss of ethylene group. After it was analyzed again by electron impact technique of mass spectrometer, its mass spectrum of PA-3 (Fig. 44) did not give the molecular ion peak, due to the breaking of molecule almostly. The possible mass fragmentation ions pattern of PA-3 was presented in Scheme 3.

Scheme 3 **The possible mass fragmentation pattern of PA-3**

m/e 516

$- CO_2$

m/e 472

m/e 393

$- H_2O$ $- H_2O$

m/e 375
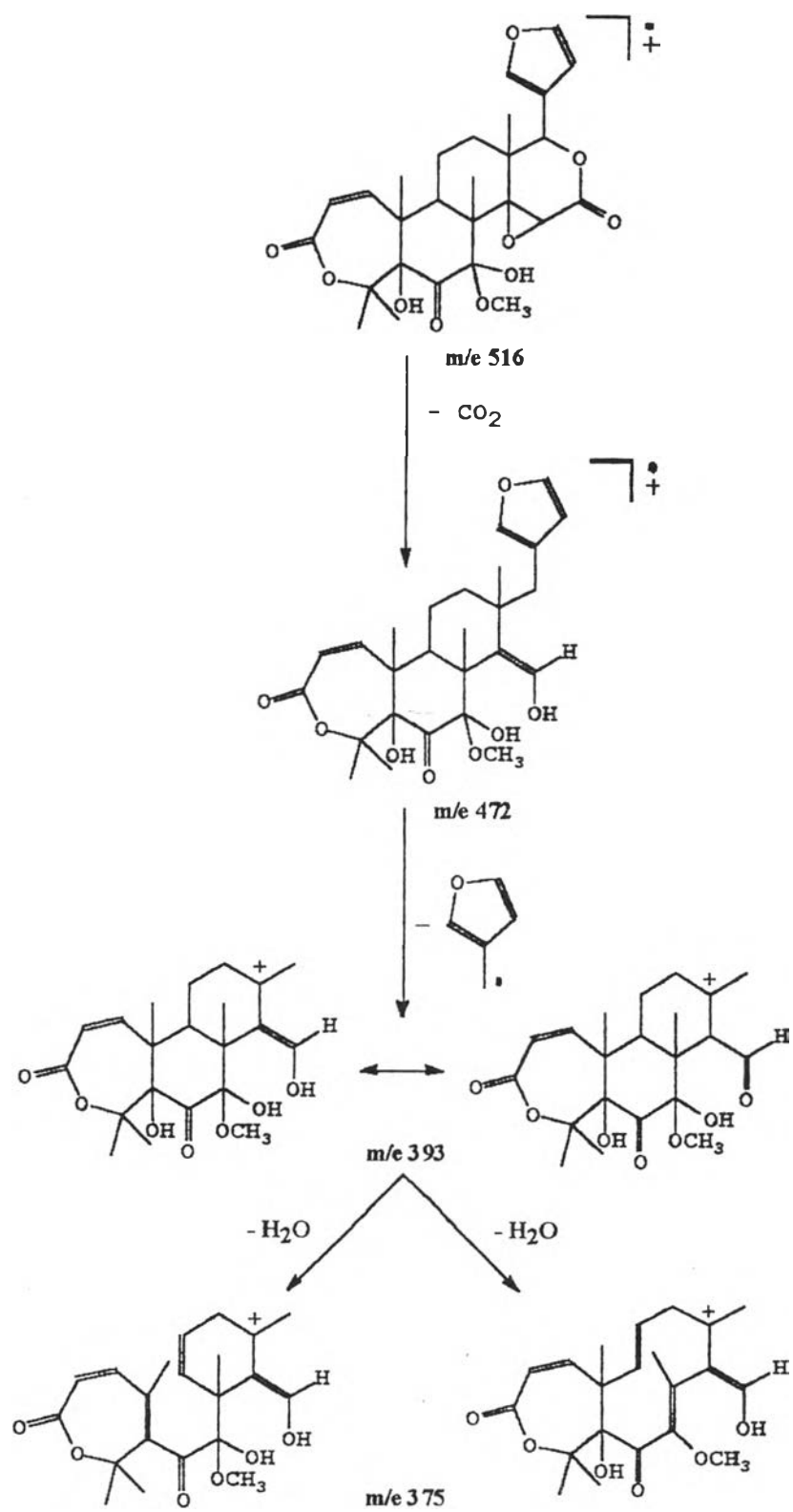
The spectral evidences and physical properties of this compound confirmed that this compound was harrisonin found recently from the root-bark of this plant by American scientist in 1976 [6]. The structure of this compound was shown below :
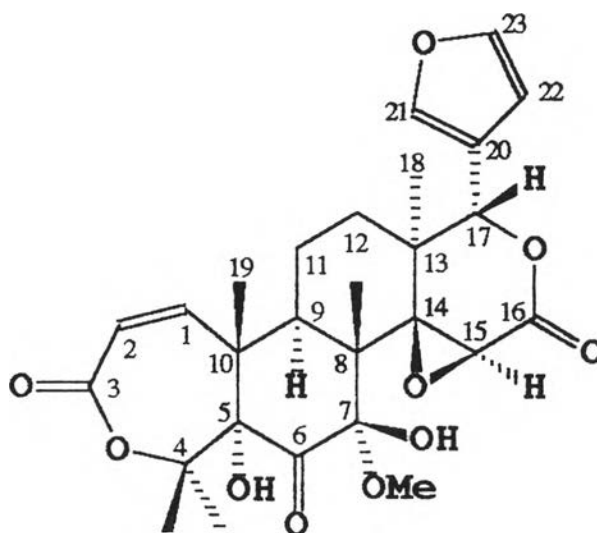
PA-3 : Harrisonin

3.4 Structural Elucidation of PA-4

PA-4 was collected from the silica gel column chromatography of fraction 71-78 by 40 % diethyl ether in n-hexane. The compound was recrystallized from a hot methanol to give a white amorphous solid, 0.22 % wt. by wt. of diethyl ether crude. It melted at 292-295 °C and R_f value of this compound was 0.40 using 20 % methanol in chloroform as a developing solvent system. This compound was soluble in methanol, ethanol which was slightly soluble in chloroform, but it does not soluble in hexane. The color tests of this compound indicated that this compound had a steroidal skeleton and unsaturation part in molecule.

The IR spectrum of PA-4 was shown in Fig. 45 and the absorption peaks are assigned in Table 17. Its IR spectrum showed important absorption bands at 3457 and 1011 cm^{-1} (O-H and C-O stretching vibration of alcohol), 2986, 2953 and 2903 cm^{-1} (C-H stretching vibration), 1682 cm^{-1}, (C=C stretching vibration of olifinic), but did not appeared the peak at more than 3000 cm^{-1} due to tetrasubstituted olefin.

Table 17 The IR absorption band assignments of PA-4

Frequency	Band type	Tentative assignment
3475	s	O-H stretching vibration
2946	s	CH stretching vibration of -CH_2, -CH_3
1682	s	C=C stretching vibration
1436	m	C-H bending vibration of -CH_2, -CH_3
1375	m	C-H symmetric bending vibration of -CH_3
1011	m	C-O stretching vibration of secondary alcohol

The ^1H NMR spectrum ($CDCl_3$ + DMSO) of PA-4, Fig. 46, exhibited the signals at 0.56-2.15 ppm., which were obtained the signals of methyl, methylene and methine groups. The proton of hydroxyl group showed the singlet signals at 2.46 ppm. while the broad multiplet signal of a methine protons attacted to a carbon atom bearing a oxygen atom at 2.90 ppm. However, the ^1H NMR spectrum did not appeared the hydroxy proton of carboxylate and had not the methoxy proton too so that this compound a should be carboxylic acid compound. Further, the signal of olefinic proton did not appeared due to tetrasubstituted olefin.

The ^{13}C NMR, DEPT-135, DEPT-90 ^{13}C NMR, COSY spectra, Fig 47, 48 and 49, respectively, exhibited the carboxyl carbon at 180.4 ppm. The signals at 133.5 and 133.7 ppm. were assigned to carbon-carbon double bond of olefin (-C=C-) that it were assinged to quartenary of olefinic carbon. The signal at 78.0 ppm. could be assigned to the carbon atom with attacted to oxygen atom. The signals at 15.3-50.2 ppm. were attributed to methyl, methylene and methine carbon. The ^{1}H NMR, ^{13}C NMR, DEPT-135 and DEPT-90 ^{13}C NMR spectra, gave important information which corresponded to the information from ^{13}C NMR spectrum and could be interpreted as follows in Table 18.

Table 18 Comparison of spectroscopic data of PA-4

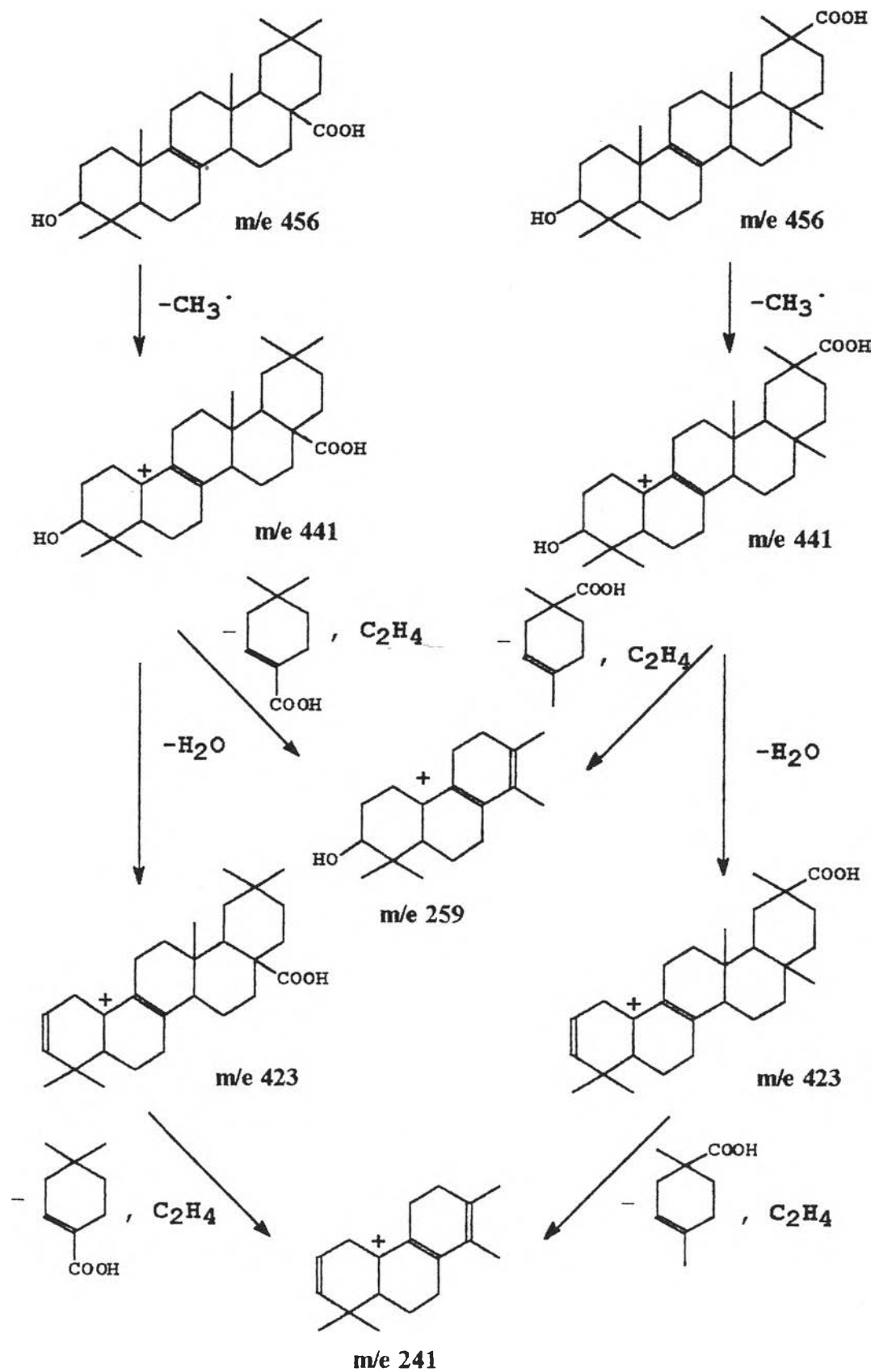| chemical shift (ppm.)				wave number (CM^{-1})	Tentative assignment
^{1}H	^{13}C	DEPT-135	DEPT-90	IR	
	15.3	15.3^{u}			CH3
	17.1	17.1^{u}			CH3
	18.8	18.8^{d}			CH2
	19.4	19.4^{u}			CH3
	20.2	20.2^{d}			CH2
	21.7	21.7^{u}			CH3
	24.7	24.7^{d}			CH2
	27.1	27.1^{d}			CH2
	27.5	27.5^{d}			CH2
	27.7	27.7^{u}			CH3
	29.4	29.4^{d}			CH2
	29.8	29.8^{d}			CH2
	30.1	30.1^{d}			CH2
	30.5				CH3
	30.8	30.8^{u}			CR4
	32.3	32.3^{u}			CH3
	34.1	34.1^{d}			CH2
	34.8	34.8^{d}			CH2
	36.6	36.6^{d}			CH2
	36.9				CR4
	37.1				CR4
	38.4				CR4
	39.6				CH
	41.4				CH
	44.4	44.4^{u}	44.4		-O-CH
	50.2	50.2^{u}	50.2		=CR2
2.90	78.0	78.0^{u}	78.0		=CR2
	133.5			1682	-COOH
	133.7				
	180.4				

The mass spectrum exhibited by chemical ionization technique (Fig. 50) did give the molecular ion peak at 457 (MH^+) which agrees with the molecular formular $C_{30}H_{48}O_3$ and the signal at 439 (MH^+-18) could be account for the loss of one hydroxyl group. After it was analyzed again by electron impact technique of mass spectrometer, its mass spectrum of PA-4 (Fig. 51) did not give the molecular ion peak, due to the breaking of molecule almostly. The peak series pattern implied a triterpenoid compound. The fragmentation ion peak at m/e 441 (M^+-15) was identified as methyl group, and then, the presence of the ion at m/e 423 (M^+-18) suggested the elimination of hydroxyl group at C-3 position. The possible mass fragmentation ions pattern of PA-4 was presented in Scheme 4.

From the spectral evidences and physical properties of this compound that the above information obviously could not proved that PA-4 possed triterpenoidal skeleton with one hydroxy group at C-3 position, carboxylic acid group, one of vinylidene moiety of tetrasubstituent carbon, seven methyl group, eleven methylene carbon, three methine carbon, and nine quaternary carbon (one carboxyl carcon). PA-4 may be one of the following compounds.
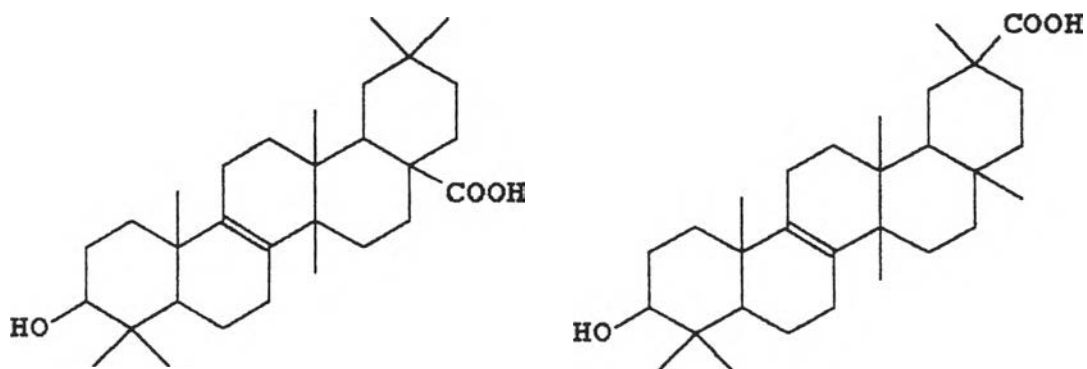
PA-4

Scheme 4 The possible mass fragmentation pattern of PA-4

m/e 456   m/e 456

$-CH_3^{\cdot}$ $-CH_3^{\cdot}$

m/e 441   m/e 441

$, C_2H_4$ $, C_2H_4$

$-H_2O$ $-H_2O$

m/e 259

m/e 423   m/e 423

$, C_2H_4$ $, C_2H_4$

m/e 241

3.5 Structural Elucidation of PA-5

PA-5 was isolated from the silica gel column chromatography of fractions 89-99 by 60 % ether in n-hexane. This compound was crystallized from ethanol to yield PA-5 as a white amorphous solid, 0.83 % wt. by wt. of diethyl ether crude. Its melted at 222-223 $^{\circ}$C. R_f value was 0.35 in 30 % methanol in chloroform system. This compound was readilt soluble in chloroform, ethyl acetate, hot methanol and hot ethanol, but slightly soluble in hexane, diethyl ether, ethanol and methanol.

The IR spectrum of PA-5 was presented in Fig. 52 and the absorption peaks were assigned in Table 19. Its IR spectrum showed important absorption bands at 3040 cm^{-1} (C-H stretching vibration of olefinic), 2974, 2950, 2889 cm^{-1} (C-H stretching vibration), 1745 cm^{-1} (C=O stretching vibration of α,β-unsaturated of lactone), 1700 cm^{-1} (C=O stretching vibration of ketone), 1626 cm^{-1} (C=C stretching vibration of olefinic), 1283 cm^{-1} (C-O-C stretching vibration), and 875 cm^{-1} (out of plane bending vibration coincided with the characteristic absorption peak of furan). The absorption peak at 1390 cm^{-1} corresponed to C-H symmetric bending vibration mode of CH$_3$ group.

Table 19 The IR absorption band assignments of PA-5

Frequency	Band type	Tentative assignment
3040	w	CH stretching vibration of =CH
2974, 2950, 2889	m	CH stretching vibration of -CH$_2$, -CH$_3$
1745	s	C=O stretching vibration of lactone
1700	s	C=O stretching vibration of ketone
1626	w	C=C stretching vibration
1536, 1506, 1455, 1422	m	C-H bending vibration of -CH$_2$, -CH$_3$
1390	s	C-H symmetric bending vibration of -CH$_3$
1283	s	C-O stretching vibration of -CO-O-C=C
875	m	C-H bending vibration of furan

The ^{1}H NMR spectrum (CDCl$_3$) of PA-5, Fig. 53, exhibited the signals at 1.10-1.49 ppm., which were the signals of angular methyl groups at side chain of C-18, C-19, C-24, C-25 and C-26.   The signals at 1.86 ppm. exhibited methylene group (-CH$_2$-) of C-11 and C-12. The double of doublet signals (J=7, 5 Hz.) at 2.10 ppm. was shown the methine group (-CH-) of C-9. The double of doublet signals at 2.58 ppm. (J=14, 5 Hz.) and 2.97 ppm. (J=14, 13 Hz.) should be of methylene group (-CH$_2$) of C-6 and the methine proton of C-5 appeared at 2.26 ppm. that this was a double of doublet signals (J=13, 5 Hz.).   The signal of a methine protons attacted to a carbon atom bearing a oxygen atom at 3.63 and 5.44 ppm. (C-15 and C-17, respectively) and doublet signals in olefinic region which should be a methine protons at 5.93 and 6.50 ppm. (2H, J=12 Hz.).   The broad singlet signals of furan ring appeared at chemical shift 6.34, 7.37 and 7.38 ppm. (3H).

The ^{13}C NMR spectrum, Fig. 54, displayed the signal at 207.4 ppm. which was corresponded to carbonyl carbon of ketone and signals at 166.8 and 166.6 ppm. could be assigned carbonyl carbon of α,β-unsaturated of lactone. The signals at 156.7 and 122.9 ppm. were assigned to α,β-unsaturated carbonyl carbon and signals at 143.1, 140.9, 120.0 and 109.7 were assigned to β-substituted furanoid carbon. The signals at 83.9 and 65.0 ppm. could be assigned to the carbon atom with three substituents group that its ought to attacted to oxygen atom and signals at 77.9 and 57.2 ppm. two substituents group which its were contacted to the oxygen atom and the signals at 16.4-49.1 ppm. were attributed to methyl, methylene and methine carbon.

The ^{13}C NMR, DEPT-135 and DEPT-90 ^{13}C NMR spectra, Fig 54-56, gave important information which corresponded to the information from ^{13}C NMR spectrum and could be interpreted as follows in Table 20.

It could be concluded that PA-5 exhibited to the ^{13}C NMR chemical shifts similar to obacunone [6]. The ^{13}C NMR chemical shifts of PA-5 and obacunone could be compared as in Table 21.

The mass spectrum of this compound exhibited by chemical ionization technique (Fig. 57) gave the molecular ion peak at m/e 455 (MH^{+}), $C_{26}H_{30}O_{7}$. After it was analyzed again by electron impact technique of mass spectrometer, its mass spectrum of PA-5 (Fig. 58) did not revealed the molecular ion peak, due to the braking of molecule almostly. The possible mass fragmentation ions pattern of PA-5 was presented in Scheme 5.

**Table 20   Comparison of spectroscopic data of PA-5**

chemical shift (ppm.)				wave number (CM^{-1})	Tentative assignment
^{1}H	^{13}C	DEPT-135	DEPT-90	IR	
	16.4	16.4 ^{u}			CH3
	16.9	16.9 ^{u}			CH3
	19.4	19.4 ^{d}			CH2
	21.0	21.0 ^{u}			CH3
	26.7	26.7 ^{u}			CH3
	31.9	31.9 ^{u}			CH3
	32.7	32.7 ^{d}			CH2
	37.4				CR
2.58,2.98	39.8	39.8 ^{d}			CH3
	43.1				CR
2.10	49.1	49.1 ^{u}	49.1		CH
	52.9				CR
2.26	53.2	53.2 ^{u}	53.2		CH
3.63	57.2	57.2 ^{u}	57.2		-O-CH
	65.0				-O-CR
5.43	77.9	77.9 ^{u}	77.9		-O-CH
	83.9				-O-CR
6.34	109.7	109.7 ^{u}	109.7		=CH
	120.0				=CR
5.93	122.9	122.9 ^{u}	122.9		=CH
7.39	140.9	140.9 ^{u}	140.9	1626	=CH
7.37	143.1	143.1 ^{u}	143.1		=CH
6.47	156.7	156.7 ^{u}	156.7		=CH
	166.6				-COOR
	166.8			1700	-COOR
	207.4			1745	-COR

Table 21   The ^{13}C NMR chemical shifts of PA-5 and Obacunone.

Carbon	Chemical shift (ppm.)	
	PA-5	Obacunone
1	156.7	156.7
2	122.9	122.8
3	166.6	166.6
4	83.9	83.9
5	53.2	53.4
6	39.8	39.9
7	207.4	207.4
8	52.9	52.9
9	49.1	49.2
10	-	-
11	-	-
12	-	-
13	-	-
14	65.0	65.2
15	57.2	57.3
16	166.8	166.8
17	77.9	78.0
18	-	-
19	-	-
20	120.0	120.2
21	140.9	141.0
22	109.7	109.8
23	143.1	143.1
24	-	-
25	-	-
26	-	-

Scheme 5 The possible mass fragmentation pattern of PA-5

m/e 454

$- CO_2$

m/e 410

m/e 331
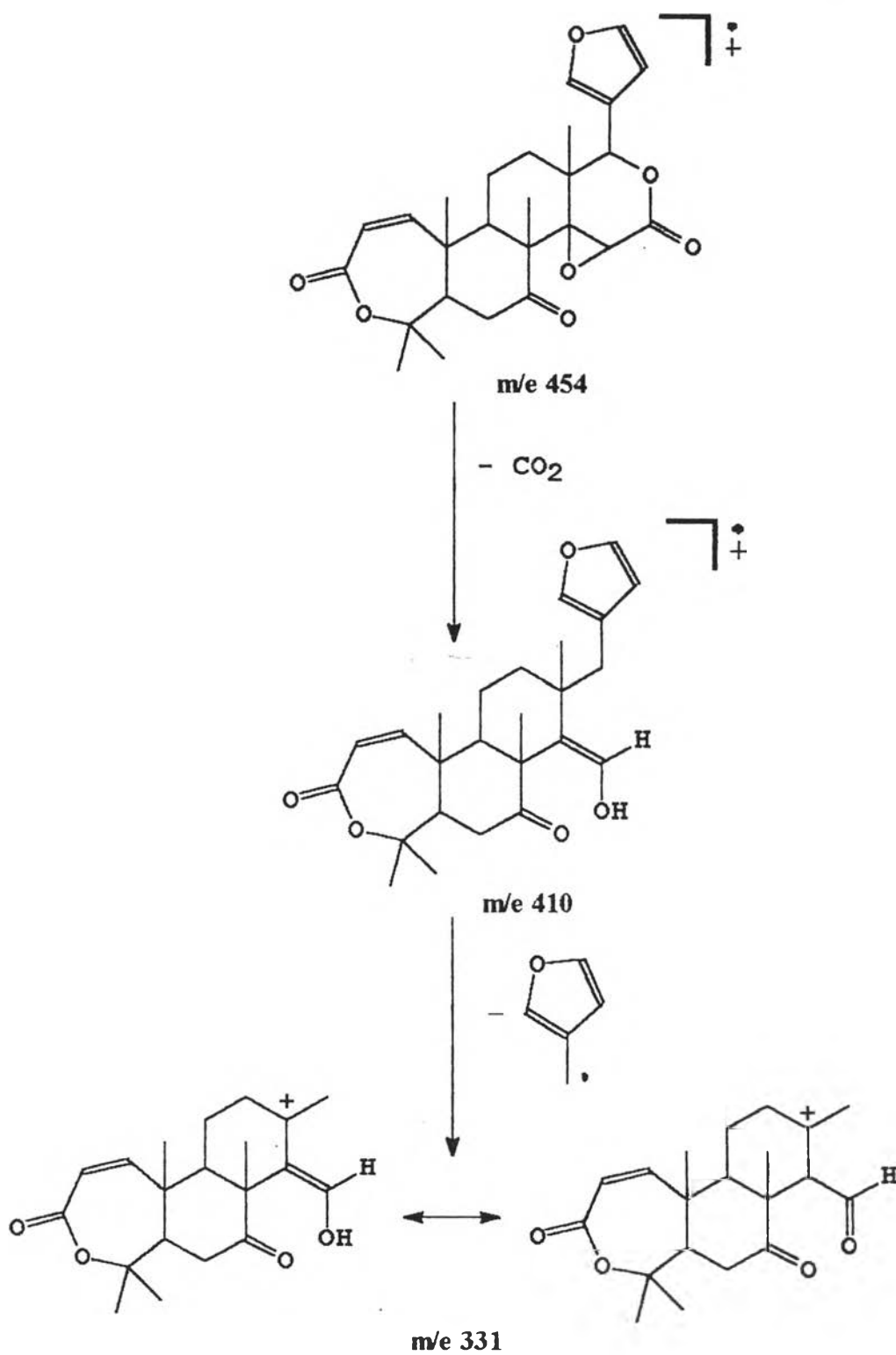
According to spectroscopic evidences and physical properties, PA-5 was identical to obacunone [6,7,8,10,11,14]. The structure of this compound was shown below :

PA-5 : Obacunone