

CHAPTER III

RESULTS AND DISCUSSION

STRUCTURAL ELUCIDATION OF SUBSTANCES SEPARATED FROM CRUDE EXTRACT OF HEXANE AND CHLOROFORM

Structural elucidation of A

A was the yellow needle, m.p. 147-149 °C and $R_f = 0.53$ (silica gel/chloroform: hexane; 1:1). The UV absorption spectrum (Fig. 1), signal at $\lambda_{max} = 342$ nm exhibited conjugated double bond. The IR spectrum is shown in Fig. 2 and absorption bands are exhibited in Table 5.

Table 5 The Infrared absorption band assignments of A

Wavenumber (cm ⁻¹)	Tentative assignment
3050	C-H stretching of C-H aromatic
2950	C-H stretching of -CH ₂ , -CH ₃
1715	C=O stretching
1630	C=C stretching of -C=C-CO
1620	C=C stretching of -C=C-Ph
1515, 1505	C-H stretching of -O-CH ₃
1445	C-H stretching of -CH ₂ -O-Ph
1280, 1250	C-O-C bending

From IR spectrum, absorption peak at 3050 cm^{-1} corresponding to CH-aromatic, absorption peak at 2950 cm^{-1} corresponding to CH-aliphatic(CH_2 , CH_3 group), absorption peak at 1715 cm^{-1} corresponding to -CO- carbonyl group of aliphatic ester, absorption peak at 1630 and 1620 cm^{-1} corresponding to -C=C- alkenyl group, absorption peak at 1515 and 1505 cm^{-1} corresponding to -O- CH_3 (C-O bond of ester group), absorption peak at 1445 cm^{-1} corresponding to CH_2 -O-Ph, absorption peak at 1280 and 1250 cm^{-1} corresponding to C-O-C(C-O bond of ester group).

From ^1H -NMR spectrum(see Fig. 3), signal at 3.70 ppm corresponding to - OCH_3 methoxy group, signals at 5.85, 6.75-6.8, and 7.30 ppm corresponding to -CH= alkene, signals at 6.76-6.8, 6.9, and 7.03 ppm corresponding to -CH= aromatic, signal at 5.95 ppm corresponding to O- CH_2 -O.

From ^{13}C -NMR(Fig. 5), ^{13}C -NMR DEPT-135(Fig. 6) and ^{13}C -NMR DEPT-90 spectrum(Fig. 7), signals at 50.5 ppm corresponding to - OCH_3 (methoxy group), signals at 100.2 ppm corresponding to O- CH_2 -O, signals at 105.0, 108.0, 119.5 and 122.5 ppm corresponding to -CH= (alkene), signals at 124.0, 139.5 and 144.0 ppm corresponding to -CH=(aromatic), signals at 129.5, 147.5 and 148.0 ppm corresponding to -C=(aromatic), signal at 166.0 ppm corresponding to -CO- (carbonyl of aliphatic ester). From ^1H and ^{13}C -NMR spectra of **A** were similar to those of methyl piperate¹⁰ all signals assigned as **Table 6** and **Table 7**.

Table 6 Comparison of chemical shifts and coupling constants of $^1\text{H-NMR}$ spectrum of **A** with those of methyl piperate

Position of hydrogen	Methyl piperate $\delta(\text{J})$	A $\delta(\text{J})$	Peak type, Number of hydrogen	Tentative assignment
13	3.70	3.70	s, 3	$-\text{OCH}_3$ methoxy
2	5.92(15.6)	5.85(15.5)	d, 1	$-\text{CH}=\text{alkene}$
3	7.36(15.6, 9.7)	7.30(15.5, 9.1)	dd, 1	$-\text{CH}=\text{alkene}$
4	6.67(9.7)	6.75-6.8(3H)	m, 1	$-\text{CH}=\text{alkene}$
5	6.75(15.6)	6.75-6.80(3H)	m, 1	$-\text{CH}=\text{alkene}$
7	7.01(1.5)	7.03(1.4)	d, 1	$-\text{CH}=\text{aromatic}$
10	6.80(7.8)	6.75-6.80(3H)	m, 1	$-\text{CH}=\text{aromatic}$
11	6.91(7.8, 1.5)	6.90(7.9, 1.4)	dd, 1	$-\text{CH}=\text{aromatic}$
12	5.98	5.95	s, 2	$\text{O}-\text{CH}_2-\text{O}$

Table 7 Comparison of chemical shifts of $^{13}\text{C-NMR}$ spectrum of **A** with those of methyl piperate

Position of carbon	Methyl piperate $\delta(\text{J})$	A $\delta(\text{J})$
1	165.70	166.0
8 or 9	147.30	148.0
8 or 9	147.10	147.5
10 or 11	143.50	144.0
10 or 11	139.60	139.5

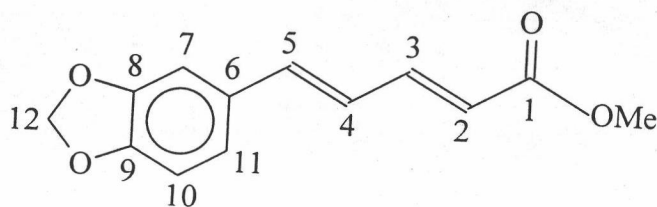
Table 7 continue

6	129.30	129.5
7	123.30	124.0
2	121.60	122.5
3 or 4 or 5	118.70	119.5
3 or 4 or 5	107.20	108.0
3 or 4 or 5	104.70	105.0
12	100.10	100.5
13	49.90	50.5

Mass spectrum(Fig. 3) displayed molecular ion at m/e 232 and others fragmentation at m/e 217($M^+ - CH_3$), 201($M^+ - OCH_3$), 173($M^+ - OCH_3 - CO$), 143($M^+ - C_3H_5O_3$), 115($M^+ - C_3H_5O_3 - CO$). Fragmentation of **A** was similar to that of methyl piperate (see **Scheme 2**)

From $^1H-^1H$ COSY (Fig. 8) and $^1H-^1H$ NOESY (Fig. 9), the signal of proton at 5.85 ppm(H-2) coupled with the signal of proton at 7.30 ppm(H-3) and coupled long range with the signal at 6.75 ppm(H-4), the signal of proton at 6.75 ppm(H-4) coupled with the signals at 7.30 ppm(H-3) and 6.76 ppm(H-5), the signals of protons at 3.70 ppm(H-13), 5.95 ppm(H-12) and 7.03 ppm(H-7) not coupled with other protons.

From the spectroscopic data and comparison of **A** with methyl piperate, they can be concluded that **A** was methyl piperate($C_{13}H_{12}O_4$). The structure is shown below.



Structural elucidation of C

C was the white needle (m.p. 146-148 °C). It was soluble in chloroform and was recrystallized from the mixture of hexane and ethylacetate. The UV absorption spectrum(Fig. 19), signals at $\lambda_{\max} = 268, 312$ nm exhibited chromophore such as C=C, aromatic or conjugated double bond. The Infrared spectrum is shown in Fig. 20 and absorption bands indicated in **Table 8**.

Table 8 The Infrared absorption band assignments of C

Wavenumber (cm ⁻¹)	Tentative assignment
3050	C-H stretching of C-H aromatic
2995-2900	C-H stretching of -CH ₂ , -CH ₃
1620	C=C stretching of -C=C-Ph
1515, 1490	C-H stretching of -O-CH ₂ -
1460	C-H stretching of -CH ₂ -O-Ph
1390	C-H bending of -CH ₃
1270, 1240	C-O-C bending
1050	C-O bending
980	C-H bending of -CH=CH-(trans)

The IR spectrum indicated that this substance contained an aromatic functional group at 3050 cm⁻¹. Characteristic of aliphatic -CH₃ at 1390 cm⁻¹ and O-CH₂-Ph at 1515 cm⁻¹, 1490 cm⁻¹ and 1460 cm⁻¹. Characteristic of aromatic alkene at 1620 cm⁻¹ and aliphatic alkene at 980 cm⁻¹. Characteristic of C-O bond at 1270 cm⁻¹, 1240 cm⁻¹ and 1050 cm⁻¹.

From $^1\text{H-NMR}$ spectrum(see Fig. 22) signals at 1.90 ppm and 2.40 ppm indicated CH_3 group, signals at 6.20 ppm and 6.50 ppm indicated C-H aliphatic alkene, signals at 6.80 ppm, 6.85 ppm, 7.00 ppm and 7.27 ppm(2 proton) indicated C-H aromatic, signal at 4.00 ppm indicated $-\text{OCH}_3$ group and signal at 5.95 ppm indicated dioxymethyl group($\text{O-CH}_2\text{-O}$)

The $^{13}\text{C-NMR}$ spectrum(Fig. 23), $^{13}\text{C-NMR DEPT-135}$ (Fig. 24) and $^{13}\text{C-NMR DEPT-90}$ (Fig. 25) signals at 9.59 ppm and 19.0 ppm indicated $-\text{CH}_3$, signal at 56.0 ppm indicated $-\text{OCH}_3$, signal at 101.0 ppm $-\text{CH}_2-$, signals at 105.0 ppm, 107.0 ppm, 108.0 ppm, 109.5 ppm, 121.0 ppm, 124.5 ppm and 131.5 ppm indicated $-\text{CH=}$ and signals at 110.5 ppm, 125.5 ppm, 133.0 ppm, 133.5 ppm, 142.0 ppm, 145.0 ppm, 147.5 ppm, 148.0 ppm and 151.0 ppm indicated $-\text{C=}$. From $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of **C** was similar to those of eupomatene²⁰ and all signals were assigned as **Table 9** and **Table 10**.

Table 9 Comparison of chemical shifts and coupling constant of $^1\text{H-NMR}$ spectrum of **C** with those of eupomatene

Position of proton	C $\delta(\text{J})$	eupomatene $\delta(\text{J})$	Type of signals, Number of proton	Tentative assignment
12	1.9(6.0)	1.9(6.0)	d, 3	$-\text{CH}_3$
14	2.4	2.4	s, 3	$-\text{CH}_3$
13	4.05	4.0	s, 3	$-\text{OCH}_3$
7'	6.0	5.95	s, 2	$\text{O-CH}_2\text{-O}$
11	6.2(15.7)	6.2(15.0)	m, 1	$-\text{CH=}$ alkene
10	6.47(6.0, 15.7)	6.5(6.0, 15.0)	dd, 1	$-\text{CH=}$ alkene
6'	6.81(8.5)	6.8	d, 1	$-\text{CH=}$ aromatic

Table 9 continue

5'	6.88(8.5)	6.85	d, 1	-CH= aromatic
4	7.10	7.0	s, 1	-CH= aromatic
6	7.27	7.26	s, 1	-CH= aromatic
2'	7.29	7.27	s, 1	-CH= aromatic

Table 10 Comparison of chemical shifts of ^{13}C -NMR spectrum of C with those of eupomatene

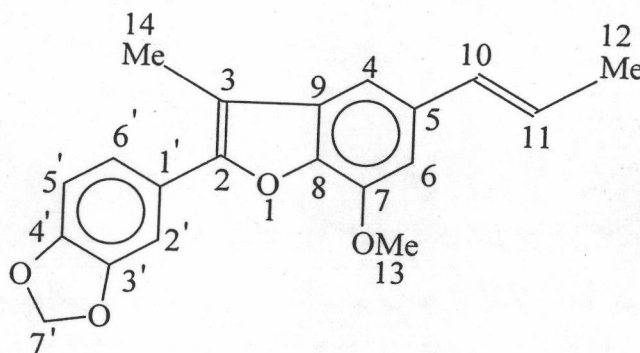
Position of carbon	Eupomatene $\delta(\text{J})$	C $\delta(\text{J})$
2	151.10	151.0
3	110.50	110.5
4	104.19	109.5
5	133.66	133.5
6	104.67	105.0
7	144.87	145.0
8	142.12	142.0
9	132.99	133.0
10	131.56	131.5
11	124.32	124.5
12	18.44	19.0
13	56.11	56.0
14	9.59	9.59
1'	125.35	125.5

Table 10 continue

2'	121.00	121.0
6'	107.28	107.0
3'	147.86	148.0
4'	147.28	147.5
5'	108.47	108.0
7'	101.25	101.0

From the mass spectrum (Fig. 21), it displayed the molecular ion peak at m/e 322. It revealed the fragmentation of molecular at m/e 295 ($M^+ - CO$), 263 ($M^+ - C_2H_3O_2$) and 161. When the mass spectrum of **C** was compared with eupomatene, fragmentation of **C** was similar to that of eupomatene (see **Scheme 3**).

From the spectroscopic data and 2D-NMR spectrum ($^1H-^1H$ COSY (Fig. 26) and $^1H-^1H$ NOESY (Fig. 27) and comparison of **C** with eupomatene, they can be concluded that **C** was eupomatene ($C_{20}H_{18}O_4$). The structure is shown below.



Structure elucidation of **B**

B was white amorphous solid (m.p. 102-105°C). It was soluble in chloroform and was recrystallized from the mixture of hexane and ethyl acetate. The

UV absorption spectrum(Fig.10), signal at $\lambda_{\text{max}}=268, 306$ nm exhibited chromophore such as aromatic, C=C or conjugated double bond. The IR spectrum is shown in Fig. 11 and absorption bands indicate in **Table 11**.

Table 11 The Infrared absorption band assignments of **B**

Wavenumber (cm^{-1})	Tentative assignment
3450, 1225	O-H stretching of phenolic
3020	C-H stretching of C-H aromatic
2950, 2880	C-H stretching of $-\text{CH}_2, -\text{CH}_3$
1625, 1610	C=C stretching of C=C-Ph
1525, 1500	C-O stretching of $-\text{OCH}_3$
1470, 1460	C-O stretching of $\text{CH}_3\text{-O-Ph}$
1300	C-H stretching of $-\text{CH}_3$
1280, 1240	C-O-C bending
980	C-H bending of $-\text{CH}=\text{CH}-$ (trans)

The IR spectrum indicated that this substance contained phenolic functional group at 3450 cm^{-1} and 1225 cm^{-1} . Absorption band at $2950\text{-}2880 \text{ cm}^{-1}$ and 1380 cm^{-1} indicated $-\text{CH}$ aliphatic group. Absorption band at 1625 cm^{-1} and 1610 cm^{-1} indicated aromatic alkene and 980 cm^{-1} indicated aliphatic alkene($-\text{CH}=\text{CH}-$ trans). Absorption band at 1470 cm^{-1} and 1460 cm^{-1} indicated $\text{CH}_3\text{-O-Ph}$ and absorption band at 1280 cm^{-1} and 1240 cm^{-1} indicated C-O-C.

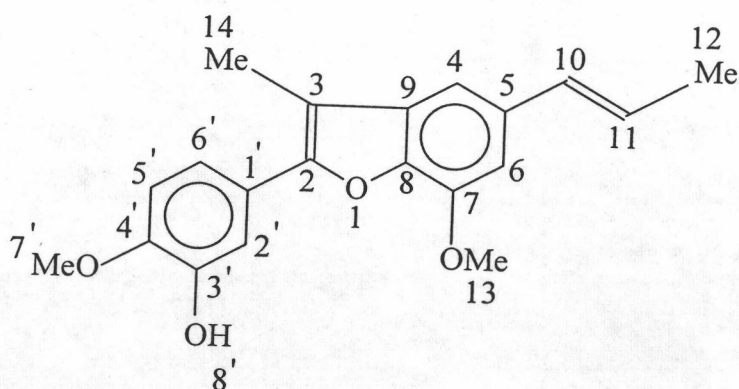
The $^1\text{H-NMR}$ spectrum(Fig. 13) displayed the proton of methoxy group($-\text{OCH}_3$) at 3.95(3 protons, singlet) ppm, the proton of phenolic group(HO-Ph) at

5.8(1proton, broad singlet) ppm and the proton of methylenedioxy (O-CH₂-O) is not shown. And the other protons were displayed similar to that of C

From ¹³C-NMR spectrum(Fig. 14), ¹³C-NMR DEPT-135(Fig. 15) and ¹³C-NMR DEPT-90 spectrum(Fig. 16), signal of dioxymethyl group(O-CH₂-O) is not shown and signal of methoxy group(-OCH₃) displayed at 56.0 ppm. And the other signals were displayed similar to that of C.

The mass spectrum(Fig. 12) displayed the molecular ion peak at m/e 324. The ¹H-¹H COSY(Fig. 17) and ¹H-¹H NOESY(Fig. 18), the signal of proton at 3.95 ppm(H-7', methoxy group) coupled long rang with the signal at 5.8 ppm(H-8', phenolic group) and coupled long range with the signal at 6.95 ppm(H-5', -CH= aromatic). And the signals of proton at 6.95 ppm(H-5') coupled with the signal of proton at 7.35 ppm(H-6', -CH= aromatic)

From the spectroscopic data and comparison of B with C, they can be concluded that B(C₂₀H₂₀O₄) was derivative of C(eupomatene)²⁰. The structure is shown below.



Structural elucidation of D

D was white needle which recrystallized from mixture of chloroform and hexane. Its melting point was 153-154 °C. From UV absorption spectrum(Fig. 28) λ_{\max} =292 nm exhibited chromophore such as aromatic or C=C. The IR spectrum was shown in Fig. 29 and absorption bands revealed in Table 12.

Table 12 The Infrared absorption band assignments of **D**

Wavenumber (cm ⁻¹)	Tentative assignment
3500-3100	O-H stretching of phenolic
3050, 2000-1700	C-H stretching of -CH aromatic
2980, 2900, 1460, 1350	C-H stretching of -CH ₂ , -CH ₃
1680	C=O stretching of ester
1640, 1620, 1600	C=C stretching of C=C aliphatic
1525	C=C stretching of C=C aromatic
1390, 1380	C-H stretching of CH ₃ -C-CH ₃
1230-1170	C-O bending
840	p-substitution of benzene ring

The IR absorption band at 1680 cm⁻¹ revealed carbonyl functional group. **D** gave negative test to NaHCO₃. No absorption band at 2720 cm⁻¹ revealed that **D** was ester. Absorption bands at 3500-3100 cm⁻¹ and 3050 cm⁻¹, 2000-1700 cm⁻¹, 840 cm⁻¹, therefore **D** was phenolic compound(p-disubstitution benzene ring), absorption band at 1640 cm⁻¹, 1620 cm⁻¹ and 1600 cm⁻¹ revealed alkene group(C=C), absorption band at 1230-1170 cm⁻¹ revealed C-O bond and absorption band at 1390 cm⁻¹ and 1380 cm⁻¹ revealed CH₃-C-CH₃

From ¹H-NMR(Fig. 31) displayed the protons of -CH₃ at 0.87, 0.90, 1.06 ppm, O-CH aliphatic at 4.75 ppm, -CH=CH- alkene at 6.27, 7.61 ppm, -CH= aromatic 6.85(2 protons), 7.41(2 protons) ppm and -OH phenolic at 7.55 ppm

From ¹³C-NMR spectrum(Fig. 32), ¹³C-NMR DEPT-135(Fig. 33) and ¹³C-NMR DEPT-90(Fig. 34) were displayed the carbon of -CH₃ at 11.5, 19.9, 20.1 ppm, -CH₂- at 27.0, 33.7, 39.0 ppm, -CH aliphatic at 45.0 ppm, -O-CH aliphatic at 81.5

ppm, -CH alkene at 115.6, 144.6 ppm, -CH= aromatic at 116.0, 116.0, 130.0, 130.0 ppm, tertiary-carbon aliphatic at 47.0, 49.0 ppm, C=O at 167.7 ppm, -C= aromatic at 126.8 ppm and O-C= aromatic at 158.3 ppm. ^1H and ^{13}C -NMR spectrum of **D** were similar to the NMR spectrum of (-)-borneol p-hydroxycinnamate²¹ as **Table 13** and **Table 14**.

Table 13 Comparison of chemical shifts and coupling constants of ^1H -NMR spectrum of **D** with those of (-)-borneol p-hydroxycinnamate

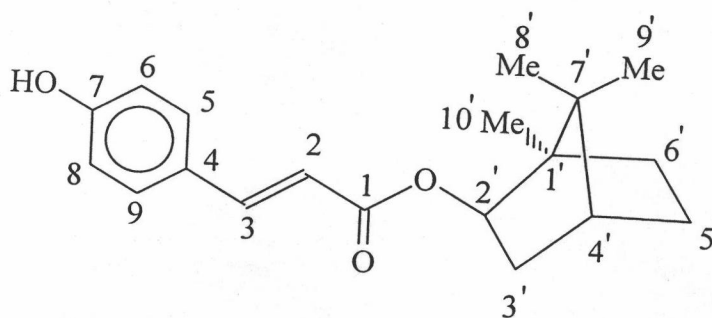
Position of proton	Peak type, Number of proton	Tentative assignment	(-)-borneol p-hydroxy cinnamate $\delta(\text{J})$	D $\delta(\text{J})$
9'	s, 3	-CH ₃	0.87	0.87
8'	s, 3	-CH ₃	0.90	0.90
10'	s, 3	-CH ₃	1.06	1.04
3', 4', 5', 6'	m, 7	-CH aliphatic	1.21-1.83	1.05-1.88
2'	t, 1	O-CH-CH ₂	4.81(6.0)	4.79(5.9)
2	d, 1	-CH= alkene	6.27(16.0)	6.27(16.0)
5, 9	d, 2	-CH aromatic	6.85(8.0)	6.85(8.0)
-OH	s, 1		6.23	7.55
6, 8	d, 2	-CH aromatic	7.42(8.0)	7.41(8.0)
3	d, 1	-CH= alkene	7.58(16.0)	7.61(16.0)

Table 14 Comparison of chemical shifts of ^{13}C -NMR spectrum of **D** with those of (-)-borneol p-hydroxycinnamate

Position of carbon	(-)-borneol p-hydroxycinnamate δ	D δ
1	167.6	167.7
2	115.6	115.6
3	144.6	144.6
4	126.8	126.8
5, 9	115.9	116.0
6, 8	130.0	130.0
7	158.3	158.3
2'	81.3	81.5
5'	27.0	27.0
4'	45.1	45.0
1'	48.9	49.0
3'	38.8	39.0
7'	46.9	47.0
6'	33.7	33.7
10'	11.5	11.5
8'	20.2	20.1

The mass spectrum(Fig. 30) displayed the molecular ion peak at m/e 300. When the mass spectrum of **D** was compared with (-)-borneol p-hydroxycinnamate, they have similar fragmentation.(see **Scheme 4**)

From ^1H - ^1H COSY (Fig. 35) and ^1H - ^1H NOESY (Fig. 36), the signal of proton at 6.27 ppm(H-2) coupled with the signal of proton at 7.61 ppm(H-3), the signal of proton at 6.85 ppm(H-5, H-9) coupled with signal of proton at 7.41 ppm(H-6, H-8) and signal of proton at 4.75 ppm(H-2') coupled with the signal of proton at 1.80 ppm From spectroscopic data and comparison of **D** with (-)-borneol p-hydroxy cinnamate, it can be concluded that **D** was (-)-borneol p-hydroxy cinnamate($\text{C}_{19}\text{H}_{24}\text{O}_3$). The structure is shown below.



Structural elucidation of E

E was yellow needle which recrystallized from mixture of chloroform and hexane. Its melting point was 102-105°C. From UV absorption spectrum (Fig.37) $\lambda_{\text{max}} = 260$ nm exhibited chromophore such as aromatic or C=C. The IR spectrum was shown in Fig. 38 and absorption bands revealed in **Table 15**.

Table 15 The Infrared absorption band assignments of **E**

Wavenumber (cm^{-1})	Tentative assignment
3100	-OH phenolic(hydrogen bonding which C=O)
3010, 870, 750	C-H stretching of C-H aromatic
2990, 2940, 980	C-H stretching of $-\text{CH}_2$, $-\text{CH}_3$
2880, 1445	C-H stretching of CH_3 -O-Ph
1680	C=O stretching(hydrogen bonding which -OH phenolic)
1630	C=C stretching of C=C-CO-
1610	C-H stretching of $-\text{CH}=\text{C}$
1430	C-H stretching of $-\text{CH}_2$ - $\text{CH}=\text{C}$
1280	C-O-C bending of Ph-O-R
1230	C-O bending
1090	C=C bending of Ph-O-C=C

From IR spectrum, absorption peak at 3100 cm^{-1} corresponding to -OH phenolic(hydrogen bonding with C=O), absorption peak at 3010, 820, 750 cm^{-1} corresponding to -CH aromatic, absorption peak at 2990, 2940, 2880, 1445, 980 cm^{-1} corresponding to -CH aliphatic, absorption peak at 1680 cm^{-1} corresponding to C=O, absorption peak at 1630, 1610 cm^{-1} corresponding to C=C and absorption peak at 1280, 1230 cm^{-1} corresponding to C-O

From ^1H -NMR spectrum (Fig. 40) display the protons of $-\text{CH}_3$ (9 protons) at 1.60, 1.75 and 2.35 ppm, protons of $-\text{CH}_2$ at 3.33 ppm, protons of $-\text{OCH}_3$ (3 protons) at 3.85 ppm, proton of $-\text{CH}(-\text{CH}_2-\text{CH}=\text{C}$, 1 proton) at 5.05 ppm, proton of $=\text{CH}-\text{CO}-$ at 5.90 ppm, proton of $-\text{CH}$ aromatic (1 proton) at 6.25 ppm and proton of $-\text{OH}$ phenolic (1 proton) at 12.6 ppm.

From ^{13}C -NMR spectrum (Fig. 41), ^{13}C -NMR DEPT-135 (Fig. 42) and ^{13}C -NMR DEPT-90 (Fig. 43) were displayed the carbons of $-\text{CH}_3(=\text{C}(\text{CH}_3)_2$, 2 carbons) at 17.5, 20.5 ppm, carbon of $-\text{CH}_3(=\text{C}-\text{CH}_3)$ at 25.5 ppm, carbon of $-\text{CH}_2$ at 21.5 ppm, carbon of $-\text{OCH}_3$ at 55.5 ppm, carbons of $-\text{CH}=(3$ carbons) at 107.5, 108 and 122 ppm, carbon of $=\text{C}-(3$ carbons) at 94.5, 104.5 and 131.0 ppm, carbon of $-\text{CO}=(4$ carbon) at 154.5, 160.5, 162.5 and 166.0 ppm and carbon of $\text{C}=\text{O}$ at 182.5 ppm. ^1H and ^{13}C -NMR spectrum of **E** were similar to the NMR spectrum of heteropeucenin-8-methyl ether²² all signals assigned as **Table 16** and **Table 17**.

Table 16 Comparison of chemical shifts and coupling constants of ^1H -NMR spectrum of **E** with those of heteropeucenin-8-methylether

Position of proton	Number and type of proton	Tentative assignment	heteropeucenin-8-methylether $\delta(\text{J})$	E $\delta(\text{J})$
15 or 16	3, s	$=\text{C}(\text{CH}_3)_2$	1.66	1.60
15 or 16	3, s	$=\text{C}(\text{CH}_3)_2$	1.79	1.75
1	3, s	$-\text{CH}_3$	2.36	2.35
12	2, d	$=\text{CH}-\text{CH}_2-$	3.33(7.2)	3.30(7.2)
11	3, s	$-\text{OCH}_3$	3.87	3.85
13	1, t	$=\text{CH}-\text{CH}_2-$	5.15(7.2)	5.05(7.2)

Table 16 continue

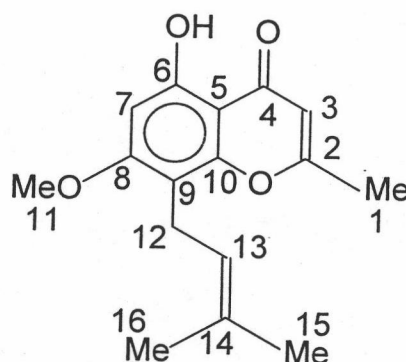
3 or 7	1, s	=CH-CO-	5.99	5.90
3 or 7	1, s	-CH= aromatic	6.36	6.25
	1, s	-OH phenolic	12.76	12.6

Table 17 Comparison of chemical shifts of ^{13}C -NMR spectrum of **E** with those of heteropeucenin-8-methylether

Position of carbon	heteropeucenin-8-methyl ether δ	E δ
4	182.89	182.5
6, 8 or 10	162.69	162.5
6, 8 or 10	160.51	160.5
6, 8 or 10	154.67	154.5
5	131.48	131.0
7	107.69	107.5
9	94.67	94.5
2	166.69	166.0
3	108.24	108.0
13	122.10	122.0
14	104.66	104.5
11	55.96	55.5
1	25.73	25.5
12	21.73	21.5
15 or 16	20.47 or 17.76	20.5 or 17.5

The mass spectrum(Fig. 39) displayed the molecular ion peak at m/e 274. When the mass spectrum of **E** was compared with heteropeucenin-8-methylether, they have similar fragmentation.(see **Scheme 5**)

From $^1\text{H}-^1\text{H}$ COSY(Fig. 44) and $^1\text{H}-^1\text{H}$ NOSEY(Fig. 45), the signal of proton at 3.3 ppm(H-12) coupled with the signal of proton at 5.05 ppm(H-13). From spectroscopic data and comparison of **E** with heteropeucenin-8-methyl ether, it can be concluded that **E** was heteropeucenin-8-methylether($\text{C}_{16}\text{H}_{18}\text{O}_4$). The structure is shown below.



Structural elucidation of F

F was white solid which recrystallized from mixture of chloroform and hexane. It's melting point was $149-150^{\circ}\text{C}$. From UV absorption spectrum(Fig. 46) $\lambda_{\text{max}}=240$ nm exhibited chromophore such as aromatic or $\text{C}=\text{C}$. The IR spectrum was shown in Fig. 47 and absorption bands revealed in **Table 18**.

Table 18 The Infrared absorption band assignments of **F**

Wavenumber (cm ⁻¹)	Tentative assignment
3050, 730	C-H stretching of C-H aromatic
1780, 1740	C=O stretching
1610	C=C stretching
1250, 1220	C-O bending of ester
1130	C-O-C bending

The IR absorption band at 3050, 730 cm⁻¹ revealed CH aromatic, absorption band at 1740 cm⁻¹ revealed C=O(ester), absorption band at 1610 cm⁻¹ revealed C=C aromatic, absorption band at 1250, 1220 and 1130 cm⁻¹ revealed C-O(C-O bond of ester).

From ¹H-NMR spectrum(Fig. 49) displayed the protons of -CH=(aromatic, 5 protons) at 7.40(2 protons), 7.52(1 proton) and 7.95 ppm(2 protons), protons of -CH₃(6 protons) at 1.93(3 protons) and 2.05 ppm(3 protons), protons of -CH₂-O(2 protons) at 4.25 and 4.50 ppm and protons of CH-O (5 protons) at 5.73, 5.01, 3.67, 3.45 and 3.09 ppm

From ¹³C-NMR(Fig. 50), ¹³C-NMR DEPT-135(Fig. 51) and ¹³C-NMR DEPT-90(Fig. 52) were displayed the carbon of -CH=(6 carbons) at 116.5, 124.0, 124.0, 127.5 and 143.0 ppm, carbon of -C=(aromatic, 1 carbon) at 118.5 ppm, carbon of -CO=(aromatic, 1 carbon) at 154.0 ppm and carbon of C=O(ester) at 160.0 ppm. ¹H and ¹³C-NMR spectrum of **F** were similar to the NMR spectrum of crotepoxide²³ all signals assigned as **Table 19** and **Table 20**.

Table 19 Comparison of chemical shifts and coupling constants of $^1\text{H-NMR}$ spectrum of **F** with those of crotepoxide

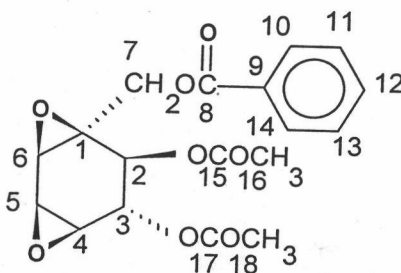
Position of proton	Peak type, Number of proton	Tentative assignment	Crotepoxide $\delta(\text{J})$	F $\delta(\text{J})$
2	d, 1	CH-O aliphatic	5.73(9.0)	5.65(9.0)
3	dd, 1	CH-O aliphatic	5.01(9.0, 1.5)	4.90(9.0, 1.4)
4	dd, 1	CH-O aliphatic	3.09(3.9, 1.5)	3.09(3.7, 1.4)
5	dd, 1	CH-O aliphatic	3.45(3.9, 2.7)	3.45(3.7, 2.5)
6	d, 1	CH-O aliphatic	3.67(2.7)	3.67(2.5)
7	Ab _q , 2	CH ₂ -O	4.23 and 4.58(12.0)	4.25 and 4.50(12.1)
10, 14	dd, 2	-CH aromatic	8.04(7.0, 1.5)	7.49(7.7, 1.4)
11, 13	ddd, 2	-CH aromatic	7.46(7.3, 7.0, 1.5)	7.40(7.3, 7.7, 1.4)
12	tt, 1	-CH aromatic	7.61(7.3, 1.5)	7.52(7.3, 1.4)
16 or 18	s, 3	-CH ₃	1.97	1.93
16 or 18	s, 3	-CH ₃	2.09	2.05

Table 20 Comparison of chemical shifts of ^{13}C -NMR spectrum of **F** with those of crotepoixide

Position of carbon	crotepoixide δ	F δ
1	59.2	59.3
2	69.4	69.4
3	70.1	70.2
4	52.3	52.5
5	47.7	48.0
6	53.3	53.5
7	62.0	62.0
8	168.2	165.0
9	128.9	129.0
10	128.1	128.2
11	129.3	129.8
12	133.0	133.0
13	129.3	129.8
14	128.1	128.2
15	169.3	169.3
16	20.1	20.0
17	169.5	169.6
18	20.2	20.0

From ^1H - ^1H COSY(Fig. 53) and ^1H - ^1H NOESY(Fig. 54), the signal of proton at 5.01 ppm(H-3) coupled with signal of protons at 5.72(H-2) and 3.09 ppm(H-4), signal of proton at 3.45 ppm(H-5) coupled with signal of protons at 3.09(H-4) and 3.67 ppm(H-6)

From spectroscopic data and comparison of **F** with crotepoxide, it can be concluded that **F** was crotepoxide ($\text{C}_{18}\text{H}_{18}\text{O}_8$). The structure is shown below.



Structural elucidation of G

G was white needle which recrystallized from mixture chloroform and hexane. It's melting point was 116-117°C. From UV absorption spectrum(Fig. 55) $\lambda_{\text{max}}=260$ nm exhibited chromophore such as aromatic or C=C. The IR spectrum was shown in Fig. 56 and absorption bands revealed in **Table 21**.

Table 21 The Infrared absorption band assignments of G

Wavenumber (cm^{-1})	Tentative assignment
3320, 1555	N-H stretching of R_2NH
3050, 2000-1700	C-H stretching of -CH aromatic
2940, 2880	C-H stretching of $-\text{CH}_2$, $-\text{CH}_3$
1670	C=O stretching
1640	C=C stretching of $\text{C}=\text{CH}-\text{CO}-\text{R}$
1620	C=C stretching of $\text{C}=\text{C}-\text{Ph}$
1445	C-H stretching of $-\text{CH}_2-\text{C}=\text{C}$
1385-1350, 1170	C-H stretching of $-\text{CH}(\text{CH}_3)_2$
1270, 1050	C-O bending of $\text{O}-\text{CH}_2-\text{O}$
1320, 1260	C-N stretching of $\text{C}-\text{NHR}$

From IR spectrum, absorption peak at 3320, 1555 cm^{-1} corresponding to NH(amine or amide), absorption peak at 3050 cm^{-1} corresponding to CH aromatic, peak at 2940, 2880 cm^{-1} corresponding to CH aliphatic, peak at 1670 cm^{-1} corresponding to C=O(amide), peak at 1640, 1620 cm^{-1} corresponding to C=C(alkene), peak at 1320, 1260 cm^{-1} corresponding to C-N and absorption peak at 1270, 1050 cm^{-1} corresponding to O-CH₂-O.

From ¹H-NMR spectrum(Fig. 58) displayed the proton of -CH₃(6 protons) at 0.95 ppm, proton of -CH₂ at 1.35 ppm(8 proton), 2.10 ppm(4 protons)and 3.10 ppm (2 protons), proton of CH(CH₃)₂ at 1.75 ppm, proton of OCH₂O at 5.85 ppm, proton of CH=(alkene, 6 protons) at 5.30-6.30 ppm and 7.15 ppm, proton of CH=(aromatic, 3protons) at 6.70 ppm(2 protons) and 6.85 ppm(1 proton) and proton of NH at 5.20 ppm

From ^{13}C -NMR spectrum(Fig. 59), ^{13}C -NMR DEPT-135(Fig. 60) and ^{13}C -NMR DEPT-90(Fig. 61) were displayed the carbon of $-\text{CH}_3$ at 20.5, 20.5 ppm, carbon of $-\text{CH}_2-$ at 29.5(4 carbons), 32.8(2 carbons) and 46.8 ppm, carbon of $-\text{CH}=\text{alkene}$ at 122.0 ppm, 128.5 ppm, 141.0 ppm, 142.5 ppm and 129.5 ppm(2 carbons), carbon of $-\text{CH}=\text{aromatic}$ at 105.5 ppm, 108.2 ppm and 120.1 ppm, carbon of $\text{O}-\text{CH}_2-\text{O}$ at 100.8 ppm, carbon of 132.5 ppm, carbon of $-\text{CH}$ aliphatic at 28.5 ppm, carbon of $-\text{CO}=\text{aromatic}$ at 146.8 ppm and 147.8 ppm. ^1H and ^{13}C -NMR spectrum of **G** were similar to the NMR spectrum of N-isobutyl-13-(3,4-methylenedioxy phenyl) trideca-2,4,12-trienamide¹⁰ all signals assigned as **Table 22** and **Table 23**.

Table 22 Comparison of chemical shifts and coupling constants of ^1H -NMR spectrum of **G** with those of N-isobutyl-13-(3,4-methylenedioxyphenyl) trideca-2,4,12-trienamide

Position of proton	Peak type, Number of proton	Tentative assignment	N-isobutyl-13-(3,4-methylene dioxypheyl)trideca-2,4,12-trie namide $\delta(\text{J})$	G $\delta(\text{J})$
23 and 24	d, 6	$-\text{CH}(\text{CH}_3)_2$	0.92(6.0)	0.95(6.3)
20	s, 2	$\text{O}-\text{CH}_2-\text{O}$	5.92	5.85
7-10	m, 8	$-\text{CH}_2-$	1.40	1.35
6, 11	m, 4	$-\text{CH}_2-$	2.17	2.10
21	t, 2	$-\text{CH}_2-$	3.16(6.0)	3.10(6.3)
2	d, 1	$-\text{CH}=\text{alkene}$	5.78	5.30-6.30
4, 5, 12	m, 3	$-\text{CH}=\text{alkene}$	6.06-6.16	5.30-6.30
13	d, 1	$-\text{CH}=\text{alkene}$	6.28	5.30-6.30

Table 22 continue

3	dd, 1	-CH= alkene	7.20	7.15
-NH	t, 1	-NH	5.65	5.30-6.30
22	m, 1	-CH(CH ₃) ₂	1.80	1.75
18, 19	m, 2	CH=aromatic	6.75(8.1)	6.70(8.0)
15	s, 1	CH=aromatic	6.90	6.85

Table 23 Comparison of chemical shifts of ¹³C-NMR spectrum of **G** with those of N-isobutyl-13-(3,4-methylenedioxyphenyl)trideca-2,4,12-trienamide

Position of carbon	N-isobutyl-13-(3,4-methylene dioxypheyl)trideca-2,4,12-trienamide δ	G δ
1	166.42	166.5
2	121.84	122.0
3	143.07	142.5
4	141.29	141.0
5	128.29	128.5
6	32.88	32.8
7	29.36	29.5
8	29.36	29.5
9	29.36	29.5
10	29.36	29.5
11	32.88	32.8
12	129.37	129.5

Table 23 continue

13	129.37	129.5
14	132.51	132.5
15	105.20	105.5
16	147.95	147.8
17	146.57	146.8
18	108.24	108.2
19	120.21	120.1
20	100.93	100.8
21	46.93	46.8
22	28.96	28.5
23	20.15	20.5
24	20.15	20.5

The mass spectrum(Fig. 57) displayed the molecular ion peak at m/e 383. When the mass spectrum of **G** was compared with N-isobutyl-13-(3,4-methylenedioxyphenyl) trideca-2,4,12-trienamide, they have similar fragmentation.(see **Scheme 6**, the fragmentation of **G**)

From ^1H - ^1H COSY(Fig. 62) and ^1H - ^1H NOESY(FIG. 63), the signal of proton at 1.75 ppm(H-22) coupled with the signal of protons at 0.95 ppm(H-23 and H-24) and 3.10 ppm(H-21) and the signal of proton at 3.10 ppm(H-21) coupled with the signal of proton at 5.20 ppm(N-H)

From spectroscopic data and comparison of **G** with N-isobutyl-13-(3,4-methylenedioxyphenyl) trideca-2,4,12-trienamide, it can be concluded that **G** was N-isobutyl-13-(3,4-methylenedioxyphenyl)trideca-2,4,12-trienamide.The structure is shown below.

