CHAPTER III
RESULTS AND DISCUSSION

Structural elucidation of the eluted compounds from crude extract of hexane, chloroform and n-BuOH of the bark of *Bombax malabaricum* are described in the following sections.

3.1 Structural elucidation of Mixture I

Mixture I was obtained as a white solid (0.042) and has a melting point of 57-58°C. This compound was separated by column chromatography of the hexane crude extract (0.06% wt. by wt. of hexane crude extract). It was soluble in hexane, dichloromethane and chloroform but insoluble in methanol and acetone. Its IR spectrum was shown in Fig. 4 and the absorption band assignments were presented in Table 3.1

Table 3.1 The IR Absorption Band Assignments of Mixture I

Wavenumber (cm^{-1})	Band type	Assignments
2925, 2860	strong, strong	C-H stretching vibration of $-CH_2-$,$-CH_3$
1470	medium	C-H bending vibration of vibration of $-CH_3$
1380	weak	C-H symmetric bending vibration of $-CH_3$
805	weak	C-H rocking vibration
725	weak	C-C bending vibration of $-(CH_2)_n-$; $n \geq 4$

IR spectrum indicated C-H stretching vibration peaks of aliphatic C-H of alkanes at 2925 and 2860 cm^{-1}. The absorption peaks at 1470 and 1380 cm^{-1} correspounded to C-H bending vibration mode of $-CH_2-$ and $-CH_3$ group, absorption peak at 805 cm^{-1} corresponded to C-H rocking vibration and absorption peak at 725 cm^{-1} indicated one or more saturated long chain of $-(CH_2)_n-$; $n \geq 4$

By comparison to IR spectrum of standard long chain aliphatic hydrocarbon, both spectra show close similarity. Mixture I was further analysed by GC technique. The chromatogram appeared as seven peaks at different retention times as shown in Table 3.2 and Fig. 5

Comparison to gas chromatogram of standard long chin aliphatic hydrocarbons (C_nH_{2n+2} ; n = 27-33) (Fig. 5) using calibration curve of log retention time with number of carbon (Fig. 6) revealed that Compound I was a mixture of seven long chain aliphatic hydrocarbons (C_nH_{2n+2}; n = 27-33)

Table 3.2 The Retention Times of Mixture I

Retention time (min)	log retention time	Number of carbon
6.26	0.80	27
8.01	0.90	28
10.22	1.01	29
13.30	1.12	30
17.12	1.23	31
22.19	1.35	32
28.59	1.46	33

Table 3.3 Name of long Chain Aliphatic Hydrocarbons in Mixture I

Molecular formula	Molecular weight	Name of substance	% Composition
$C_{27}H_{56}$	380	heptacosane	0.49
$C_{28}H_{58}$	394	octacosane	1.27
$C_{29}H_{60}$	408	nonacosane	39.07
$C_{30}H_{62}$	422	triacontane	4.55
$C_{31}H_{64}$	436	hentriacontane	37.97
$C_{32}H_{66}$	450	dotriacontane	4.81
$C_{33}H_{68}$	464	tritriacontane	11.84

Then, Mixture I was a mixture of heptacosane ($C_{27}H_{56}$), octacosane ($C_{28}H_{58}$), nonacosane ($C_{29}H_{60}$), tricontane ($C_{30}H_{62}$), hentriacontane ($C_{31}H_{64}$), dotriacontane ($C_{32}H_{66}$) and tritriacontane ($C_{33}H_{68}$). Nonacosane and hentriacontane were the major components, 39.07% and 37.97% , respectively while heptacosane was the trace component, 0.49 %. The structure of Mixture I was shown below :

$$CH_3 - (CH_2)_n - CH_3 ; n = 27 - 33$$

3.2 Structural elucidation of Compound II

Compound II appeared as white needle-like crystals from column chromatography of hexane crude extract and chloroform crude extract and melting point was 137-138 ^{o}C. The color test of this compound suggested the presence of a steroidal skeleton gave a green color (positive test) with Liebermann-Burchard's reagent.

The IR spectrum of Compound II (Fig. 10) indicated important absorption bands and assigned as shown in Table 3.4 . The IR revealed the presence of hydroxyl group at 3590 - 3066 cm, unsaturated C = C at 1690 cm^{-1}, absorption band at 1059 cm^{-1} indicated the C - O stretching vibration and absorption band of unsaturated system at 800 cm^{-1}.

Table 3.4 The IR Absorption band assignments of Compound II

Wavenumber (Cm^{-1})	Band type	Assignments
3590 – 3066	strong, broad	O-H stretching vibration of R-OH
2935 , 2869	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1690	medium	C=C stretching vibration
1460	weak	C-H bending vibration of $-CH_2-$,$-CH_3$
1059	weak	C-O stretching vibration
800	weak	C-H out of plane bending vibration of trisubstituted vinyl (R_1 R_2 C=CHR_3)

The ^{1}H - NMR spectrum (Fig. 11) exhibited the signals of protons at 0.66 - 2.30 ppm corresponding to the signals of methyl (-C$\underline{H}_3$) , methylene (-C$\underline{H}_2$-) and methine (-C$\underline{H}$-) protons. The chemical shift at 3.52 ppm indicated the proton attached to a carbon bearing a hydroxy group (C$\underline{H}$-OH) and the signal at 5.34 - 5.36 ppm indicated the presence of olefinic proton (-C$\underline{H}$=C-) .

^{13}C-NMR spectrum (Fig. 12) showed 26 signals between 11.85 - 56.79 ppm which indicated sp^3 carbon in -C$\underline{H}_3$, -C$\underline{H}_2$ - and -C$\underline{H}$- group. Other signals were detected at 71.82 ppm which was the signal of a -$\underline{C}$H—OH group at 121.70 and 140.78 ppm which were the signals of sp^2 carbons of $\underline{C}$H=Cand CH=$\underline{C}$.

The information obtained from the color test , IR spectrum, H^1- NMR spectrum and ^{13}C-NMR spectrum suggested this compound should be a steroidal compound having a hydroxy group.

Compound II was analyzed by GC technique. Chromatogram was shown in Fig. 13 and was used to identify compound II by comparison the chromatogram of compound II with that of the standard mixture of steroids including campesterol , stigmasterol and β-sitosterol. The retention times of standard steroids were 17.58, 18.32 and 20.73 min respectively. The retention time of Compound II was 21.60 min which revealed that Compound II might be β-sitosterol. Table 3.5 shown the retention time of Compound II and standard steroids.

Table 3.5 The retention time of Compound II and Standard steroids

Compound	Retention time (min)
campesterol	17.58
stigmasterol	18.32
β-sitosterol	20.73
Compound II	21.60

EI mass spectra (Fig. 11) revealed a molecularion peak at M^+ = 414 which also corresponded to the molecular formular $C_{29}H_{26}O$ of β-sitosterol.

Comparison of the ^{13}C-NMR spectrum of Compound II and β-sitosterol (16) was also carried out to confirm the structure as presented in Table 3.6

Table 3.6 ^{13}C-NMR spectrum of Compound II compared with β-sitosterol

Possition	Chemical shift (ppm)	
	β-sitosterol	Compound II
1	37.4	37.3
2	31.8	31.7
3	71.9	71.8
4	42.4	42.3
5	104.9	104.8
6	121.8	121.7
7	32.0	33.9
8	32.0	31.9
9	50.3	50.2
10	36.6	36.5
11	21.1	21.1
12	39.9	39.8
13	42.4	42.3
14	56.8	56.8
15	24.3	24.3
16	28.2	28.2
17	56.2	56.1
18	11.9	11.9
19	19.4	19.4
20	36.2	36.1
21	19.1	19.1
22	34.0	34.0

Table 3.6 (continued)

Possition	Chemical shift (ppm)	
	β-sitosterol	Compound II
23	29.3	29.2
24	50.3	50.1
25	26.2	26.2
26	18.8	18.8
27	19.8	19.8
28	23.1	23.1
29	11.9	11.8

These results confirmed that Compound II was β-sitosterol.

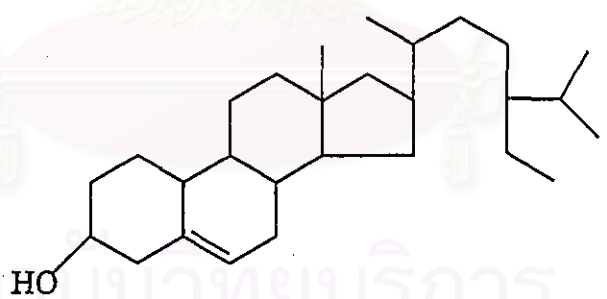


Figure 3 β-sitosterol

3.3 Structural elucidation of Compound III

Compound III was a white amorphous solid, m.p 256-258°C (dec.) having an R_f value 0.44 (silica gel, 10% methanol in chloroform). The color test of Compound III with Liebermann - Burchard's reagent gave a green color (positive test) which suggested the presence of a steroidal skelaton.

The IR spectrum of this compound was presented in Fig. 15 and the absorption was presented in Table 3.7. The absorption band assignments were shown a broad band of a hydroxy group (O-H) at 3400 cm^{-1}, C-O stretching vibration of a glycosidic linkage at 1020-1075 cm^{-1} and an anomeric axial C-H deformation of β-sugar was observed at 804 cm^{-1}.

Table 3.7 The IR absorption band assignments of Compound III

Wavenumber (cm^{-1})	Band type	Assignments
3400	broad , strong	O-H stretching vibration of R-OH
2945, 2870	strong	C-H stretching vibration of $-CH_2-$,$-CH_3$
1650	weak	C=C stretching vibration
1460 , 1380	medium	C-H bending of $-CH_2-$,$-CH_3$
1050	broad , strong	C-O stretching vibration
800	weak	C-H out of plane bending vibration of $R_1R_2 = CHR_3$

The ^{1}H-NMR spectrum (Fig. 16) showed signals of proton at 0.66-2.50 ppm which exhibited proton of methyl, methylene and methine protons of the steroid. The chemical shift at 5.32 ppm indicated olefinic proton (C<u>H</u>=C) and the signals at 2.95-3.70 were assigned to the proton of the sugar, a doublet at 4.25 ppm assigned to the proton of β-D-glucose.

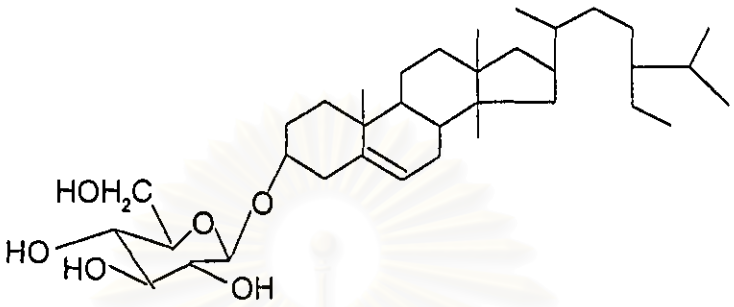
The ^{13}C-NMR spectrum (Fig. 14) showed two signals of olefinic carbons at 140.6 (CH=<u>C</u>) and 121.1 ppm (<u>C</u>H=C). The signals at 11.65-56.73 ppm which were similar to the carbon signals of β-sitosterol. Furthermore, the signals at 100.9 , 76.9 , 76.7, 73.6 , 70.4 and 61.40 ppm. Corresponding to the signals of β-D-glucose were also observed (17). The ^{13}C-NMR spectrum of Compound III was compared with that of β-sitosteryl-3-O-β-D-glucopyranoside and they were closely resembled (Table 3.8).

Table 3.8 ^{13}C-NMR spectrum of Compound III Compared with β-sitosteryl-3-O-β-D- glucopyranoside (only the sugar part shown)

position	chemical shift (ppm)	
	β-sitosteryl-3-O-β-D-glucopyranoside (sugar part)	Compound III
G1	100.7	100.9
G2	73.4	73.6
G3	76.9	76.9
G4	70.1	70.4
G5	76.7	76.7
G6	61.1	61.4

The EI mass spectrum (Fig. 15) did not show molecular ion (M^+) peaks. The dominant fragmentation were found at *m/z* 414 , 396 , 381 , 329 , 303 , 273 , 255 , 231 , 145 , 107 , 81 and 55. This fragmentation pattern was similar to the fragmentation of β-sitosterol.

All these results confiremed that Compound III was β-sitosteryl -3-O-β-D-glucopyranoside.



β-sitosteryl-3-O-β-D-glucopyranoside

3.4 Structural elucidation of Compound IV

Compound IV was pale purple amorphous solid, m.p. 215- 216° C. This compound was tesed with Liebermann-Burchard's reagent and showed a green color, which is characteristic of the presence of a steroidal skeleton.

The IR spectrum of Compound IV (Fig. 19) indicated an important absorption band of a hydroxyl group at 3336-3060 cm^{-1} and a absorption band of unsaturated C=C at 1641 cm^{-1}. The principle IR absorption bands can be assigned as shown in Table 3.9.

Table 3.9 The IR absorption band assignments of Compound IV

Wavenumber (cm^{-1})	Band type	Assignments
3336-3060	strong, broad	O-H stretching vibration of R-OH
2953,2871	strong	C-H stretching vibration of $-CH_3-,-CH_2$
1641	weak	C=C stretching vibration
1455,1382	strong	C-H bending of $-CH_3-,-CH_2$
1070	weal	C-O stretching vibration
826	weak	C-H out of plane bending vibration of $R_1R_2C = CH_2$

The H^1-NMR spectrum (Fig. 20-22) showed the signals of protons at 0.6-2.4 ppm corresponding to the signals of methyl ($C\underline{H}_3$) , methylene ($-C\underline{H}_2-$) and methine ($-C\underline{H}-$) protons. The chemical shift at 3.2 ppm (1H, dd, J = 11.3, 4.9 H_2) indicated the carbinol proton ($C\underline{H}$-OH) and the signal at 7.68 and 4.78 (1H each, d, 2.4 H_2) indicated the 2 olefinic protons ($-C\underline{H}_2=$)

^{13}C-NMR spectrum (Fig. 23) and DEPT pulse sequence (at 90° and 135°) indicated that this compound consisted of 30 carbons containing 7 methyl carbons (CH_3), 11 methylene carbons (-C<u>H</u>$_2$-) , 6 methine carbons (-C(-H)-) and 6 quarternary carbons (-C(-)-).

The mass spectrum (EI) (Fig. 27) showed molecular ion peak (M^+) at m/z = 426.

From the results of color test , IR, ^{1}H-NMR , ^{13}C-NMR and MS suggested that compound IV was a pentacyclic triterpenoid and might be one of structures indicated in Fig. 4 and the melting point of these compounds were shown in table 3.12.

From the Library search of Data band of Mass spectrum (Fig. 28) showed that the fragmentation pattern was similar to that of Lupeol.

The comparision of ^{1}H and ^{13}C-NMR of this compund and Lupeol (table 3.10 and 3.11) also showed that the signals were similar to that of Lupeol (36).

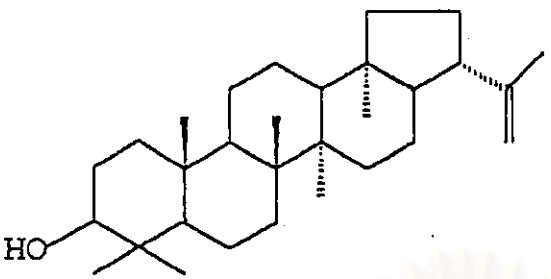
From the results described above and the fragmentation pattern of mass spectrum (Fig. 5) compound IV was Lupeol as shown below.

Table 3.10 The ^{1}H-NMR chemical shifts (δ, ppm) of Compound IV compared with Lupeol.

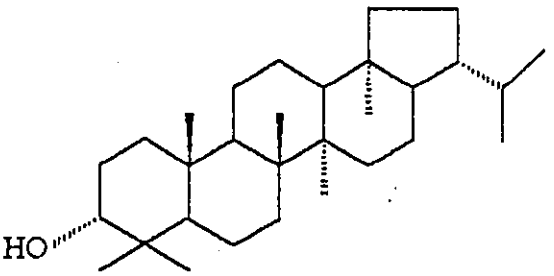
Compound	Chemical shift (δ, ppm)			
	Olefinic proton	Carbinol proton	Vinylic methyl proton	Methyl proton
Compound IV	4.54,4.67 (2H)	3.16(1H)	1.66(3H)	0.74,0.77,0.81,0.93 ,0.95,1.01 (3H each)
Lupeol (32)	4.68,4.78 (2H)	3.13(1H)	1.70(3H)	0.78(3H),0.80 (3H),0.84(3H),0.98 (6H) , 1.05 (3H)

Table 3.11 The ^{13}C-NMR chemical shifts (δ, ppm) of Compound IV compared with Lupeol

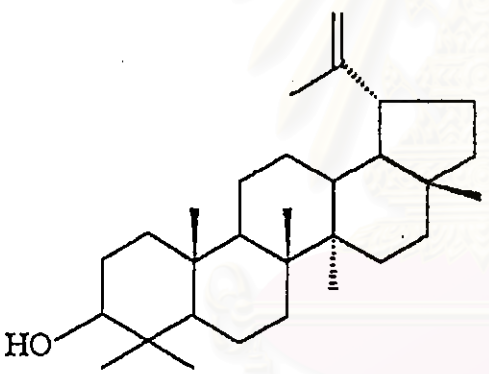
^{13}C-NMR	Chemical shift (δ, ppm)	
	Compound IV	Lupeol (36)
Olefinic carbon	109.31,150.90	109.2,150.6
Carbinol carbon	78.99	78.8
methyl, methylene		14.5,15.4., 15.9, 16.1, 18.0, 18.3, 19.3, 20.9, 25.1, 27.4, 27.4, 28.0, 29.8, 34.2, 35.5, 37.1, 38.0, 38.7, 38.8, 39.9, 40.8, 42.8, 42.9, 47.9, 48.2, 50.4,55.2



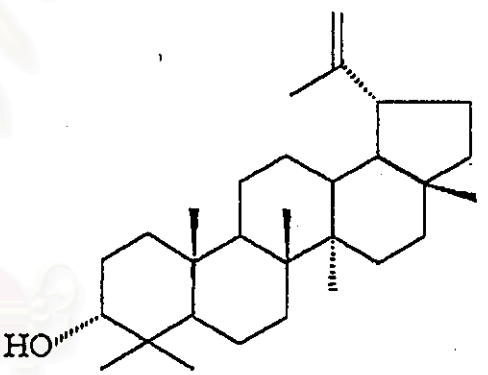
[1] Moretenol



[2] 3-Epimoretenol



[3] Lupeol



[4] 3-Epilupeol

Figure 4 The structures of Moretenol, 3-Epimoretenol, Lupeol and 3-Epilupeol.

Table 3.12 The melting point of Compound IV compared those of with Moretenol, 3-Epimoretenol, Lupeol and 3-Eqilupeol

Compound	Melting point (^{o}C)	Reference
(1) Moretenol	236	33,34
(2) 3-Epimoretenol	223	34
(3) Lupeol	215-216	33
(4) 3-Epilupeol	202	35
Compound IV	215-216	-

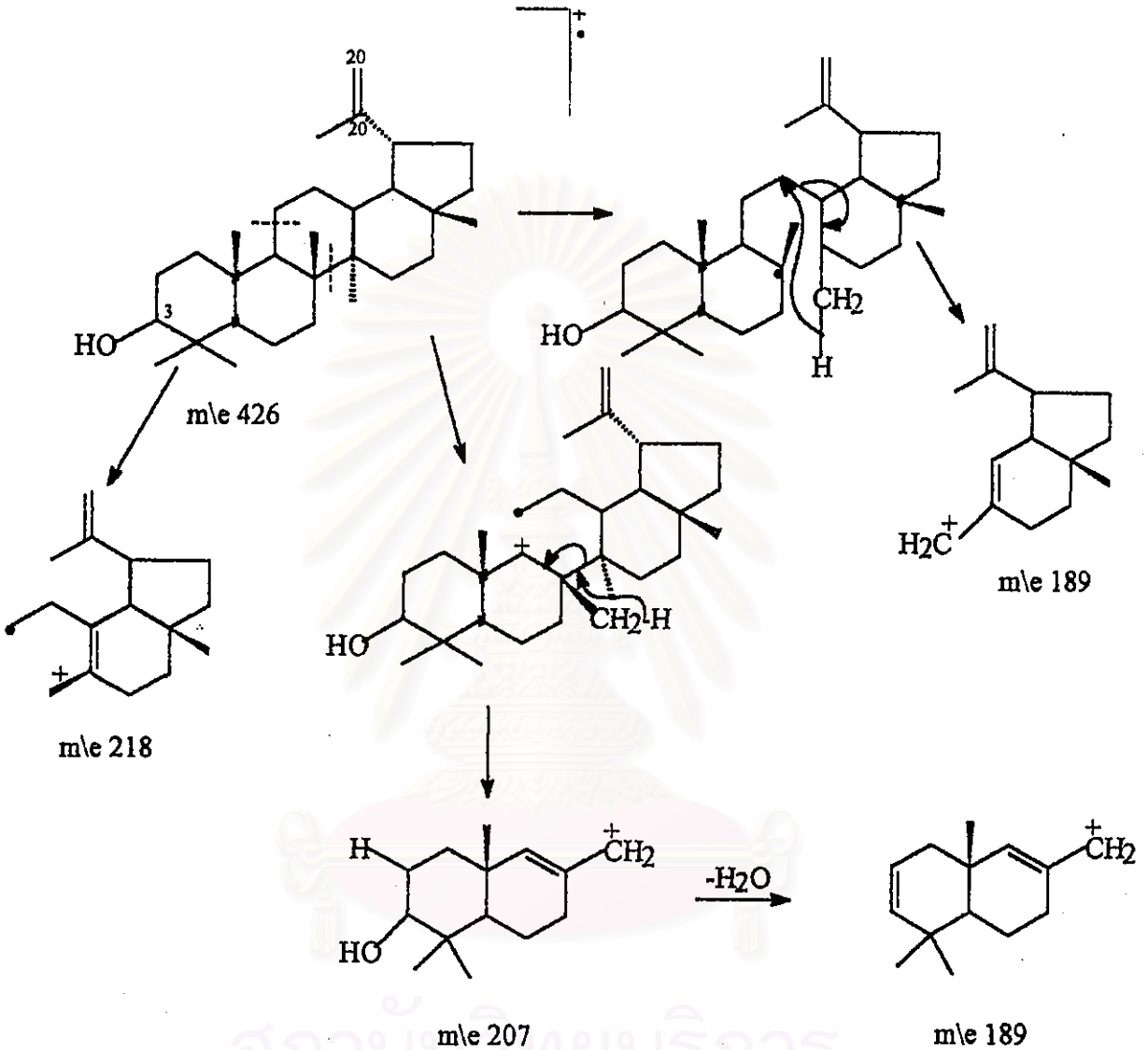


Figure 5 The fragmentation pattern of Compound IV.

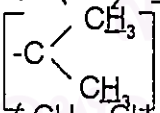
3.5 Structural elucidation of Compound V

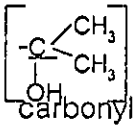
Compound V was a yellow oil, b.p 208-210°C and its R_f value was 0.52 (silica gel : 5% methanol in chloroform).

The IR spectrum (Fig. 21) showed a broad absorption band of a hydroxy group (O-H) at 3445 cm^{-1}, C-O stretching vibration of an ester group at 1050-1090 cm^{-1}

Table 3.13 The IR absorption band assignments of Compound V

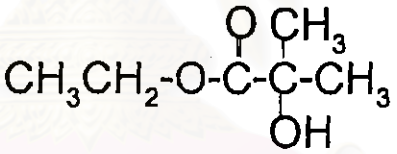
Wavenumber (cm^{-1})	Band type	Assignments
3445	Strong	O-H stretching vibration of R-OH
2950,2850	strong,medium	C-H stretching vibration of $-CH_2-$,$-CH_3$
1485	weak	C-H bending of $-CH_2-$,$-CH_3$
1775	strong	C=O stretching vibration of -C(=O)-O-
1090	strong	C-O stretching vibration

From ^{1}H-NMR spectrum (Fig. 22) the signal of methyl protons of (CH_2-CH_3) appeared as a triplet at 1.25 ppm and methyl protons of  appeared as a singlet at 2.04 ppm. The signal of methylene protons ($-CH_2-$ CH_3) appeared as a quartet at 4.12 ppm. The signal of the hydroxyl proton did not reveal due to, it was under base line.

The ^{13}C-NMR spectrum (Fig. 23) and DEPT 135 spectrum (Fig. 24) showed signals of methyl group ($-CH_3$) at 14.06 ppm and 10.88 ppm ,the methylene group ($-CH_2-$) at 60.28ppm. The signal of quarternary carbon atom  at 78 ppm (overlapped with the solvent peak) and the signal of a carbonyl group (-C(=O)-O-) at 171.07 ppm.

The EI mass spectrum (Fig. 25) revealed the molecular ion (M^++1), 113. The spectrum exhibited the dominant fragmentation ion peaks at *m/z* 132 and others minor fragments at 57 and 73. The fragmentation pattern was as shown in Figure 6.

The spectroscopic data, confirmed that this compound was 2-hydroxy-2-methyl propanoic acid, ethyl ester, and its structure was represented by :



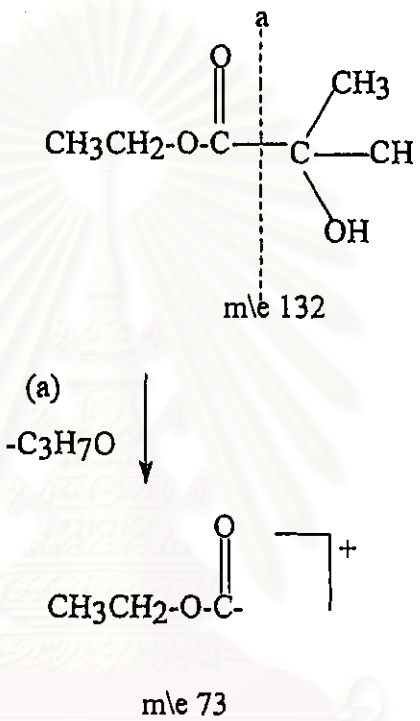


Figure 6 The fragmentation pattern of Compound V.