CHAPTER III

RESULTS AND DISCUSSION

3.1 Extraction

3.1.1 The Extraction and Partial Purification

The crude benzene extract from sugar cane rind by this method (scheme 2.1) gave the average 10.1% wt/wt of raw material. The precipitate I and II were 51.3 and 4.0 % weight by weight of the crude extract by average, respectively. Both of them were separated and identified by the GC-MS analysis and could be concluded that their chemical constituents were similar. The results of identification of the GC-MS analysis were discussed in part 3.2.

3.1.2 The Extraction by Soxhlet Extraction Apparatus

3.1.2.1 Extraction from Sugar Cane Rinds

The quantities of crude extracts of each sample were different. Fraction 1A, the crude extracts of n-hexane were 10.9-20.8% wt/wt of raw material while Fraction 2, the chloroform extracts were 0.7-2.5%. Fraction 3 was isolated only from the F147 and the F153 varieties and yielded 0.06-0.13%. The F153 variety gave the highest quantity of crude extract from both hexane (20.8%) and chloroform (2.5%) and the F147 variety gave the highest yield of Fraction 3 (0.13%).

3.1.2.2 Extraction from Filter Cakes

The quantities of crude extract from filter cakes extracted with n-hexane and benzene were 5.3-17.8% and 6.0-11.7%, in the order given. The filter cake from Khumphawapi Factory gave the highest yield(17.8% and 11.7%) while the Mitr Siam Factory gave the lowest yield (5.3% and 6.0%).

The different yield of extraction may occur because of the varieties of sugar cane and the process of production.

3.2 Separation and Identification of Chemical Constituents

3.2.1 The Chemical Constituents of Precipitate I and II

Both Precipitate I and II were separated and identified by the GC-MS analysis. The results showed that the chemical constituents in both of them were similar. They consisted of 10 compounds which showed in gas chromatogram in Fig. 2.2 and 2.3. Each peak in chromatograms was identified by mass spectroscopy.

3.2.1.1 Structural Elucidation of Peak 1

Peak 1 appeared in gas chromatograms at retention time (t_R) 6.5 min. It was trace component in Precipitate I and II. The mass spectrum of peak 1 (Fig.3) gave the molecular ion, M^+, at m/e 354 (calcd. for $C_{24}H_{50}O$). The other peaks appeared at m/e 43, 57, 71, and 85 which were the pattern of straight chain hydrocarbons (33). Thus, the peak 1 should be saturated long chain aliphatic alcohols, $C_{24}H_{49}OH$.

3.2.1.2 Structural Elucidation of Peak 2

Peak 2 showed in gas chromatograms at t_R 7.0 min. It was a small peak in both chromatograms. The mass spectrum of peak 2 (Fig.4) showed the ion peak at m/e 362 and the pattern of saturated long chain aliphatic hydrocarbons at m/e 43, 57, 71, and 85. The ion peak at m/e 362 was expected to be M-H_2O of saturated long chain aliphatic aldehydes (34). From the results, the molecular formula was $C_{26}H_{52}O$ (MW 380). Thus, peak 2 should belong to saturated long chain aliphatic aldehyde, $C_{25}H_{51}CHO$.

3.2.1.3 Structural Elucidation of Peak 3

Peak 3 was a trace composition in gas chromatograms, t_R 8.7 min. The mass spectrum of this peak (Fig.5) showed the M^+ at m/e 382 (calcd. for $C_{26}H_{54}O$). The other ions were the pattern of saturated long chain hydrocarbons. From the results, this compound was identified as saturated long chain aliphatic alcohol, $C_{26}H_{53}OH$.

3.2.1.4 Structural Elucidation of Peak 4

Peak 4, the second major peak, occured in gas chromatograms at t_R 10.5 min. The mass spectrum of this peak (Fig.6) showed M^+-H_2O ion peak at m/e 390 and other peaks were the pattern of saturated long chain hydrocarbons. It could be concluded that the molecular formula should be $C_{28}H_{56}O$ (MW 408) and this peak should belong to saturated long chain aliphatic aldehyde, $C_{27}H_{55}CHO$.

3.2.1.5 Structural Elucidation of Peak 5

Peak 5 was the first major peak in gas chromatograms, t_R 12.0 min. The mass spectrum of peak 5 (Fig.7) only showed the pattern of saturated long chain hydrocarbons. From the correlation standard curves (Fig. 49 and 50) of alcohols and aldehydes which constructed by the 4 compounds elucidated before. This compound should be a saturated long chain alcohol, molecular formula $C_{28}H_{58}O$ (MW 410). From these results, this peak should belong to saturated long chain aliphatic alcohol, $C_{28}H_{57}OH$.

3.2.1.6 Structural Elucidation of Peak 6

The peak 6, the third major peak in gas chromatograms had t_R 14.8 min. and the mass spectrum (Fig.8) showed the pattern of saturated long chain hydrocarbons and ion peak at m/e 418 which expected to be M^+-H_2O of long chain aliphatic aldehydes. These results and the correlation standard curve (Fig.49) indicated that this peak should belong to saturated long chain aliphatic aldehyde, $C_{29}H_{59}CHO$ (MW 436).

3.2.1.7 Structural Elucidation of Peak 7

Peak 7 appeared in gas chromatograms at t_R 16.5 min. The mass spectrum of peak 7 (Fig.9) only indicated the pattern of saturated long chain hydrocarbons. From the correlation standard curve (Fig. 50), this peak should belong to saturated long chain aliphatic alcohol, $C_{30}H_{61}OH$ (MW 438).

3.2.1.8 Structural Elucidation of Peak 8

The peak 8 was shown in gas chromatograms at t_R 21.0 min. The mass spectrum (Fig.10) showed the pattern of saturated long chain hydrocarbons and ion peak at m/e 446 which expected to be M^+-H_2O of long chain aliphatic aldehydes, MW 464. From these results, this peak should belong to $C_{31}H_{63}CHO$ (MW 464) which was comfirmed by the correlation standard curve (Fig.49).

3.2.1.9 Structural Elucidation of Peak 9

Peak 9 in both precipitations of gas chromatograms, t_R 24.0 min., showed the mass spectrum (Fig.11) of the saturated long chain hydrocarbons pattern. From correlation standard curve of saturated long chain alcohols (Fig.50), this compound corresponded to $C_{32}H_{65}OH$ (MW 466).

3.2.1.10 Structural Elucidation of Peak 10

Peak 10 appeared in gas chromatogram at t_R 30.5 min. The mass spectrum of this peak (Fig.12) indicated the pattern of saturated long chain hydrocarbons. However, another ion peak at m/e 474 which was expected to be M^+-H_2O of long chain aldehyde, MW 492. Thus, this peak should belong to $C_{33}H_{67}CHO$ which confirmed by the correlation standard curve of aldehydes (Fig.49).

The compositions of each compound in Precipitation I and II are shown in Table 3.1.

From the results, it was indicated that the extraction and partial purification could not separated the mixture of saturated long chain aliphatic aldehydes from the mixture of saturated long chain aliphatic alcohols. So, the chromatography technique was introduced.

Table 3.1 % Compositions in Precipitation I and II

Compound	% in Precipitation I	% in Precipitation II
$C_{24}H_{49}OH$	0.4	1.0
$C_{25}H_{51}CHO$	0.4	1.0
$C_{26}H_{53}OH$	2.9	4.7
$C_{27}H_{55}CHO$	17.5	18.4
$C_{28}H_{57}OH$	36.5	29.4
$C_{29}H_{59}CHO$	16.0	16.4
$C_{30}H_{61}OH$	8.2	7.3
$C_{31}H_{63}CHO$	6.9	10.7
$C_{32}H_{65}OH$	1.5	2.8
$C_{33}H_{67}CHO$	9.7	8.4

3.2.2 The Chemical Constituents of Fraction 1A and 1B

Fraction 1A and 1B were extracted with n-hexane from sugar cane rinds and filter cakes, in the order given. Separated by quick column and purification, it was found that both fractions consisted of the same 5 substances as follows.

3.2.2.1 Structural Elucidation of Substance 1

Substance 1 was isolated from crude Fraction 1A and 1B by silica gel quick column and recrystallized from ethylacetate or acetone to form bright white plate. The melting points and quantities were different in each material as showed in Table 2.14. The highest percentage yield of substance 1 was obtained from filter cake of the United Farmer & Industry Factory (1.1%).

This substance showed a spot at Rf 0.82 using 50 % $CHCl_3$-hexane as a developing solvent. It gave negative results to Liebermann-Burchard's, $FeCl_3$, Br_2 in CCl_4, and 2,4-DNP reagents which indicated that it was not composed of steroidal skeleton, phenolic group, unsaturated part and carbonyl as functional groups, respectively.

That the IR spectrum of substance 1 (Fig.13) being similar to paraffin oil indicated that this substance should be a saturated long chain aliphatic hydrocarbon. The IR absorption band assignments of Substance 1 are given in Table 3.2 (35,36).

Table 3.2 The IR absorption band assignments of Substance 1

Frequency (cm^{-1})	Band type	Tentative assignments
2940	s	C-H stretch. of CH_3-
2860	s	C-H stretch. of $-CH_2-$
1470	s	C-H bend. of CH_3-, $-CH_2-$
720	m	C-H rock. mode of $-CH_2-$ for chain > 4 carbons

The GC analysis data (Fig. 14a - 14d and 15a - 15e) showed that Substance 1 was in fact a mixture of 11-13 saturated long chain aliphatic hydrocarbons by construction of correlation standard curve between logarhithm of retention times and numbers of carbon atom* (37). The standard curves were presented in Fig. 51 and 52. The retention time of all peaks shown in Table 2.15 and 2.16 corresponded to number of carbon as 24-35.

Therefore, Substance 1 supported by physical properties and spectroscopic data was a mixture of long chain aliphatic hydrocarbons. The structure of Substance 1 is shown below:

$CH_3\,(CH_2)_n\,CH_3$ n = 22,23,24,25,26,27,
28,29,30,31,32,33

Substance 1

The compositions of saturated long chain aliphatic hydrocarbons contained in Substance 1 of each sample is given in Table 3.3 - 3.5.

From Table 3.3, the major compositions of Substance 1 in sugar cane rinds of the 3 varieties were $C_{27}H_{56}$, $C_{29}H_{60}$, $C_{33}H_{68}$, and $C_{31}H_{64}$, respectively. The table 3.4 indicated that the major compositions of Substance 1 in filter cakes of Mitr Phol and Mitr Siam Factory were $C_{33}H_{68}$, $C_{31}H_{64}$, $C_{29}H_{60}$, and

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* The standard saturated long chain aliphatic hydrocarbons obtained from the mixture of long chain aliphatic hydrocarbons separated from Rhizophora apiculata Bl.

$C_{35}H_{72}$, respectively. In Table 3.4, the major compositions of this substance in filter cakes from Khumphawapi Factory were $C_{33}H_{68}$, $C_{31}H_{64}$, $C_{29}H_{60}$, and $C_{27}H_{56}$, respectively. In the same Table, $C_{27}H_{56}$, $C_{29}H_{60}$, $C_{31}H_{64}$, and $C_{33}H_{68}$ were the major components in filter cake from The United Farmer & Industry Factory.

From these results, it was indicated that the major composition of saturated long chain aliphatic hydrocarbon contained in most of sample was $C_{33}H_{68}$, except from filter cake of The United Farmer&Industry Factory. $C_{27}H_{56}$ was found to be the major composition of The United Farmer&Industry Factory.

Table 3.3 The composition of saturated long chain aliphatic hydrocarbons contain Substance 1 from sugar cane rinds

Name	Formula	% Composition		
		Q83	F147	F153
pentacosane	$CH_3-(CH_2)_{23}-CH_3$	0.7	0.8	0.4
hexacosane	$CH_3-(CH_2)_{24}-CH_3$	2.8	2.1	0.9
heptacosane	$CH_3-(CH_2)_{25}-CH_3$	38.9	31.0	31.9
octacosane	$CH_3-(CH_2)_{26}-CH_3$	1.9	1.5	1.6
nonacosane	$CH_3-(CH_2)_{27}-CH_3$	22.6	18.8	26.8
triacontane	$CH_3-(CH_2)_{28}-CH_3$	1.1	1.0	1.0
hentriacontane	$CH_3-(CH_2)_{29}-CH_3$	13.8	13.2	15.8
dotriacontane	$CH_3-(CH_2)_{30}-CH_3$	1.2	1.4	1.2
tritriacontane	$CH_3-(CH_2)_{31}-CH_3$	15.0	23.5	17.6
tetratriacontane	$CH_3-(CH_2)_{32}-CH_3$	0.3	0.8	0.4
pentatriacontane	$CH_3-(CH_2)_{33}-CH_3$	1.8	5.9	2.4

Table 3.4 The composition of saturated long chain aliphatic hydrocarbons contain in Substance 1 from filter cakes

Name	Formula	% Composition			
		Mitr Phol	Mitr Siam	Khumpha-wapi	United Farmer& Industry
tetracosane	$CH_3-(CH_2)_{22}-CH_3$	-	-	-	0.1
pentacosane	$CH_3-(CH_2)_{23}-CH_3$	0.4	0.1	0.2	0.7
hexacosane	$CH_3-(CH_2)_{24}-CH_3$	0.8	0.2	0.6	1.2
heptacosane	$CH_3-(CH_2)_{25}-CH_3$	7.1	6.2	12.1	28.5
octacosane	$CH_3-(CH_2)_{26}-CH_3$	1.7	1.0	1.7	2.1
nonacosane	$CH_3-(CH_2)_{27}-CH_3$	12.6	13.3	15.6	19.0
triacontane	$CH_3-(CH_2)_{28}-CH_3$	3.0	2.3	2.2	1.6
hentriacontane	$CH_3-(CH_2)_{29}-CH_3$	21.4	21.3	20.0	17.6
dotriacontane	$CH_3-(CH_2)_{30}-CH_3$	4.6	4.1	3.8	1.9
tritriacontane	$CH_3-(CH_2)_{31}-CH_3$	36.1	35.6	32.3	19.5
tetratriacontane	$CH_3-(CH_2)_{32}-CH_3$	1.7	2.6	1.9	1.1
pentatriacontane	$CH_3-(CH_2)_{33}-CH_3$	10.8	13.3	9.6	6.7

3.2.2.2 Structural Elucidation of Substance 2

Substance 2 were collected from crude hexane fraction, Fraction 1A and 1B, by silica gel quick column. It was white amorphous substance after purified by recrystallization from n-hexane. The quantities and melting points of each sample were given in Table 2.17. This substance showed a spot at Rf 0.64 on TLC using 50% $CHCl_3$-hexane as a developing solvent.

The IR spectrum of Substance 2 (Fig.16), assigned in Table 3.5 ,indicated that this substance should be a saturated long chain aliphatic aldehyde (38).

Table 3.5 The IR absorption band assignments of Substance 2

Frequency (cm^{-1})	Band type	Tentative assignments
2940	s	C-H stretch. of CH_3-
2860	s	C-H stretch. of $-CH_2-$
2750	w	aldehydic C-H stretch.
1725	s	$-\overset{|}{C}=O$ stretch. of aldehyde
730,720	m	C-H rock. of $-CH_2-$ for chain > 4 carbons

The ^1H NMR spectrum (Fig. 17) showed the singlet signal at 9.75 ppm.(1H) which corresponded to aldehydic proton ($O=C-\underline{H}$). The triplet signal at 2.42 ppm.(2H) corresponded to methylene protons next to aldehydic group ($-C\underline{H}_2-CHO$) and 1.25 ppm corresponded to methylene interlinking system

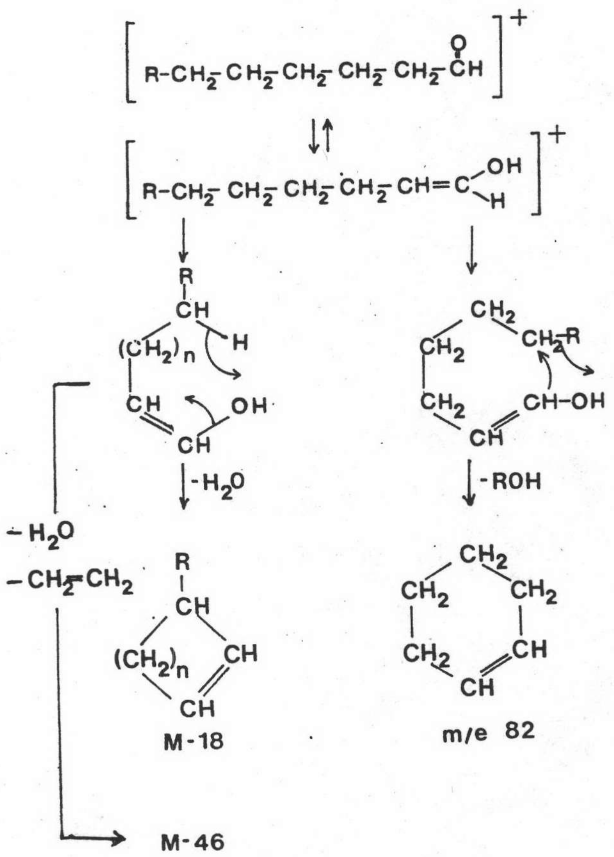
$(CH_3-(\underline{CH}_2)_n-)$. The triplet signal at 0.88 ppm.(3H) corresponded to methyl protons $(\underline{CH}_3-(CH_2)_n-)$ (39).

The ^{13}C NMR spectrum (Fig. 18) indicated the important carbon signal characteristic of aldehydic carbon at 202.78 ppm.(40). The other signals at 14.08 ppm. (methyl carbon) and 22.18-43.94 ppm. corresponded to methylene carbons.

The GC-MS analysis of Substance 2 from F153 variety had 2 main peaks in gas chromatogram (Fig.19). The first peak showed at t_R 5 min. and the second one occured at t_R 7 min. The mass spectrum of first peak (Fig.20) gave the molecular ion peak, M^+, at m/e 408 (Calcd. for $C_{28}H_{56}O$) together with fragmentation ion peaks at m/e 362, 348, and other peaks corresponded to $-CH_2-$ lost (m/e 14) step by step. This fragmentation pattern supported the existance of long chain saturated compounds (41). The ion peaks at 390 (M-18) and 362 (M-46) were found to be the characteristic of saturated long chain aliphatic aldehydes (34) of which fragmentation ions were shown in scheme 3.1 (34). The mass spectrum of second peak (Fig.21) gave the molecular ion peak, M^+, at m/e 436 (calcd. for $C_{30}H_{60}O$) and ion peaks at m/e 418 (M-18) and 390 (M-46) which were the characteristic of saturated long chain aldehydes. The other peaks corresponded to pattern of long chain saturated hydrocarbons.

From the spectral data, the 2 main peaks in GC-MS analysis should be $C_{27}H_{55}CHO$ and $C_{29}H_{59}CHO$, respectively.

Scheme 3.1 The mass fragmentation pattern of saturated long chain aliphatic aldehydes



Moreover, this substance was confirmed by synthesis of semicarbazone derivative which was proved by IR spectrum (Fig.24). The IR absorption band assignments of this derivative are displayed in Table 3.6 (42).

Table 3.6 The IR absorption band assignments of semicarbazone derivative of Substance 2

Frequency (cm^{-1})	Band type	Tentative assignments
3455	m	N-H stretch. of 2° amide
3280, 3180	m	N-H stretch. of 1° amide
2920, 2855	s	C-H stretch. of CH_3-,$-CH_2$-
1680	s	$-\overset{\vert}{C}=O$ stretch. of amide band I
1630	w	N-H bend. of amide band II
1510	m	C=N stretch. vibration
1470	s	C-H bend. of CH_3-,$-CH_2$-
1355	w	C-N stretch. of 1° amide
770, 630	m	N-H wagging out-of-plane
720	s	C-H rock. of $-CH_2$- for chain > 4 carbons

The GC analysis data (Fig. 22a-22d and 23a-23e) indicated that Substance 2 was a mixture of saturated long chain aliphatic aldehyde. By using construction of correlation standard curve between log of retention time and number of carbon atom (Fig. 53 - 54). The results of GC analysis and composition of Substance 2 of each sample were different which are shown in Table 3.7 and 3.8.

From these results, the structure of Substance 2 was confirmed as a mixture of saturated long chain aliphatic aldehyde which is shown below:

$$CH_3(CH_2)_nCHO \qquad n = 20,21,22,---,32$$

Substance 2

From Table 3.7, substance 2 in sugar cane rinds consisted of 4-8 compounds. The major compositions of Substance 2 in Q83 variety were $C_{27}H_{55}CHO$, $C_{29}H_{59}CHO$, $C_{33}H_{67}CHO$, and $C_{31}H_{63}CHO$, respectively. In F153 variety, the major compositions were $C_{27}H_{55}CHO$ and $C_{29}H_{59}CHO$ while F147 variety had the major compositions as $C_{24}H_{49}CHO$, $C_{27}H_{55}CHO$ and $C_{26}H_{53}CHO$, respectively. The other compositions were trace.

From Table 3.8, Substance 2 separated from filter cake of the Mitr Phol Factory composed of 8 compounds and the major compositions were $C_{27}H_{55}CHO$, $C_{29}H_{59}CHO$, $C_{33}H_{67}CHO$, and $C_{31}H_{63}CHO$, respectively. The substance 2 from The United Farmer&Industry Factory's filter cake consisted of 8 compounds with $C_{24}H_{49}CHO$, $C_{28}H_{57}CHO$, $C_{30}H_{61}CHO$, and $C_{26}H_{53}CHO$ as major compositions. From Khumphawapi Factory, 8 compounds were isolated from Substance 2 which $C_{28}H_{57}CHO$ and $C_{27}H_{55}CHO$ were identified as major compositions, respectively.

From GC analysis, it was indicated that the major compositions in sugar cane rinds of each variety and filter cakes from each factory were different. Nevertheless, the difference of chemical compositions should be occured from the difference of sugar cane varieties and agricultural area.

Table 3.7 The composition of saturated long chain aliphatic aldehyde contain in Substance 2 from sugar cane rinds

Name	Formula	% Composition		
		Q83	F147	F153
tricosanal	$CH_3-(CH_2)_{21}-CHO$	-	2.8	-
tetracosanal	$CH_3-(CH_2)_{22}-CHO$	-	-	-
pentacosanal	$CH_3-(CH_2)_{23}-CHO$	-	51.4	2.1
hexacosanal	$CH_3-(CH_2)_{24}-CHO$	-	-	1.8
heptacosanal	$CH_3-(CH_2)_{25}-CHO$	-	15.1	1.1
octacosanal	$CH_3-(CH_2)_{26}-CHO$	51.9	16.8	63.4
nonacosanal	$CH_3-(CH_2)_{27}-CHO$	-	5.5	4.8
triacontanal	$CH_3-(CH_2)_{28}-CHO$	23.3	2.4	18.0
hentriacontanal	$CH_3-(CH_2)_{29}-CHO$	-	6.0	-
dotriacontanal	$CH_3-(CH_2)_{30}-CHO$	11.4	-	4.9
tritriacontanal	$CH_3-(CH_2)_{31}-CHO$	-	-	-
tetratriacontanal	$CH_3-(CH_2)_{32}-CHO$	13.5	-	4.0

Table 3.8 The composition of saturated long chain aliphatic aldehyde contain in Substance 2 from filter cakes

Name	Formula	% Composition		
		Mitr Phol	United Farmer& Industry	Khumphawapi
docosanal	$CH_3-(CH_2)_{20}-CHO$	-	-	1.1
tricosanal	$CH_3-(CH_2)_{21}-CHO$	-	0.9	1.2
tetracosanal	$CH_3-(CH_2)_{22}-CHO$	0.6	1.3	1.4
pentacosanal	$CH_3-(CH_2)_{23}-CHO$	3.0	24.2	5.9
hexacosanal	$CH_3-(CH_2)_{24}-CHO$	-	-	2.7
heptacosanal	$CH_3-(CH_2)_{25}-CHO$	2.9	18.4	8.5
octacosanal	$CH_3-(CH_2)_{26}-CHO$	29.1	5.6	10.6
nonacosanal	$CH_3-(CH_2)_{27}-CHO$	-	23.2	68.5
triacontanal	$CH_3-(CH_2)_{28}-CHO$	22.1	-	-
hentriacontanal	$CH_3-(CH_2)_{29}-CHO$	9.8	22.6	-
dotriacontanal	$CH_3-(CH_2)_{30}-CHO$	14.6	-	-
tritriacontanal	$CH_3-(CH_2)_{31}-CHO$	-	3.8	-
tetratriacontanal	$CH_3-(CH_2)_{32}-CHO$	18.0	-	-

3.2.2.3 Structural Elucidation of Substance 3

Substance 3 was obtained from Fraction 1A and 1B and recrystallized with the mixture of chloroform and hexane to give bright white needle crystal, the Rf value 0.13 using chloroform as a mobile phase. The quantities and melting points of this substance from each sample were shown in Table 2.20. It gave deep green colour with Liebermann-Burchard's reagent and decolourized Br_2 in CCl_4 . These results indicated the possibility of unsaturated steroids (43).

The IR spectrum (Fig.25) gave the absorption bands of 2° alcohol at 3430 and 1050 cm^{-1} and bands at 1670-1630 cm^{-1} which might due to the C=C stretching vibration. The absorption bands at 840 and 800 cm^{-1} (probably C-H out of plane bending) coincided with the characteristic absorption peaks of trisubstituted olefin. The IR absorption band assignments of this substance are presented in Table 3.9 (42,44).

Table 3.9 The IR absorption band assignments of Substance 3

Frequency (cm^{-1})	Band type	Tentative assignments
3430	b	O-H stretch. vibration
2960-2840	s	C-H stretch. of $-CH_3$,$-CH_2-$
1650	w	C=C stretch. vibration
1460	m	C-H bend. of $-CH_3$, $-CH_2-$

(continued)

Table 3.9 (continued)

Frequency (cm^{-1})	Band type	Tentative assignments
1380	m	C-H in plane bend. of trisubstituted of olefin
1050	m	C-O stretch. of 2° alcohol
970,960	m	C-H out of plane bend. of trans configuration
840,800	w	C-H out of plane bend. of trisubstituted olefin

Both the colour tests and the IR spectral data supported a steroidal structure.

The ^{1}H NMR spectrum of Substance <u>3</u> (Fig.27) showed the signals at 0.70-1.06 ppm., which were the signals of angular methyl groups of C-18 and C-19 and methyl groups at side chain that were C-21, C-26, C-27, and C-29. The signal at 1.51-2.30 ppm. indicated methylene group (CH_2) and methylnic group (CH) of steroids. The multiplet signal at 3.46 ppm. was the proton of hydroxyl group while the doublet doublet signal at 5.08 ppm. was the signal of disubstituted vinyl protons (H-22 and H-23). The last signal at 5.32 ppm. was the signal of trisubstituted vinyl proton (H-6).

The ^{13}C NMR spectrum (Fig.28) showed the signals which corresponded with the ^{13}C NMR spectrum of B-sitosterol and stigmasterol that is shown in Table 3.10 (46).

Table 3.10 The ^{13}C NMR chemical shift comparision between β-sitosterol, stigmasterol and Substance 3

Carbon position	Chemical shift (ppm.)		
	β-sitosterol	stigmasterol	substance 3
1	37.1	37.4	37.29
2	31.8	31.7	31.60
3	71.9	71.8	71.69
4	42.4	42.4	42.28
5	140.9	140.0	140.76
6	121.8	121.7	121.64
7	32.0	31.9	31.87
8	32.0	31.9	31.87
9	50.3	50.3	50.18
10	36.6	36.6	36.48
11	21.1	21.1	21.09
12	39.9	39.8	39.73
13	42.4	42.4	42.28
14	56.8	57.0	56.79
15	24.3	24.4	24.29
16	28.2	28.9	28.24,28.89
17	56.2	56.0	55.98
18	11.9	12.2	11.83,12.21
19	19.4	19.4	19.36

(continued)

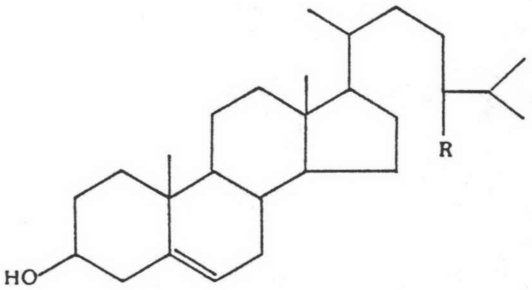
Table 3.10 (continued)

Carbon position	Chemical shift (ppm.)		
	β-sitosterol	stigmasterol	substance <u>3</u>
20	36.2	40.5	36.15,40.43
21	19.1	21.1	18.99,21.09
22	34.0	138.4	33.99,138.27
23	29.3	129.4	29.22,129.28
24	50.3	51.3	50.21
25	26.2	31.9	26.13,31.87
26	18.8	19.0	18.99
27	19.8	21.1	19.79,21.20
28	23.1	25.4	23.09,25.37
29	11.9	12.0	11.83,12.04

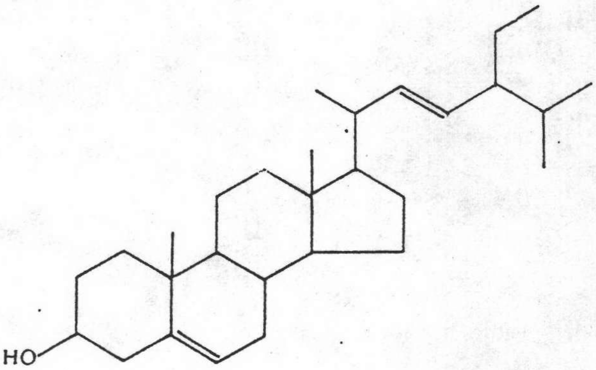
The MS spectrum (Fig.26) showed the expected molecular ion peak for β-sitosterol at m/e 414.0 and also revealed the fragmentation pattern of C_{29} steroids. However, two significant peaks at m/e 412.0 and m/e 400.0 could not be accounted by the β-sitosterol structure. Since the loss of two atoms of hydrogen from the molecular ion is highly unlike, there must be a second component corresponding to the fragmentation ion peak at m/e 412.0. Similarly, the loss of a methylene fragment from the m/e 414.0 and a unit of 12 from the ion m/e 412.0 could be ruled out, therefore, the ion at m/e 400.0 indicated the presence of a third compound. TLC was unable to separate this mixture though it is changed various developing solvent systems. The possible mass fragmentation pattern of Substance 3 is shown in Scheme 3.2 (47).

The presence of β-sitosterol in plants was frequently accompanied by one or more than steroids (48) such as stigmasterol and campesterol. From the GC analysis of Substance 3 compared with the authentic campesterol, stigmasterol, cholesterol, and β-sitosterol, it was found that substance 3 corresponded to campesterol, stigmasterol, and β-sitosterol.

So, Substance 3 was a mixture of β-sitosterol, stigmasterol, and campesterol. The structure of these three steroids were given as follow:



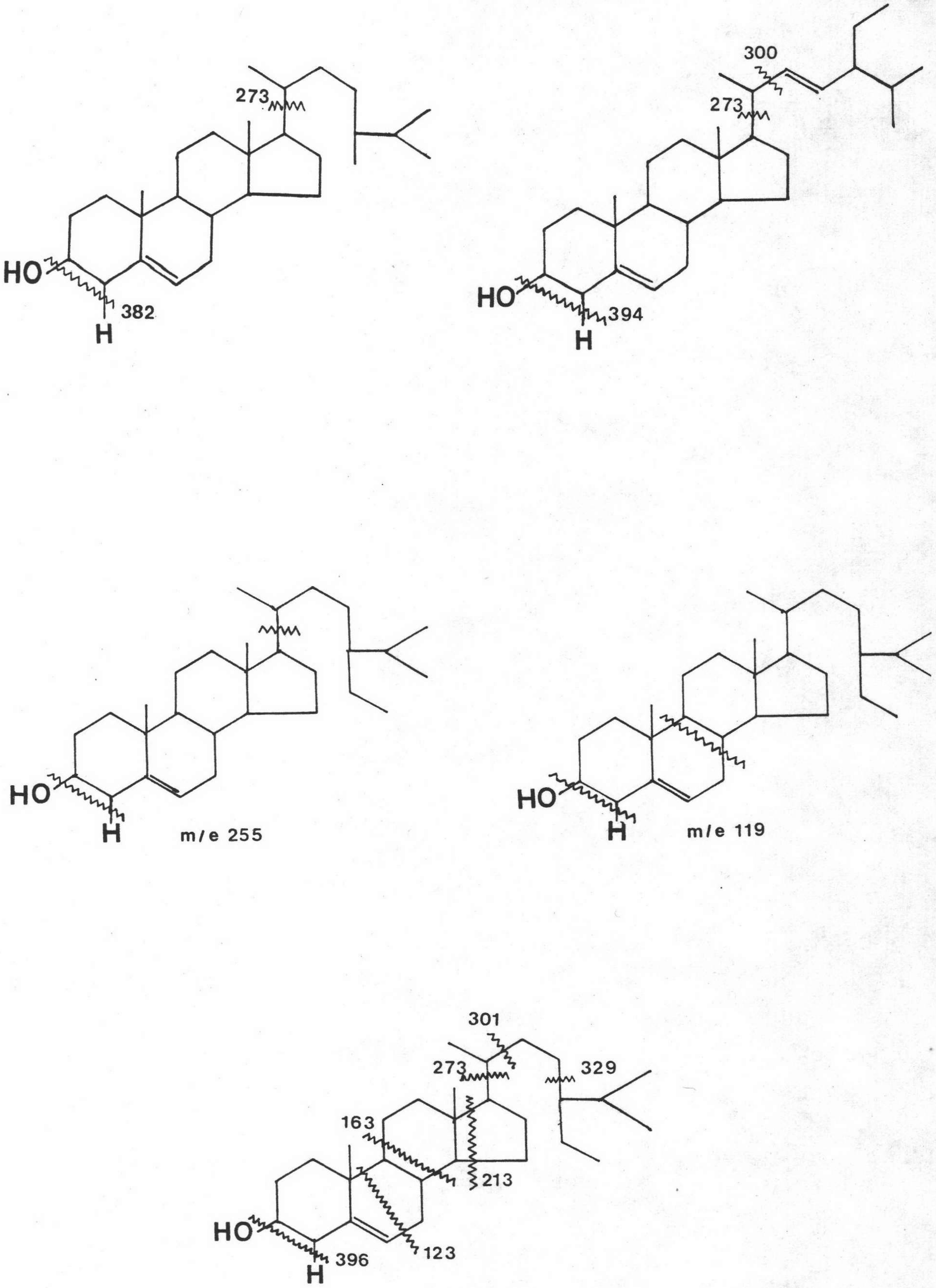
R=Et, β-sitosterol
R=Me, campesterol



stigmasterol

Substance 3

Scheme 3.2 The possible mass fragmentation pattern of Substance 3



The composition of steriods in Substance 3 of various filter cakes were shown in Table 3.11.

Table 3.11 The composition of steroids in Substance 3 separated from filter cakes

Compound	% Composition of steroids			
	Mitr Phol	Mitr Siam	Khumphawapi	United Farmer&Industry
campesterol	8.5	14.0	9.6	7.3
stigmasterol	68.3	55.5	68.8	76.8
β-sitosterol	23.2	30.4	21.6	15.8

3.2.2.4 Structural Elucidation of Substance 4

Substance 4, after recrystallization with hexane, was white amorphous substance, Rf 0.24 by chloroform as a solvent. It gave a negative test to Liebermann-Burchard's reagent and could not change the colour of Br_2 in CCl_4. These results indicated that substance 4 was not triterpenoids, steroids, nor unsaturated compound.

The IR spectrum (Fig.30) illustrated the absorption peaks at 3320 and 1070 cm^{-1}. These peaks were the characteristic of 1° alcohol. The other absorption peaks were similar to saturated long chain aliphatic compound. Thus, this substance should be a saturated long chain aliphatic primary alcohol. The IR absorption band assignments of this substance are appeared in Table 3.12 (42,44).

Table 3.12 The IR absorption band assignments of Substance 4

Frequency (cm^{-1})	Band type	Tentative assignments
3320	b	O-H stretch. vibration
2940,2860	s	C-H stretch. of $-CH_3$, $-CH_2-$
1470	m	C-H bend. of $-CH_3$, $-CH_2-$
1070	m	C-O stretch. of 1° alcohol
730,720	w	C-H rock. mode of $-CH_2-$ for carbon > 4

The GC analysis data indicated that Substance 4 was a mixture of saturated long chain aliphatic primary alcohol by using the plotted standard correlation curve between log of retention time and the number of carbons in the authentic primary long chain alcohol sample*(Fig.55 and 56). The results of GC analysis indicated 7-11 peaks on gas chromatogram (Fig.31) that are shown in Table 3.13-3.14.

From Table 3.13 and 3.14, the major composition in both of sugar cane rinds and filter cakes was $C_{30}H_{61}OH$ (triacontanol) about 64.7-88.6%. The other minor ones

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* The standard saturated long chain aliphatic alcohols obtained from the mixture of long chain aliphatic alcohols separated from Rhizophora apiculata Bl. and authentic $C_{30}H_{61}OH$ (Fluka)

were $C_{32}H_{65}OH$ (dotriacontanol) 4.5-11.4% and $C_{29}H_{59}OH$ (nonacosanol) 2.1-8.6%.

Table 3.13 The composition of saturated long chain aliphatic alcohol contain in Substance 4 from sugar cane rinds

Name	Formula	% Composition		
		Q83	F147	F153
pentacosanol	$CH_3-(CH_2)_{24}-OH$	0.1	-	-
hexacosanol	$CH_3-(CH_2)_{25}-OH$	0.4	0.2	0.05
heptacosanol	$CH_3-(CH_2)_{26}-OH$	0.3	0.2	0.1
octacosanol	$CH_3-(CH_2)_{27}-OH$	2.3	4.3	2.8
nonacosanol	$CH_3-(CH_2)_{28}-OH$	8.6	5.4	2.1
triacontanol	$CH_3-(CH_2)_{29}-OH$	71.4	80.5	88.6
hentriacontanol	$CH_3-(CH_2)_{30}-OH$	4.8	3.1	1.8
dotriacontanol	$CH_3-(CH_2)_{31}-OH$	10.3	5.4	4.5
tritriacontanol	$CH_3-(CH_2)_{32}-OH$	1.0	0.6	-
tetratriacontanol	$CH_3-(CH_2)_{33}-OH$	0.4	-	-
pentatriacontanol	$CH_3-(CH_2)_{34}-OH$	0.3	0.2	-

Table 3.14 The composition of saturated long chain aliphatic alcohol contain in Substance 4 from filter cakes

Name	Formula	% Composition			
		Mitr Phol	Mitr Siam	Khumpha-wapi	United Farmer& Industry
hexacosanol	$CH_3-(CH_2)_{25}-OH$	0.1	0.1	-	0.2
heptacosanol	$CH_3-(CH_2)_{26}-OH$	0.2	0.2	0.2	0.4
octacosanol	$CH_3-(CH_2)_{27}-OH$	4.0	6.9	2.4	5.5
nonacosanol	$CH_3-(CH_2)_{28}-OH$	5.9	3.3	2.7	8.1
triacontanol	$CH_3-(CH_2)_{29}-OH$	64.7	79.1	83.6	64.7
hentriacontanol	$CH_3-(CH_2)_{30}-OH$	4.3	0.5	-	4.8
dotriacontanol	$CH_3-(CH_2)_{31}-OH$	11.4	6.4	8.0	9.2
tritriacontanol	$CH_3-(CH_2)_{32}-OH$	2.4	0.4	-	2.4
tetratriacontanol	$CH_3-(CH_2)_{33}-OH$	6.0	2.9	3.0	3.8
pentatriacontanol	$CH_3-(CH_2)_{34}-OH$	1.0	0.2	-	0.9

From the spectral data and GC analysis, the structure of Substance 4 is shown below:

$$CH_3\ (CH_2)_n\ OH \qquad n = 24, 25, \_\_\_, 34$$

Substance 4

3.2.2.5 Structural Elucidation of Substance 5

Substance 5, white amorphous substance, gave positive results with $NaHCO_3$ which indicated that this substance should have carboxylic group in its structure.

The IR spectrum (Fig.32) showed the absorption bands at 2920, 2860, 1460, 730, and 720 cm^{-1} which were the characteristic of saturated long chain aliphatic compound. The absorption band at 1710 cm^{-1} was the characteristic of $-\overset{|}{C}=O$ of carboxylic acid and the absorption band at 940 cm^{-1}, was the O-H bending of carboxylic acid. So, Substance 5 should be a saturated long chain aliphatic acid. The IR absorption band assignments of this substance are shown in Table 3.15 (42,44).

Table 3.15 The IR absorption band assignments of Substance 5

Frequency (cm^{-1})	Band type	Tentative assignments
3300-3000	b	O-H stretch. vibation
2920,2860	s	C-H stretch. of CH_3-,-CH_2-
1710	s	$-\overset{|}{C}=O$ stretch. of carboxylic acid
1430-1410	m	O-H bend. vibration
1300-1200	m	C-O stretch. vibration
940	b,m	O-H out-of-plane bend.
730,720	s	C-H rock. mode of -CH_2- for carbon > 4

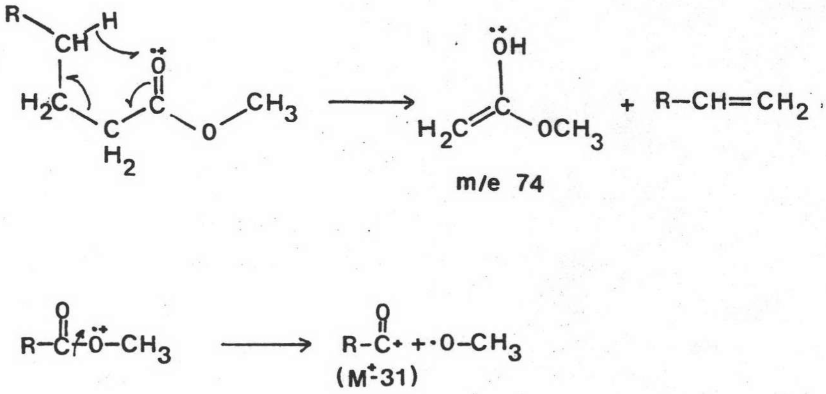
Substance 5 was changed into methyl ester derivative by diazomethane which was confirmed by the absorption bands of IR spectrum (Fig.33) shown in Table 3.16 (42,44).

Table 3.16 The IR absorption band assignments of methyl ester derivative of Substance 5

Frequency (cm^{-1})	Band type	Tentative assignments
2920,2840	s	C-H stretch. of CH_3-,-CH_2-
1740	m	$-\overset{|}{C}=O$ stretch. of ester
1460	m	C-H bend. of CH_3-,-CH_2-
1170	w	C-O stretch. of methyl ester
730,720	m	C-H rock. mode of -CH_2- for carbon > 4

The GC-MS analysis of methyl ester derivatives showed 3 main peaks in gas chromatogram (Fig.34) appeared at t_R 6.3, 11.3 and 20.0, respectively. The mass spectrum of first peak (Fig.35) gave the molecular ion peak, M^+, at m/e 410 (calcd. for $C_{27}H_{54}O_2$). The fragmentation ion peaks at m/e 379 (M^+-31), 367(M^+-43) and 74 were the fragmentation pattern of methyl ester of long chain aliphatic acid.(Scheme 3.3) The other peaks were the pattern of long chain ester lost each CH_2 (45,49). The mass spectrum of second peak (Fig.36) showed the M^+ at m/e 438 (calcd. for $C_{29}H_{58}O_2$). The other fragmentation ion peaks at m/e 407 (M^+-31), 395 (M^+-43), 74 were identified as the fragmentation pattern of methyl ester of long chain acid. The third peak showed the molecular ion peak of MS spectrum (Fig.37) at m/e 466 (calcd. for $C_{31}H_{62}O_2$). The other fragmentation ions indicated the pattern of methyl ester of long chain aliphatic acid. Thus, the three peaks in gas chromatogram of GC-MS analysis should be $CH_3(CH_2)_{24}COOCH_3$, $CH_3(CH_2)_{26}COOCH_3$ and $CH_3(CH_2)_{28}COOCH_3$, respectively.

Scheme 3.3 The mass fragmentation pattern of saturated long chain aliphatic carboxylic acids



The GC analysis of methyl ester derivative of substance 5 from each sample indicated that it was a mixture of long chain aliphatic methyl ester. By the correlation standard curve between log t_R and number of carbons (Fig.57), the composition of Substance 5 of various samples are shown in Table 3.17-3.18.

From the spectral data and GC analysis, the structure of Substance 5 is shown below:

$$CH_3\,(CH_2)_n\,COOH \qquad n = 20,22,23,---,32$$

Substance 5

Table 3.17 The composition of saturated long chain aliphatic acids contain in Substance 5 from sugar cane rinds

Name	Formula	% Composition		
		F147	F153	Q83
docosanoic acid	$CH_3(CH_2)_{20}COOH$	-	0.8	-
tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	-	1.7	-
pentacosanoic acid	$CH_3(CH_2)_{23}COOH$	-	0.8	-
hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	3.6	5.7	2.1
heptacosanoic acid	$CH_3(CH_2)_{25}COOH$	2.4	7.8	2.8
octacosanoic acid	$CH_3(CH_2)_{26}COOH$	59.4	59.4	54.1
nonacosanoic acid	$CH_3(CH_2)_{27}COOH$	-	2.6	-
triacontanoic acid	$CH_3(CH_2)_{28}COOH$	16.7	13.4	19.3
dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$	8.4	4.2	9.9
tetratriacontanoic acid	$CH_3(CH_2)_{32}COOH$	9.4	3.7	11.8

Table 3.18 The composition of saturated long chain aliphatic acids contain in Substance 5 from filter cake

Name	Formula	% Composition			
		Mitr Phol	Mitr Siam	Khumphawapi	United Farmer& Industry
tetracosanoic acid	$CH_3(CH_2)_{22}COOH$	0.1	0.6	-	0.8
pentacosanoic acid	$CH_3(CH_2)_{23}COOH$	0.2	0.2	-	0.5
hexacosanoic acid	$CH_3(CH_2)_{24}COOH$	2.2	2.6	2.7	3.8
heptacosanoic acid	$CH_3(CH_2)_{25}COOH$	2.7	3.1	3.3	4.1
octacosanoic acid	$CH_3(CH_2)_{26}COOH$	50.6	49.0	63.2	52.8
nonacosanoic acid	$CH_3(CH_2)_{27}COOH$	1.2	1.5	1.0	-
triacontanoic acid	$CH_3(CH_2)_{28}COOH$	18.8	19.2	17.4	22.9
hentriacontanoic acid	$CH_3(CH_2)_{29}COOH$	0.7	0.5	-	-
dotriacontanoic acid	$CH_3(CH_2)_{30}COOH$	14.0	11.4	7.0	8.2
tritriacontanoic acid	$CH_3(CH_2)_{31}COOH$	-	0.6	-	-
tetratriacontanoic acid	$CH_3(CH_2)_{32}COOH$	9.6	11.2	5.4	6.8

From the GC analysis, it was found that octacosanoic acid ($C_{27}H_{55}COOH$) was the major composition (49.0-63.2%) in substance 5 from both sugar canes and filter cakes from all sources. Triacontanoic acid ($C_{29}H_{59}COOH$) was the second major component varied from 13.4-22.9% and the minor components were dotriacontanoic acid ($C_{31}H_{63}COOH$) 4.2-14.0% and tetratriacontanoic acid ($C_{33}H_{67}COOH$) 3.7-11.8%.

3.2.3 The Chemical Constituents of Fraction 2

Fraction 2 was obtained from extraction of sugar cane rind with chloroform (Scheme 2.2). This fraction was separated by silica gel column chromatography (Table 2.13) to give 2 substances, Substance 6 and Compound 7.

3.2.3.1 Structural Elucidation of Substance 6

Substance 6 was isolated from column by 50% $CHCl_3$-hexane to $CHCl_3$. It was white amorphous solid, m.p. 75-77°C. The Rf value was similar to Substance 4.

The IR spectrum (Fig.39) showed the same absorption band as Substance 4's. Thus, this substance should be a saturated long chain aliphatic primary alcohol.

The GC analysis results displayed 6 peaks. From the correlation curve between log t_R and number of carbons (Fig.58), they should be $C_{26}H_{53}OH$, $C_{27}H_{55}OH$, $C_{28}H_{57}OH$, $C_{30}H_{61}OH$, and $C_{32}H_{65}OH$. The percentage of each composition of alcohols is shown in Table 3.19.

Table 3.19 The composition of saturated long chain aliphatic alcohols contain in Substance 6

Name	Formula	% composition in substance 6
hexacosanol	$CH_3-(CH_2)_{25}-OH$	3.2
heptacosanol	$CH_3-(CH_2)_{26}-OH$	0.8
octacosanol	$CH_3-(CH_2)_{27}-OH$	85.1
nonacosanol	$CH_3-(CH_2)_{28}-OH$	2.0
triacontanol	$CH_3-(CH_2)_{29}-OH$	8.0
dotriacontanol	$CH_3-(CH_2)_{31}-OH$	0.9

3.2.3.2 Structural Elucidation of Compound 7

Compound 7 was separated from column by $CHCl_3$ to 25% MeOH-$CHCl_3$. After recrystallization, it was green needle-shaped crystal, m.p. 274-276°C. From the chemical reaction of Liebermann-Burchard's reagent, it gave green solution which indicated that it should be a steroidal compound.

The IR spectrum (Fig.41) showed the absorption bands of hydroxyl group at 3350, 1355-1260 cm^{-1} and the absorption bands of carbonyl group of ketone conjugated with double bond at 1655-1580 cm^{-1}. Thus, Compound 7 should have hydroxyl group and carbonyl group which conjugated with double bond in its structure. The mass spectrum (Fig.43) showed the peak which was expected to be the molecular ion peak at m/e 330.

However, from the available data, it was not enough informations to elucidate the structure of Compound 7.

3.2.4 The Chemical Constituents of Fraction 3

Compound 8 was isolated from chloroform extract solution, Fraction 3 (Scheme 2.2). After recrystallization with a mixture of chloroform and acetone, it gave pale brown needle crystal, m.p. 149-151°C. It was a polar compound because of solubility in water and DMSO and gave positive results with $FeCl_3$ which indicated that it should be phenolic or enolic compound (50).

From elemental analysis and mass spectrum which indicated the M^+ at m/e 142, the molecular formula of this compound should be $C_6H_6O_4$ (%C 50.70, %H 4.22, %O 45.07).

The IR spectrum (Fig.44) displayed the absorption bands of alcohol at 3280, 3180, 1350, 1230 cm^{-1}, the band of alkene at 3100 and 1700 cm^{-1}, the band of ketone at 1665-1590, and the band of ether at 1150 and 1080 cm^{-1}. These results suggested that this compound should be composed of hydroxyl group, carbonyl group, olefinic group, and C-O-C of ether which was similar to the one of kojic acid (54). The IR absorption band assignments of Compound 8 are given in Table 3.20 (42,44).

Table 3.20 The IR absorption band assignments of Compound 8

Frequency (cm^{-1})	Band type	Tentative assignments
3280,3180	b,s	O-H stretch. vibration
3100	w	C-H stretch.of olefin
2940,2860	w	C-H stretch. of alkane
1700	w	C=C stretch. mode of unconjugated olefin
1665-1590	s	$-\overset{\vert}{C}=O$ stretch. of ketone conjugated with C=C
1350	m	O-H bend. vibration
1230	s	C-O stretch. of alcohol
1150,1080	s	C-O-C stretch. of ether

The UV (H_2O) spectrum (Fig.45) indicated the λ_{max} at 232 nm. (log ε = 3.97) and 266 nm. (log ε = 3.89) which indicated that there should be a non-conjugated unsaturation and a carbonyl functional group as it chromophore.

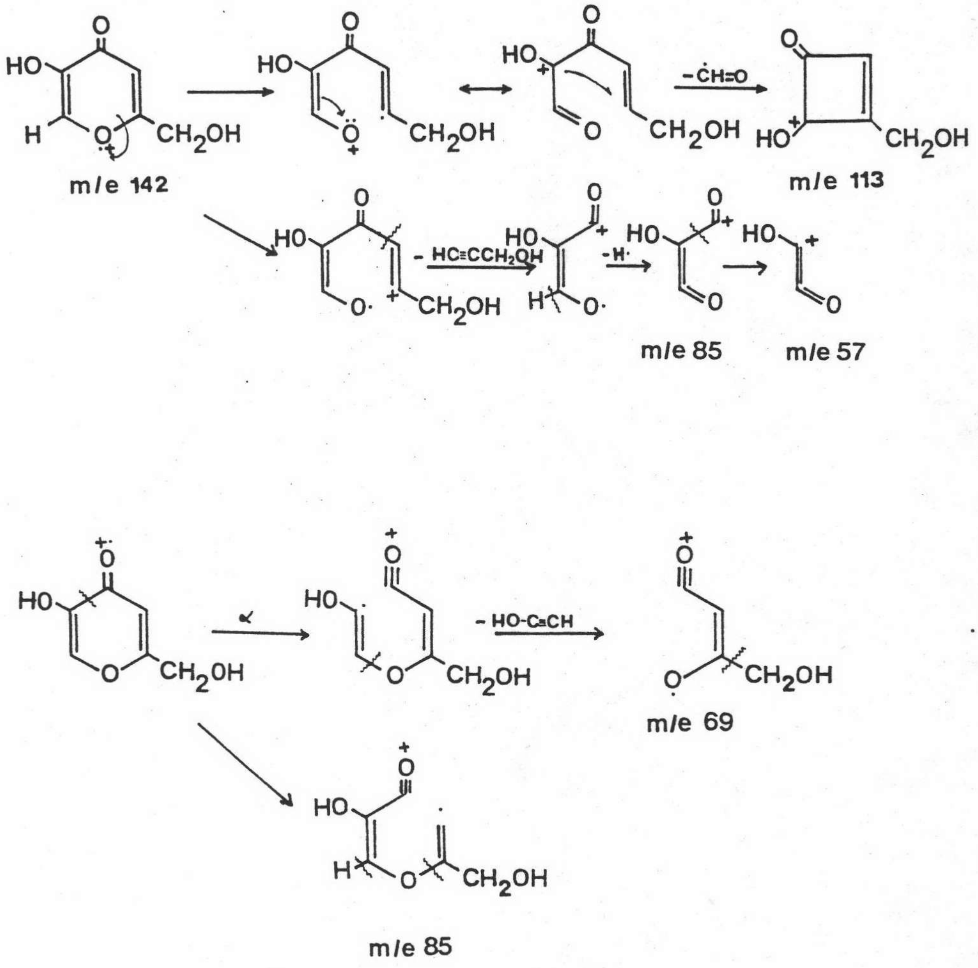
The ^{13}C NMR spectrum (Fig.48) indicated the important carbon signal corresponded to the ^{13}C NMR of kojic acid shown in Table 3.21 (51).

Table 3.21 The comparision of ^{13}C NMR chemical shift between kojic acid and Compound 8

Carbon composition	Chemical shift (ppm.)	
	kojic acid	compound 8
2	167.8	168.4
3	109.6	109.7
4	173.6	174.6
5	145.4	145.9
6	139.0	138.4
7	no reported	60.2

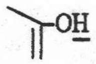
The mass spectrum of Compound 8 (Fig.46) showed the expected molecular ion, M^{+},at m/e 142 and the other fragmentation ions at m/e 113, 97, 85, 69, 57, and 39 which corresponded to kojic acid (52). Since the fragmentations of kojic acid had not been assigned, so they were proposed as follow (Scheme 3.4):

Scheme 3.4 The possible mass fragmentation pattern of Compound 8

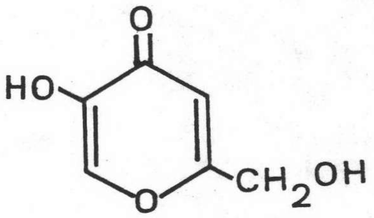


The ^1H NMR spectrum (Fig.47) showed the signals which was assigned in Table 3.22 (53).

<u>Table 3.22</u> The ^1H NMR signal assignments of Compound <u>8</u>

Chemical shift (ppm.)	Type and number of proton	tentative assignments
4.34	d, 2H	$-C\underline{H_2}-OH$
5.52	t, 1H	$-CH_2-O\underline{H}$
6.45	s, 1H	$-O-C\underline{H}=C-$
7.79	s, 1H	$O=C-C\underline{H}=C-$
8.48	s, 1H	

From the physical properties, chemical properties, and spectroscopic data, compound <u>8</u> was proved to be kojic acid (5-hydroxy-2-(hydroxymethyl)-4-pyrone)



Compound <u>8</u>