CHAPTER II

EXPERIMENTS

2.1 Materials

2.1.1 Filter Cakes

Samples of filter cake were collected from 4 factories which were Mitr Phol, Mitr Siam, Khumphawapi, and United Farmer&Industry. Each filter cake was sun-dried before being used in the experiment. Details of collecting samples are given in Table 2.1.

Table 2.1 Details about filter cakes

Name of factories	Collected time	Varieties of sugar cane
Mitr Phol	Jan. 1988	F140, F147, Q83
Mitr Siam	Jan. 1988	F140, F147, F156, Q83
Khumphawapi	Jun. 1987	F154, Q82, Q83, Pindar
United Farmer&Industry	Jan. 1988	F154, F140, F147, F156 Q83, Q96, Q82

2.1.2 Sugar Cane Rinds

The sugar cane rinds were collected from Cholburi Province. The sugar cane varieties used in this research were

F147, F153, and Q83. These three varieties were the same age, 18 months.

2.2 Instruments and Equipments

2.2.1 Soxhlet Extraction Apparatus(29)

For continuous extraction of a solid by a hot solvent, it is better to use a Soxhlet extraction apparatus such as that shown in Fig.2.1.

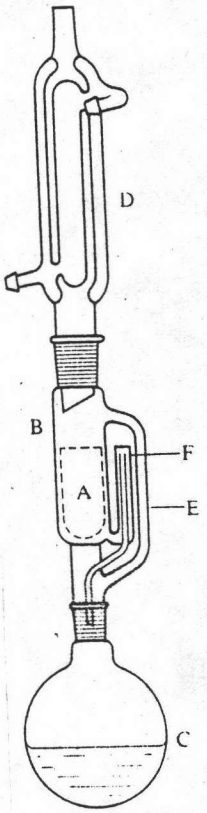


Figure 2.1

Soxhlet extraction apparatus

The solid substance is placed in the porous thimble A (made of tough filter-paper) and the latter is placed in the inner tube of the Soxhlet apparatus. The apparatus is then fitted to a round-bottomed flask C of appropiate size containing the solvent and boiling chips, and to a reflux condenser D (preferably of the double surface type). The solvent is gently boiled; the vapor passes up through the tube E, is condensed by the condenser D, and the solvent falls into the top of the tube F, it is siphoned over into the flask C, and thus removes the portion

of the substance which it has extracted in A. The process is repeated automatically until complete extraction is succeeded.

2.2.2 Rotary Film Evaporator(30)

This instrument is used for the rapid removal of a large quantity of volatile solvent from a solution of an organic compound (i.e.,from a solvent extraction process). Evaporation is conducted under reduced pressure (a water pump is the most convenient).

2.2.3 Infrared Spectra (IR)

The IR spectra were recorded on either a Shimadzu Spectrophotometer Model IR 440 or a Perkin-Elmer Model IR 781 Infrared Spectrophotometer. Solid samples were examined by incorporating the sample into a pellet of potassium bromide (KBr).

2.2.4 Ultraviolet and Visible Spectra (UV and VIS)

The UV and VIS spectra were determined by a Shimadzu UV-VIS Spectrophotometer Model 240 (wavelength range: 190-900 nm. with double beam photometric system).

2.2.5 Mass Spectra (MS)

The mass spectra were obtained by a Jeol Mass Spectrometer Model JMS-DX-300/JMA 2000 at 70 eV.

2.2.6 Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H NMR and ^{13}C NMR)

The ^{1}H and ^{13}C NMR spectra were performed using a

Jeol Fourier Transform NMR Spectrometer Model FX902. Tetramethylsilane (TMS) was used as an internal standard. The chemical shifts (δ) were given in ppm. down field from the TMS.

2.2.7 Elemental Analyses

The elemental analyses were made by using a Perkin Elmer CHNO Analyzer Model 240C.

2.2.8 Gas Chromatography (GC)

The GC analysis was carried out by using a Shimadzu Gas Chromatograph Model GC-R1A.

2.2.9 Gas Chromatography-Mass Spectra (GC-MS)

The GC-MS analysis was determined by either a Shimadzu GC-MS QP-1000 or a JMS-DX300 GC-Mass Spectrometer, Jeol.

2.2.10 Melting Point (m.p.)

The melting points were obtained on a Fisher-John apparatus and were uncorrected.

2.3 Chemical Reagents

2.3.1 Solvent

All solvents used in this research were commercial grade which were purified by distillation. They were n-hexane, chloroform, methanol, acetone, benzene, and ethyl acetate.

2.3.2 Silica Gel

The silica gels used in this research were silica gel 60G 7731 (for TLC) and silica gel 60 (70-230 mesh) (for CC). They belong to Merck Co.Ltd.

2.4 Color Tests

2.4.1 Liebermann-Burchard's Test

To a solution of the sample to be tested (2-3 mg.) in 0.5 mL. chloroform, a few drops of acetic anhydride was added, followed by one drop of concentrated sulfuric acid. Development of the colour after a few minutes suggests the presence of steroids or triterpenoids.

2.4.2 Other Reagents

Other reagents for color tests, such as 2,4-DNP, 5%$FeCl_3$, Br_2 in CCl_4, $KMnO_4$ and etc. were carried out and observed by following the textbook of Practical Organic Chemistry and the Systematic Identification of Organic Compounds.

2.5 Physical Separation Techniques

2.5.1 Quick Column Chromatography

This method is especially useful for separating large quantities of complex mixtures which were obtained from natural resources into fractions. Because of its speed and separating power, it can also be used for separating the reaction mixture.

Packing the column

The column was a glass column of 10.0 cm. diameter with sintered glass frit. Silica gel was added to the column and distributed evenly over the surface. The vacuum was applied (water pump) and the silica gel was allowed to settle. Any cracks were pressed with a glass rod and more silica gel was added to give a packed bed of 4.0 cm. or less. When the bed was compressed, the application of vacuum was continued and the column was ready to be packed with extract.

Preparing the extract

The extract was dissolved in small amount of chloroform, then, mixed with silica gel. The mixture was dried on water bath or in the air.

Separating the extract on the column

Before adding the extract, it was ensured that the surface of the column was smooth. Then, the extract was added and distributed over the surface like packing the column. When the column was ready, 500 ml. n-hexane was added gently and quickly to the top of the column and the fractions were collected. Polarity of solvents was changed from n-hexane to a mixture of chloroform-hexane, chloroform, a mixture of chloroform-methanol and methanol, respectively.

2.5.2 Thin-layer Chromatography (TLC)

The 0.25 mm. thin-layer chromatoplate was prepared in the following manner:

A mixture of silica gel (25.0 g) and water (50.0 mL.) was stirred until it became slurry, then, it was

applied to glass plates (20x20 cm.) using a Desaga spreader. After being dried at room temperature for an hour, the plates were heated at 125°C for 30 min., cooled and stored in a dessicator, ready to be used.

Before testing, Two lines were drawn on each plate, one 2.0 cm. from one edge; this line was referred to the base line. The other line, the upper line, was 14.0 cm. above and parallel to the first line. The solution containing the compounds to be investigated was applied as small spots along the base line of the plate at 1.0 cm. interval, and after the solvent had evaporated, the plate is placed in a glass tank filled to a depth of 1.0 cm. with the eluting solvent and the tank was covered with a glass cover. The eluting solvent moved up the plate immediately. The rate of solvent moving became slower as the distance between the solvent front and the base line increased. The time taken for the solvent front to reach the upper line varied according to the solvents used; time between 20 to 45 min. was generally encountered. When the solvent front had reached the upper line, the plate was removed from the tank; the solvent was allowed to evaporate, and the plate was detected with a suitable detector such as UV, I_2 and 25% sulfuric acid to reveal the compounds.

2.5.3 Column Chromatography (CC)

Merck's silica gel 70-325 mesh ASTM for column chromatography was used as adsorbents. The ratio of the mixture to be separated to the adsorbent used was approximately 1 : 20-30 by weight.

Packing the column

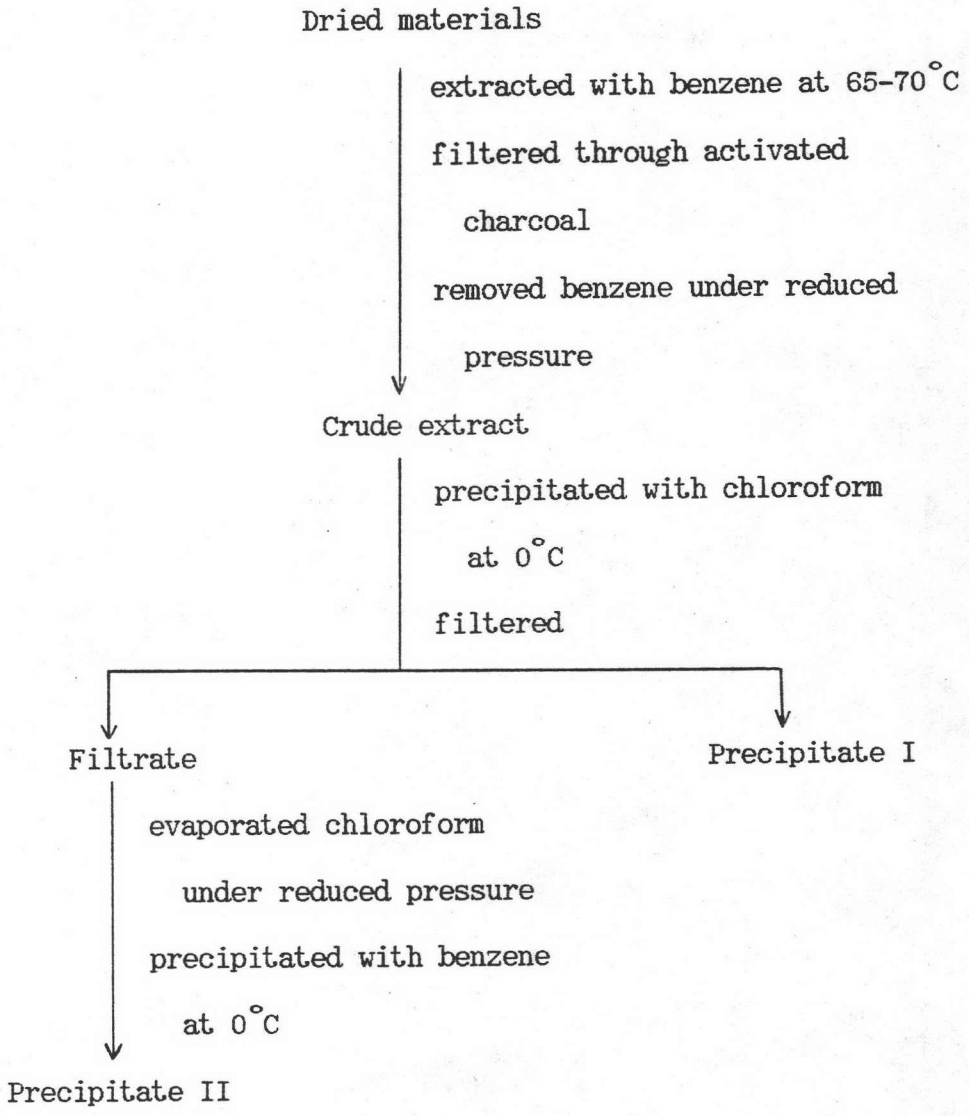
Pre-adjust the stopcock so that it was almost closed, but still allow the solvent to drip through. Allowed approximately 0.5 cm. of solvent to remain in the column when the slurry of the silica gel was poured. After the bed has settled, a paper disk, prepared from 3 mm. paper was inserted in the column. The sample was dissolved in the solvent as a 10% (w/v) solution and it was introduced when the solvent level was just above the paper disk in the column. If the material was not soluble in the developing solvent, one could always dissolve the sample in which it was soluble. The solution was next added to minimum quantity of adsorbent in a boiling flask and then removed the solvent in vacuum. The dried powder was sprinkled on the top of the column and the disk. Some adsorbent was usually added to the top of the column prior to the adding of the eluent.

2.6 Extraction

2.6.1 Extraction and Partial Purification

Dried rinds of F147 variety were extracted with benzene at 65-70°C. The benzene solution was filtered through actived charcoal, then, the benzene was removed under reduced pressure. The crude extract was dissolved in chloroform and cooled in refrigerator. The precipitation separated from the chloroform solution was filtered, and the chloroform in filtrate was evaporated under reduced pressure. The residue from filtrate was precipitated in benzene. This method was shown in Scheme 2.1.

Scheme 2.1 Extraction and Partial Purification



The result of extraction were shown in Table 2.2.

Table 2.2 The result of extraction and partial purification

No.	Weight of dried rind (g)	Benzene crude extract		Precipitate I		Precipitate II	
		wt(g)	%wt/wt**	wt(g)	%wt/wt*	wt(g)	%wt/wt*
1	5	0.42	8.4	0.23	54.8	0.02	4.7
2	5	0.55	11.0	0.24	43.6	0.03	5.4
3	10	1.08	10.8	0.60	55.6	0.02	1.8

* % wt of precipitation by wt of crude extract

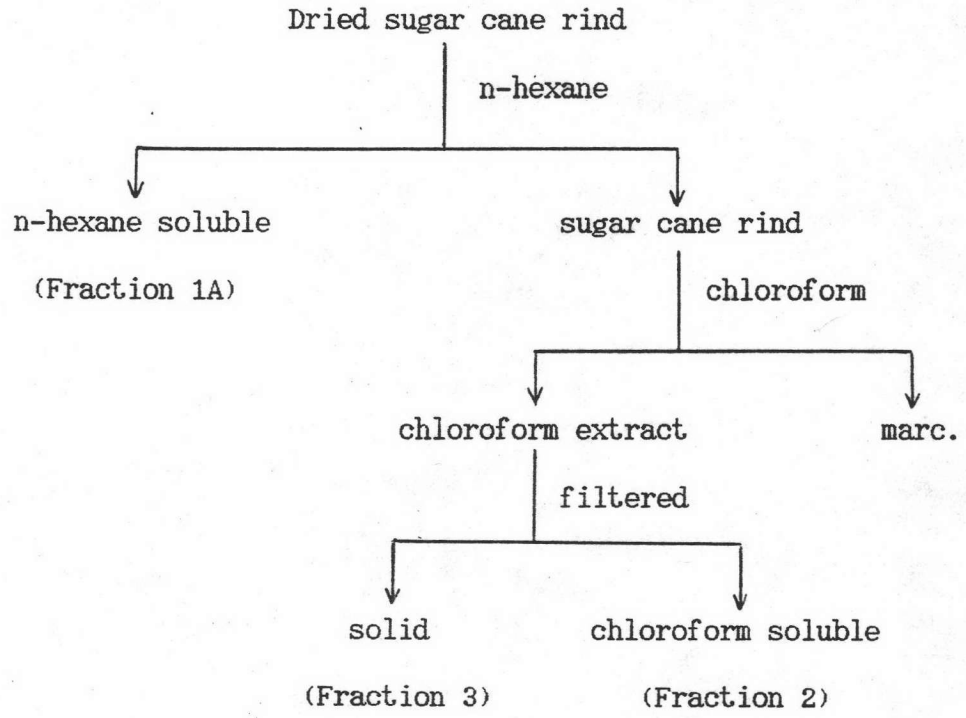
** % wt of crude extract by wt of dried rind

2.6.2 Extraction by Soxhlet Extraction Apparatus

2.6.2.1 Extraction of sugar cane rinds

The sun-dried sugar cane rinds were extracted with n-hexane and chloroform by Soxhlet extraction apparatus, respectively. After extraction, the n-hexane solutions were concentrated under reduced pressure yielded Fraction 1A. The chloroform solutions were filtered to separate some solid which divided before concentrating, Fraction 3. The filtrates were concentrated under reduced pressure yielded Fraction 2. The various extraction was process as shown in Scheme 2.2.

Scheme 2.2 Extraction by Soxhlet extraction apparatus



The quantities of Fraction 1A, Fraction 2, and Fraction 3, of each variety are shown in Table 2.3.

Table 2.3 The quantities of Fraction 1A, 2 and 3 of each variety of sugar cane

Varieties	wt of dried sugar cane rind (g)	Fraction 1A		Fraction 2		Fraction 3	
		wt(g)	%wt/wt	wt(g)	%wt/wt	wt(g)	%wt/wt
F147	299.5	44.6	14.9	5.4	1.8	0.4	0.13
F153	177.5	37.0	20.8	4.4	2.5	0.1	0.06
Q83	181.8	19.9	10.9	1.2	0.7	-	-

2.6.2.2 Extraction of Filter Cakes

The sun-dried filter cakes were extracted with n-hexane or benzene by Soxhlet extraction apparatus. Next, they were concentrated in reduced pressure Fraction 1B. The quantities of crude extracts are shown in Table 2.4 and 2.5.

Table 2.4 The extraction of filter cakes with n-hexane

Name of factory	wt of dried filter cake (g)	wt of crude extract (g)	%yield (wt/wt)	Remarks
Mitr Phol	110	11.0	10.0	yellow solid
Mitr Siam	113	6.0	5.3	yellow solid
Khumphawapi	126	22.4	17.8	thick yellow solid
United Farmer&Industry	105	7.7	7.3	yellow solid

Table 2.5 The extraction of filter cake with benzene

Name of factory	wt of dried filter cake (g)	wt of crude extract (g)	%yield (wt/wt)	Remarks
Mitr Phol	250	25.7	10.3	thick brown solid
Mitr Siam	250	15.0	6.0	green-yellow solid
Khumphawapi	1010	119.0	11.7	yellow solid
United Farmer&Industry	276	19.7	7.1	yellow solid

2.7 Separation and Identification of Precipitate I and II

The precipitate I and II were separated and identified by the GC-MS (condition: column 1 m, ID 3 mm packing OV-1 5% wt support chromosorb W, column temp. 240-280°C, temp. rate 5°C/min., injection temp. 300°C). The results were shown in Fig. 1-12.

2.8 Separation of Fraction 1A and Fraction 1B

The 10 g crude extract was chromatographed on silica gel using quick column chromatography technique to separate into small fractions due to their properties. First, the column was eluted with n-hexane, and then increased polarity by increasing

percentage of chloroform. Finally, the column was stipped with methanol. The eluted solution was collected approximately 500 mL. for each fraction. Each one was monitored by using TLC technique and the equivalent fractions were combined. The results of separation are presented in Table 2.6-2.12.

Table 2.6 The results of separation Fraction 1A of F147 variety

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-3	colourless oil (Sub. 1)	0.15
	4-28	pink solid (Sub.2,4)	4.35
5% $CHCl_3$-hexane	29-30		
10% $CHCl_3$-hexane	31-52	yellow solid (Sub.4,5)	2.29
20% $CHCl_3$-hexane	53-54		
	55-66		
30% $CHCl_3$-hexane	67-79	yellow solid (Sub.4)	0.78
	80		
50% $CHCl_3$-hexane	81-96	yellow solid (Sub.4)	1.34
70% $CHCl_3$-hexane	97-102		
	103-115	green solid	0.41
$CHCl_3$	116-130	green solid	0.40
10% MeOH-$CHCl_3$	131-135		
50% MeOH-$CHCl_3$	136-137	dark green solid	0.72
MeOH	138-139		

Table 2.7 The results of separation Fraction 1A of F153 variety

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1	colourless oil (Sub.1)	0.04
	2	yellow oil	trace
	3-15	colourless solid	
5% $CHCl_3$-hexane	16-18	(Sub.2)	3.12
	19-30		
10% $CHCl_3$-hexane	31-45	pink solid (Sub.4,5)	3.27
20% $CHCl_3$-hexane	46-63		
	64-68	green solid (Sub.4)	0.3
	69-73		
30% $CHCl_3$-hexane	74-80	green solid (sub.4)	0.51
	81-88		
50% $CHCl_3$-hexane	89-95	green solid (sub.4)	0.48
	96-100		
70% $CHCl_3$-hexane	101-110	green solid	4.51
$CHCl_3$	111-120		
10% MeOH-$CHCl_3$	121		
	122		
30% MeOH-$CHCl_3$	123-124	green solid	0.15
MeOH	125	-	-

Table 2.8 The results of separation Fraction 1A of Q83 variety

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1	colourless oil (Sub.1)	0.13
	2-16		
5% $CHCl_3$-hexane	17-30	colourless solid	0.92
10% $CHCl_3$-hexane	31-33	(Sub.2,4,5)	
	34-46		
20% $CHCl_3$-hexane	47-50	yellow solid (Sub.2,4)	2.16
	51-55	yellow solid (Sub.4)	0.18
	56-60		
30% $CHCl_3$-hexane	61-67	yellow solid (sub.4)	0.53
	68-70		
50% $CHCl_3$-hexane	71-85	yellow solid (sub.4)	0.93
70% $CHCl_3$-hexane	86-96		
$CHCl_3$	97-105	green solid (sub.4)	0.52
	106		
10% MeOH-$CHCl_3$	107-109	brown-green solid	0.46
	110		
50% MeOH-$CHCl_3$	111-112	-	-
MeOH	113-114		

Table 2.9 The results of separation Fraction 1B of filter cake from Mitr Phol Factory

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-2	colourless oil (Sub.1)	0.23
	3-12	pale yellow solid	
5% $CHCl_3$-hexane	13-23	(Sub.2,4,5)	1.67
	24-28	pale yellow solid	
10% $CHCl_3$-hexane	29-39	(Sub.4)	2.16
	40-45	pale yellow solid	
20% $CHCl_3$-hexane	46-61	with needle crystals	1.16
30% $CHCl_3$-hexane	62-65	(Sub.3,4)	
	66-80	pale yellow solid(Sub.4)	1.00
	81		
50% $CHCl_3$-hexane	82-96	pale yellow solid	0.99
70% $CHCl_3$-hexane	97-98	(Sub.4)	
	99-111		
$CHCl_3$	112-127	yellow solid	2.25
10% MeOH-$CHCl_3$	128-129		
	130-138	yellow solid	0.24
	139	-	-
30% MeOH-$CHCl_3$	140-143	-	-
50% MeOH-$CHCl_3$	144-145	-	-
MeOH	146-147	-	-

Table 2.10 The results of separation Fraction 1B of filter cake from Mitr Siam Factory

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-4	yellow oil (Sub.1)	0.21
	5-11		
5% $CHCl_3$-hexane	12-25	yellow solid(Sub.4,5	1.71
10% $CHCl_3$-hexane	26-39	yellow solid (Sub.4)	2.36
	40	yellow solid with	
20% $CHCl_3$-hexane	41-55	needle crystals	1.21
30% $CHCl_3$-hexane	56-60	(Sub.3,4)	
	61-69		
50% $CHCl_3$-hexane	70-84		
70% $CHCl_3$-hexane	85-100	green solid (Sub.4)	0.27
$CHCl_3$	100-114		
10% MeOH-$CHCl_3$	115		
	116-120	brown-green solid	4.00
30% MeOH-$CHCl_3$	121-122	-	-
50% MeOH-$CHCl_3$	123-124	-	-
MeOH	125	-	-

Table 2.11 The results of separation Fraction 1B of filter cake from Khumphawapi Factory

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-3	pale yellow solid (Sub.1)	0.08
	4-10	pale yellow solid (Sub.2,5)	0.74
	11-32		
5% $CHCl_3$-hexane	33-46	yellow solid (Sub.4)	0.58
10% $CHCl_3$-hexane	47-56		
	57-63		
20% $CHCl_3$-hexane	64-112		
30% $CHCl_3$-hexane	113-122	yellow solid (Sub.4)	0.32
50% $CHCl_3$-hexane	123-134		
	135-143	yellow solid	
70% $CHCl_3$-hexane	144-149	(Sub.4)	2.49
	150-159		
$CHCl_3$	160-170	yellow solid (Sub.4)	1.21
5% MeOH-$CHCl_3$	171-174		
10% MeOH-$CHCl_3$	175-178		
20% MeOH-$CHCl_3$	179-182		
30% MeOH-$CHCl_3$	183-186	thick brown solid	3.12
50% MeOH-$CHCl_3$	187-190		
70% MeOH-$CHCl_3$	191-194		
MeOH	195-198		

Table 2.12 The results of separation Fraction 1B of filter cake from United Farmer&Industry

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-14	colourless solid	
5% $CHCl_3$-hexane	15-28	(Sub.1,2,5)	2.16
	29-30		
10% $CHCl_3$-hexane	31-51	pale yellow solid	1.60
20% $CHCl_3$-hexane	52-62	(Sub.4)	
	63-66	pale yellow solid	
30% $CHCl_3$-hexane	67-83	with needle crystals	0.41
		(Sub.3,4)	
	84-89		
50% $CHCl_3$-hexane	90-113	pale yellow solid	2.21
70% $CHCl_3$-hexane	114-131	(Sub.4)	
	131-140		
$CHCl_3$	141-160		
10% MeOH-$CHCl_3$	162-168	brown and thick	3.39
30% MeOH-$CHCl_3$	169-173	solid	
50% MeOH-$CHCl_3$	174-178		
MeOH	179-180		
	181	-	-

2.9 Separation of Fraction 2

The crude chloroform extract, Fraction 2, of all varieties (6 g) was chromatographed on silica gel using column chromatography technique to separate into small fractions. It was eluted with n-hexane, n-hexane-chloroform, chloroform, and chloroform-methanol, respectively. 20-50 mL. of each fraction was collected and combined by TLC. The results were shown in Table 2.13.

Table 2.13 The results of separation Fraction 2 of all varieties

Eluents	Fraction No.	Remarks	Weights (g)
n-hexane	1-10		
25% $CHCl_3$-hexane	11-19	pale yellow oil	0.81
50% $CHCl_3$-hexane	20		
	21-29		
75% $CHCl_3$-hexane	30-38	yellow solid	0.48
$CHCl_3$	39-44	(Sub.6)	
	45-47	yellow needle	
25% MeOH-$CHCl_3$	48-49	crystals (cpd.7)	0.21
	50-54	yellow solid	0.50
50% MeOH-$CHCl_3$	55-71	(Sub.6)	
	72-110	pale yellow solid	0.38
	111-119	(Sub.6)	
75% MeOH-$CHCl_3$	120-160	thick dark solid	4.73
MeOH	161-177		

2.10 Purification and Properties of Substances Separated from Fraction 1A and 1B

2.10.1 Substance 1

The colourless amorphous product was obtained from the combination of initial n-hexane fractions (from Table 2.6-2.12). It was purified by recrystallization from acetone or ethyl acetate to afford substance 1 as bright white plates. The substance 1 showed spot on TLC plate at Rf value 0.82 (solvent: 50%$CHCl_3$-hexane). The quantities and melting points of substance 1 of each sample are shown in Table 2.14.

Table 2.14 The quantities and melting points of Substance 1 of each sample

Materials	Weight (g)	% by wt *	m.p.(°C)
1. Rind			
F147	0.10	1.0	62-64
F153	0.04	0.4	49-51
Q83	0.09	0.9	61-63
2. Filter cake			
Mitr Phol	0.02	0.2	60-63
Mitr Siam	0.03	0.3	63-65
Khumphawapi	0.05	0.5	52-55
United Farmer& Industry	0.11	1.1	55-59

* % weight of subs.1 by weight of crude extract (Fraction 1)

This substance was soluble in n-hexane, chloroform and slightly soluble in ethyl acetate and acetone. It gave negative results to Liebermann-Burchard's, 2,4-DNP, $FeCl_3$, and Br_2 in CCl_4.

The IR spectrum (Fig. 13) showed the absorption bands at ν_{max}^{KBr} (cm^{-1}): 2940, 2860 (C-H stretch.), 1470 (C-H bend.), and 720 (C-H rock.).

The GC analysis (Fig. 14a-14d and 15a-15e) indicated 11 - 13 peaks on gas chromatogram at various retention times that are shown in Table 2.15 and 2.16.

Table 2.15 The retention times of various peaks from gas chromatograms of substance 1 separated from rind

No. of peak	Retention times (min.)			
	Std.	F147	F153	Q83
1		1.44	-	1.43
2		1.70	1.70	1.69
3		2.03	2.04	2.03
4	2.48(C_{27})	2.47	2.46	2.45
5	3.00(C_{28})	2.99	3.00	2.97
6	3.65(C_{29})	3.67	3.65	3.63
7	4.50(C_{30})	4.50	4.50	4.47
8	5.53(C_{31})	5.55	5.53	5.50
9	6.81(C_{32})	6.85	6.85	6.80
10	8.43(C_{33})	8.52	8.46	8.40
11		10.52	10.56	10.50
12		13.02	13.03	12.93

condition: column OV-1 2%, col.temp. 280°C, inj.temp. 300°C, N_2 flow rate 50 mL/min.

Table 2.16 The retention times of various peaks from gas chromatograms of substance 1 separated from filter cake

No.	Retention times (min.)				
	Std.	Mitr Phol	Mitr Siam	Khumphawapi	United Farmer&Industry
1		2.90	2.91	2.85	2.90
2		3.60	3.63	3.55	3.61
3	4.59(C_{27})	4.50	4.54	4.45	4.54
4	5.77(C_{28})	5.69	5.72	5.62	5.71
5	7.27(C_{29})	7.17	7.24	7.10	7.24
6	9.17(C_{30})	9.07	9.16	9.00	9.14
7	11.73(C_{31})	11.54	11.66	11.47	11.67
8	14.77(C_{32})	14.60	14.72	14.54	14.74
9	18.77(C_{33})	18.74	18.96	18.57	18.94
10		23.60	23.69	23.57	23.87
11		30.20	30.42	29.97	30.41

condition: column OV-1 2%, col.temp. 260°C, inj.temp. 290°C

N_2 flow rate 48 mL/min.

2.10.2 Substance 2

Substance 2 was white amorphous substance which was recrystallized from n-hexane (from Table 2.6-2.12). It gave the Rf value 0.64 by using 50%$CHCl_3$-hexane as a developing solvent. It was soluble in chloroform and slightly soluble in hexane. The quantities and melting points of this substance from each sample are presented in Table 2.17.

Table 2.17 The quantities and melting points of substance 2 of each sample

Materials	Weight (g)	% by wt *	m.p.(°C)
1. Rind			
F147	0.26	2.6	64-67
F153	2.33	23.3	64-66
Q83	0.62	6.2	63-65
2. Filter cake			
Mitr Phol	0.24	2.4	78-79
Mitr Siam	-	-	-
Khumphawapi	0.07	0.7	69-73
United Farmer&Industry	0.01	0.1	61-66

* % weight of subs.2 by weight of crude extract (Fraction 1)

The IR spectrum (Fig.16) showed the absorption bands at ν_{max}^{KBr} (cm^{-1}): 2940, 2860 (C-H stretch.), 2750 (C-H stretch. of aldehyde), 1725 (-C=O of aldehyde),and 730, 720 (C-H rock.).

The ^{1}H NMR ($CDCl_3$) spectrum (Fig.17) showed the signals at chemical shift δ (ppm.): 9.75, 2.42, 1.55, 1.25, and 0.88.

The ^{13}C NMR spectrum (Fig.18) indicated the carbon signals at chemical shift δ (ppm.): 202.78, 43.94, 31.91, 29.69, 29.36, 29.20, 22.70, 22.18, and 14.08.

The GC-MS spectra (Fig.19 - 21) of Substance 2 from F153 variety showed 2 main peaks at retention time (t_R) 5.0 and 7.0 min. The first peak gave the important signals at m/e 408, 390, and 326. The second peak gave the important signals at m/e 436, 418, and 390.

The GC analysis (Fig. 22a - 22d and 23a - 23e) showed various peaks on gas chromatograms at retention times revealed in Table 2.18-2.20.

Table 2.18 The retention times of various peaks from gas chromatograms of substance 2 separated from rind

No. of peak	Retention times (min.)			
	Std.	F147	F153	Q83
1		3.40	-	-
2		5.34	5.35	-
3		-	7.12	-
4		8.49	8.42	-
5	11.30(C_{28})	11.35	11.35	11.38
6		13.55	13.42	-
7	18.10(C_{30})	18.29	18.22	18.28
8		21.55	-	-
9	29.10(C_{32})	-	29.22	29.28
10	46.63(C_{34})	-	46.89	46.88

condition: column SE-30, col.temp. 270°C, inj.temp. 300°C
N_2 flow rate 50 mL/min.

Table 2.19 The retention times of various peaks from gas chromatograms of substance 2 separated from filter cake

No.	Retention times (min.)		
	Std.	Mitr Phol	United Farmer&Industry
1		-	3.20
2		3.91	3.99
3		4.92	5.03
4		7.84	7.98
5	10.62(C_{28})	10.67	10.88
6		-	13.75
7	16.99(C_{30})	17.07	-
8		19.94	20.41
9	27.25(C_{32})	27.41	-
10		-	32.75
11	43.65(C_{34})	43.94	-

condition: column SE-30, col.temp. 270°C, inj.temp. 300°C

N_2 flow rate 60 mL/min.

Table 2.20 The retention times of various peaks from gas chromatograms of substance 2 separated from filter cake

No.	Retention times (min.)	
	Std.	Khumphawapi
1		2.88
2		3.59
3		4.48
4		5.64
5		7.11
6		8.94
7	11.96(C_{28})	12.14
8		14.18
9	19.13(C_{30})	-
10	30.59(C_{32})	-
11	48.93(C_{34})	-

condition: column SE-30, col.temp. 270°C, inj.temp. 300°C

N_2 flow rate 50 mL/min.

The semicarbarzone derivative of substance 2 (31)

The crude hexane extract (10 g) was dissolved in the mixture of 200 mL. benzene and 200 mL. ethanol and warmed on water bath. Added semicarbazide reagent, prepared from 1.5 g semicarbazide hydrochloride and 1.5 g sodium acetate in ethanol 50 mL. and filtered, the solution was cooled in ice bath. The solid crystallized out was filtered, washed with water and ethanol, and then dried, to yield Fraction a. The filtrate and washing solution were extracted with chloroform. The extract was dried, Fraction b. The fraction b was separated by silica gel column eluted with chloroform. The semicarbazone was recrystalized by chloroform which gave white amorphous substance, m.p. 108-112°C.

The IR spectrum (Fig.24) showed the important absorption bands at $\nu_{max}^{KBr}(cm^{-1})$: 3455 (N-H stretch.of 2°amide), 3280 and 3180 (N-H stretch. of amide), 2920 and 2855 (C-H stretch. of hydrocarbon), 1680 (-C=O stretch.of amide.band I), 1630 (N-H bend. of amide, band II), 1590 (N-H deformation of amine), 1510 (C=N stretch.), 1470 (C-H bend.), 1135 (C-N stretch. of 1°, 2° or 3°aliphatic amine), 770 and 630 (N-H wagging out-of-plane), 720 (C-H rock. of long chain > 4C).

2.10.3 Substance 3

Substance 3 was obtained from combination of fractions eluted from the silica gel column with 10%-30% $CHCl_3$-hexane (Table 2.9-2.12). The substance was recrystallized with the mixture of chloroform and hexane which gave bright white needle crystals. It gave the Rf value 0.13 with chloroform as solvent. The quantities and melting points of the substance from

each sample were shown in Table 2.21.

The substance was soluble in chloroform and slightly soluble in n-hexane. It gave a deep color with Liebermann-Burchard's and decolourized Br_2 in CCl_4 reagents.

The IR spectrum (Fig.25) showed the important absorption bands at ν_{max}^{KBr} (cm^{-1}): 3430 (O-H stretch.), 3030 (C-H strech. of $R_1R_2=CHR_3$), 1670-1630 (C=C), and 1050 (C-O).

The mass spectrum (Fig.26) gave the important fragmentation ion peaks at m/e (%rel int.) 414.0 (83.82), 412.0 (100.00), and 400.0 (58.69) (calcd. for $C_{29}H_{50}O$, $C_{29}H_{48}O$, and $C_{28}H_{48}O$, respectively) together with other vital peaks at m/e 396.0 (26.20), 382.0 (21.96), 329.0 (18.89), 303.0 (20.60), 273.0 (34.91), 255.0 (61.03), 213.0 (31.22).

The ^1H NMR ($CDCl_3$) spectrum (Fig.27) showed the significant signals at chemical shift δ (ppm.): 5.32 (2H,d, J=4.39 Hz, olefinic protons), 5.08 (1H,t,J=5.86 Hz, olefinic proton), 3.46 (m) and other signals around 2.30 to 0.70 ppm..

The ^{13}C NMR ($CDCl_3$) spectrum (Fig.28) illustrated the carbon signals at chemical shift δ(ppm.): 140.76, 138.27, 129.28, 121.64 (olefinic carbons), 71.69 (carbon attached to oxygen atom) together with signals around 56.79 to 11.83 ppm.

Table 2.21 The quantities and melting points of substance 3 of each sample

Materials	Weight (g)	% by wt *	m.p. (°C)
1. Rind			
F147	trace	trace	-
F153	trace	trace	-
Q83	trace	trace	-
2. Filter cake			
Mitr Phol	0.01	0.1	150-153
Mitr Siam	0.27	2.7	144-147
Khumphawapi	0.03	0.3	149-152
United Farmer&Industry	0.08	0.8	150-154

* % weight of subs.3 by weight of crude extract (Fraction 1)

The GC analysis (Fig.29a-29f) indicated three peaks on gas chromatogram at retention times shown in Table 2.22-2.23.

Table 2.22 The retention times of various peaks from gas chromatograms of substance 3 separated from filter cake*

No.	Retention times (min.)		
	std.	Mitr Phol	Mitr Siam
1 (choresterol)	13.40	-	-
2 (campesterol)	17.13	17.14	17.16
3 (stigmasterol)	18.20	18.12	18.33
4 (β-sitosterol)	20.86	20.82	20.89

Table 2.23 The retention times of various peaks from gas chromatograms of substance 3 separated from filter cake*

No.	Retention times (min.)		
	Std.	Khumphawapi	United Farmer&Industry
1 (chloresterol)	14.97	-	-
2 (campesterol)	19.17	19.26	19.38
3 (stigmasterol)	20.64	20.56	20.78
4 (β-sitosterol)	23.64	23.52	23.72

*condition: column OV-1 2%, col.temp. 255°C, inj.temp. 290°C

N_2 flow rate 40 mL/min.

2.10.4 Substance 4

Substance 4 was eluted from silica gel column by 5-70% chloroform-hexane (Table 2.6-2.12). It was recrystallized by hexane which gave white amorphous substance, having Rf value 0.24 using chloroform as solvent. It was soluble in hot chloroform. This substance gave negative results with Liebermann-Burchard's and Br_2 in CCl_4. The quantities and melting points of the substance from each sample are shown in Table 2.24.

Table 2.24 The quantities and melting points of substance 4 of each sample

Materials	Weight (g)	% by wt*	m.p.(°C)
1. Rind			
F147	4.63	46.3	76-80
F153	3.03	30.3	78-79
Q83	4.11	41.1	77-80
2. Filter cake			
Mitr Phol	3.12	31.2	76-80
Mitr Siam	2.75	27.5	78-79
Khumphawapi	3.17	31.7	76-80
United Farmer&Industry	2.93	29.3	77-79

* % weight of subs.4 by weight of crude extract (Fraction 1)

The IR spectrum (Fig.30) indicated the absorption bands at ν_{max}^{KBr} (cm^{-1}): 3320 (b,O-H stretch.), 2940 and 2860 (C-H stretch.), 1470 (C-H bend.), 1070 (C-O), and 730, 720 (C-H rock.).

The GC analysis (Fig.31a-31i) showed 7-11 peaks in gas chromatograms at retention times that are shown in Table 2.25-2.27.

Table 2.25 The retention times of various peaks from gas chromatograms of substance 4 separated from rind

No. of peak	Retention times (min.)			
	Std.	F147	F153	Q83
1		-	-	1.82
2		2.47	2.47	2.45
3		3.21	3.27	3.18
4		3.64	3.66	3.65
5		4.77	4.83	4.75
6	5.68(C_{28})	5.47	5.53	5.43
7	6.99(C_{29})	7.30	7.33	7.25
8	8.41(C_{30})	8.25	8.54	8.43
9	10.58(C_{31})	11.25	-	11.20
10	12.98(C_{32})	-	-	13.43
11	16.28(C_{33})	17.44	-	17.31
12	20.41(C_{34})	-	-	-

condition: column OV-1 2%, col.temp. 280°C, inj.temp. 300°, N_2 flow rate 50 mL/min.

Table 2.26 The retention times of various peaks from gas chromatograms of substance 4 separated from filter cake

No.	Retention times (min.)			
	Std.	Mitr Phol	Mitr Siam	United Farmer&Industry
1		2.47	2.50	2.51
2		3.22	3.25	3.26
3		3.64	3.64	3.68
4		4.78	4.80	4.81
5	5.68(C_{28})	5.48	5.50	5.51
6	6.99(C_{29})	7.31	7.34	7.35
7	8.41(C_{30})	8.46	8.49	8.53
8	10.58(C_{31})	11.26	11.32	11.33
9	12.98(C_{32})	13.14	13.19	13.30
10	16.28(C_{33})	17.41	17.49	17.46
11	20.14(C_{34})	-	-	-

condition: column OV-1 2%, col.temp. 280°C, inj.temp. 300°C

N_2 flow rate 50 mL/min.

Table 2.27 The retention times of various peaks from gas chromatograms of substance 4 separated from filter cake

No.	Retention times (min.)	
	Std.	Khumphawapi
1		3.68
2		4.24
3		5.64
4	6.57(C_{28})	6.38
5	8.19(C_{29})	-
6	9.90(C_{30})	9.94
7	12.50(C_{31})	-
8	15.40(C_{32})	15.54
9	19.34(C_{33})	-
10	24.04(C_{34})	-

condition: column OV-1 2%, col.temp. 280°C, inj.temp. 300°C
N_2 flow rate 60 mL/min.

2.10.5 Substance 5

Substance 5 was isolated from silica gel column by mixture of chloroform-hexane (Table 2.6-2.12). It was white amorphous by recrystallization with hexane. It was soluble in hot chloroform. The substance gave positive result with $NaHCO_3$. The quantities and melting points of each sample are shown in Table 2.28.

Table 2.28 The quantities and melting points of substance 5 of each sample

Materials	Weight (g)	% by wt *	m.p.(°C)
1. Rind			
F147	1.50	15.0	75-77
F153	0.10	1.0	74-75
Q83	0.19	1.9	78-80
2. Filter cake			
Mitr Phol	0.07	0.7	79-81
Mitr Siam	0.52	5.2	75-77
Khumphawapi	0.45	4.5	79-81
United Farmer&Industry	0.28	2.8	76-78

* % weight of subs.5 by weight of crude extract (Fraction 1)

The IR spectrum (Fig.32) illustrated important absorption peaks at ν_{max}^{KBr} (cm^{-1}): 2920 and 2860 (C-H stretch.), 1710 (-C=O), 1460 (C-H bend.), 1410 (O-H bend.), 1300 (C-O of alcohol), 940 (O-H out-of-plane bend.), and 730,720 (C-H rock.).

The methyl ester derivative of Substance 5 (32)

Substance 5 (0.1 g) was dissolved in small amount of anhydrous ether, cooled in ice, and added the ethereal solution of diazomethane (32) in small portions until the solution acquired a pale yellow colour. The solvent was evaporated and purified the product by crystallization. The methyl ester gave Rf value 0.55 (solvent: 50% $CHCl_3$-hexane).

The IR spectrum (Fig.33) indicated the important absorption bands at ν_{max}^{KBr} (cm^{-1}): 2920,2840 (C-H stretch.), 1740 (C=O stretch.), 1460 (C-H bend.), 1170 (C-O stretch.).

The GC-MS spectra (Fig.34-37) of methyl ester derivative of Substance 5 showed 3 main peaks. The first peak gave the molecular ion peak at m/e 410. The second one gave the molecular ion peak at m/e 438. The third one gave the molecular ion peak at m/e 466.

The GC analysis of methyl ester derivative of Substance 5 from each sample (Fig.38a-38g) showed 6-9 peaks on gas chromatograms at retention times shown in Table 2.29-2.31.

Table 2.29 The retention times of various peaks from gas chromatograms of methyl ester derivative of substance 5 separated from sugar cane rinds.

No.	Retention time (min.)		
	F147	F153	Q83
1	-	4.80	-
2	-	7.46	-
3	-	10.19	-
4	13.11	13.16	13.53
5	17.24	17.16	17.56
6	22.24	22.16	22.73
7	-	29.09	-
8	37.31	37.36	38.39
9	63.04	63.09	64.86
10	105.71	106.03	108.73

condition: column OV-1 2% 0.3 mm.x 2 m., col.temp. 250°C, inj.temp. 320°C, N_2 flow rate 55 ml/min.

Table 2.30 The retention times of various peaks from gas chromatograms of methyl ester derivative of substance 5 separated from filter cake

No.	retention time (min.)		
	Mitr Phol	Mitr Siam	United Farmer&Industry
1	8.10	7.73	7.67
2	10.46	10.55	10.47
3	13.50	13.58	13.52
4	17.53	17.71	17.62
5	23.03	23.58	22.82
6	29.70	29.98	-
7	38.50	38.91	38.36
8	50.23	50.65	-
9	64.90	65.31	64.89
10	-	84.78	-
11	108.90	109.85	108.89

condition: column OV-1 2% 0.3 mm.x 2 m., col.temp. 250°C, inj.temp. 320°C, N_2 flow rate 55 mL/min.

Table 2.31 The retention times of various peaks from gas chromatograms of methyl ester derivative of substance 5 separated from filter cake (Khumphawapi)

No.	retention time (min.)
1	12.52
2	16.25
3	20.95
4	27.42
5	35.15
6	59.22
7	99.48

condition: column OV-1 2% 0.3 mm.x 2 m., col.temp. 250°C, inj.temp. 320°C, N_2 flow rate 60 mL/min.

2.11 Purification and Properties of Substances Separated from Fraction 2

2.11.1 Substance 6

Substance 6 was eluted from silica gel column by 50% $CHCl_3$-hexane to $CHCl_3$ (Table 2.13). It was recrystallized by hexane to give white amorphous substance (0.23 g), the Rf value 0.24 using $CHCl_3$ as developing solvent. The melting point of this substance was 75-77°C and it dissolved in hot $CHCl_3$.

The IR spectrum (Fig.39) illustrated the absorption bands at ν_{max}^{KBr} (cm^{-1}): 3320 (b, O-H stretch.), 2940 and 2860 (C-H stretch.), 1470 (C-H bend.), 1070 (C-O) and 730, 720 (C-H rock.).

The GC analysis (Fig.40) showed 5 peaks in gas chromatograms at retention times shown in Table 2.32 .

Table 2.32 The retention times of various peaks from gas chropmatogram of substance 6

No.	retention time (min.)	
	Std. cpd.	Compound 6
1	-	3.56
2	-	4.42
3 (C_{28})	5.46	5.40
4 (C_{29})	6.78	-
5 (C_{30})	8.33	8.39
6 (C_{31})	10.44	-
7 (C_{32})	12.94	13.20
8 (C_{33})	16.21	-
9 (C_{34})	20.18	-

condition: column OV-1 2%, col.temp. 280°C, inj.temp. 300°C, N_2 flow rate 50 mL/min.

2.11.2 Compound 7

Compound 7 was eluted from silica gel column by $CHCl_3$-25% MeOH in $CHCl_3$ (Table 2.13). It was recrystallized with chloroform to give green needle crystals, m.p. 274-276 °C. This compound was soluble in acetone. It gave green solution with Liebermann-Burchard's reagent.

The IR spectrum (Fig. 41) of this compound indicated the important absorption bands at ν_{max}^{KBr} : 3350 (b,O-H stretch.), 2950 (C-H stretch.), 1655-1560, 1500-1435, 1355, 1330, 1260, 1165, 1115, 1025, and 830.

The UV spectrum (Fig.42) showed the λ_{max} at 345 nm.

The mass spectrum (Fig.43) showed the molecular ion peak, M^+, at m/e 330.

2.12 Purification and Properties of Compound 8 separated from Fraction 3

Compound 8 was the solid part separated from the crude chloroform extract solution of sugar cane rinds. It gave pale brown needle crystals by recrystallization with a mixture of chloroform and acetone. The compound gave the melting point 149-151°C and was soluble in DMSO and water. The yield of the compound is shown in Table 2.33. This compound gave positive results with $FeCl_3$.

Table 2.33 The quantities of compound 8 of sugar cane rinds

varieties	Weight of rinds	Weight of Cpd.8	% of Cpd.8
F147	98.60	0.40	0.4
F153	62.00	0.08	0.1

The elemental analysis found %C 49.54 and %H 4.18 (calcd. for $C_6H_6O_4$, C=50.7%, H=4.22%).

The IR spectrum (Fig.44) showed the important bands at $\nu_{max}^{KBr}(cm^{-1})$: 3280-3180 (b,O-H stretch.), 3100, 2940, 2860 (C-H stretch.), 1700,1665-1590, 1350, 1230, 1150, and 1080.

The UV (H_2O) spectrum (Fig.45) indicated the λ_{max} at 232 and 266 nm.

The mass spectrum (Fig.46) showed the molecular ion peak, M^+, at m/e 142 (calcd. for $C_6H_6O_4$) and other fragmentation ion peaks at m/e 113, 97, 85, 69, 57, and 39.

The ^1H NMR (DMSO-d_6+$CDCl_3$) spectrum (Fig.47) showed the signals at δ (ppm.): 8.48, 7.79, 6.45, 5.52, 4.34.

The ^{13}C NMR (DMSO-d_6+$CDCl_3$) spectrum (Fig.48) showed the signals at δ (ppm.): 174.55, 168.43, 145.89, 138.36, 109.70, and 60.19.