

CHAPTER IV

RESULTS AND DISCUSSION

From the hexane extract of dried powdered stem bark of *Croton oblongifolius* Roxb., three compounds were isolated. In addition, the presence of the steroids β -sitosterol, stigmasterol and campesterol, and long chain alcohols were detected.

To determine the chemical structures of each isolated compound, interpretation of their UV, IR, MS and NMR data, and subsequently comparison of these values with those reported in the literature could be employed.

1. Structure Determination of Compound COY4

Compound COY4 was obtained as colorless crystals (6.23 g) and had a melting point of 137-141°C.

The EI-MS of compound COY4 (Figure 2) exhibited a molecular ion at m/z 302, consistent with a molecular formula of $C_{20}H_{30}O_2$.

The FT-IR spectrum of compound COY4 (Figure 3) was summarized in Table 2.

Table 2. The IR absorption band assignments of compound COY4

Wave number (cm^{-1})	Tentative assignment
3500-2500	O-H stretching
1703	C=O stretching
1277	C-O stretching
1391	O-H bending
1598, 989	conjugated double bond
1646, 900	C=CH ₂

The FT-IR spectrum indicated the presence of a carboxylic group with a broad absorption band between 2500-3500 cm^{-1} and a strong absorption band at 1703 cm^{-1} due to the carboxylic acid carbonyl stretching. The vibration peaks at 1598 and 989 cm^{-1} corresponded to conjugated double bond. The absorption peaks at 1646 and 900 cm^{-1} indicated the presence of $\text{C}=\text{CH}_2$.

The ^1H -NMR spectrum (Figures 4-5) of compound COY4 showed two methyl groups attached to two quaternary carbons (0.76 and 1.16 ppm), one olefinic methyl group (1.78 ppm) and six olefinic protons (5.31, 6.79, 5.19, 5.09, 4.50 and 4.84 ppm).

The ^{13}C -NMR spectrum (Figure 6) showed 20 lines. The presence of carboxylic moiety was supported by ^{13}C resonance at δ_{C} 184.89 ppm. Six signals of olefinic carbons appeared at 147.8, 133.8, 131.8, 131.3, 113.3 and 108.3 ppm.

The DEPT-90 experiment (Figure 7), indicated the presence of two sp^2 methine carbons at 133.8 and 131.8 ppm and two saturated methine carbons at 57.2 and 49.3 ppm.

The DEPT-135 spectrum (Figure 8) showed eight methylene carbons at 113.3, 108.3, 38.1, 37.6, 37.0, 26.6, 21.9 and 18.4 ppm and three methyl carbons at 19.7, 16.4 and 14.7 ppm, indicating that the carbon signals at 147.8, 131.3, 47.5 and 38.8 ppm were quaternary carbons.

Compound COY4 showed a molecular ion with m/z 302 ($\text{C}_{20}\text{H}_{30}\text{O}_2$) which indicated DBE of 6. The data from 2D-NMR techniques, including COSY (Figure 17, Table 3), HMQC (Figures 9-11, Table 3) and HMBC (Figures 12-16, Table 3) were used to assist the interpretation of the chemical structure of compound COY4.

The protons directly attached to carbons in compound COY4 were assigned by examining the HMQC spectrum (Figures 9-11, Table 3).

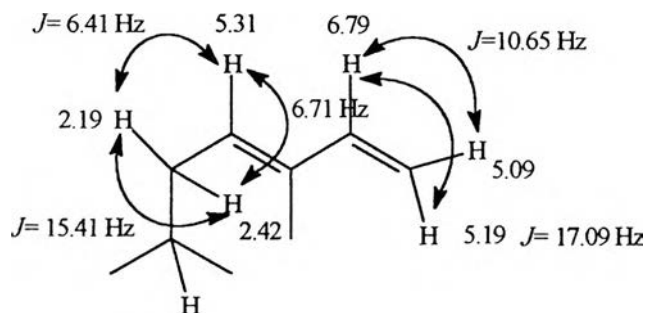
The long range ^1H - ^{13}C correlations were obtained by analysis of the HMBC spectrum (Figures 12-16, Table 3).

Table 3. The HMQC, HMBC and COSY spectral data of compound COY4

position	δ_C^a	δ_H	HMBC (H to C)	COSY
1	38.1 t	1.20 dt ($J = 5.7, 12.2$) 1.80 (overlap)		H-2 (1.62)
2	18.4 t	1.62 (2H, overlap)	C-3, C-4	H-1 (1.80), H-3 (1.63)
3	37.0 t	1.63 (overlap) 1.80 (overlap)	C-4, C-5	H-2 (1.62)
4	47.5 s	-	-	-
5	49.3 d	1.99 dd ($J = 2.9, 12.5$)	C-1, C-4, C-6, C-19, C-20, C-18	H-6 (1.36)
6	26.6 t	1.36 dddd ($J = 2.8, 5.3, 10.9, 21.1$) 1.48 dq ($J = 4.3, 13.4$)	C-7, C-8 C-5, C-7	H-5 (1.99), H-6 (1.36), H-7 (2.35) H-6 (1.36)
7	37.6 t	2.08 dt ($J = 3.6, 12.8$) 2.35 ddd ($J = 2.1, 4.3, 12.8$)	C-6, C-8	H-6 (1.36), H-7 (2.35) H-7 (2.08)
8	147.8 s	-	-	-
9	57.2 d	1.82 (overlap)	C-1, C-5, C-7, C-8, C-10	H-11 (2.19)
10	38.8 s	-	-	-
11	21.9 t	2.19 ddd ($J = 5.7, 11.4, 22.3$) 2.42 dd ($J = 6.5, 15.4$)	C-9, C-12, C-13 C-8, C-12, C-13	H-9 (1.82), H-12 (5.31) H-12 (5.31)
12	131.8 d	5.31 dd ($J = 6.7, 7.0$)		H-11 (2.42), H-11 (2.19)
13	131.3 s	-	-	-
14	133.8 d	6.79 dd ($J = 6.7, 10.7$)	C-12, C-13, C-16	H-15 (5.09), H-15 (5.19)
15	113.3 t	5.09 d ($J = 10.7$) 5.19 d ($J = 17.1$)	C-12, C-13 C-12, C-13, C-14	H-14 (6.79) H-14 (6.79)
16	19.7 q	1.78 s	C-12, C-13, C-14	
17	108.3 t	4.50 s 4.84 s	C-7, C-8, C-9 C-7, C-9	H-17 (4.84) H-17 (4.50)
18	184.9 s	-	-	-
19	16.4 q	1.16 s	C-3, C-4, C-18	
20	14.7 q	0.76 s	C-1, C-5, C-9, C-10	

^a Carbon type as determined by DEPT experiments

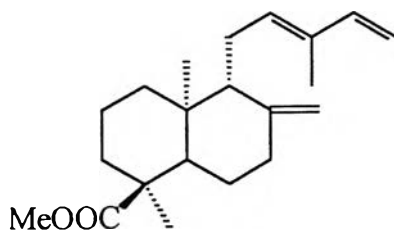
The shifts of δ_{H} 6.79 (H-14), 5.09 (d, H-15, $J=10.68$ Hz) and 5.19 (d, H-15, $J=17.09$ Hz) ppm and by following the HMBC spectrum indicated that this terminal double bond was conjugated with another one, therefore partial structure of compound COY4 could be obtained.



[134]

According to Table 3, the chemical structure of compound COY4 could be assigned.

By comparison with the previous reported value, the stereochemistry of compound COY4 was ascertained as an *ent*-labdane based on its negative optical rotation (Bevan, Ekong and Okogun, 1968) and the carbon chemical shifts of compound COY4 were similar to 12,13-*E*-ozic acid methyl ester [135] (Bohlmann, 1980) as shown in Table 4.

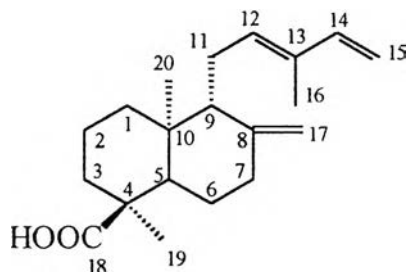


[135]

Table 4. Carbon chemical shift assignments of compound COY4 and 12,13*E*-ozic acid methyl ester

Carbon	Chemical shift (δ) ppm	
	Compound COY4	12,13 <i>E</i> -ozic acid methyl ester
1	38.1	37.7
2	18.4	18.6
3	37.0	38.3
4	47.5	47.8
5	49.3	57.5
6	26.6	26.7
7	37.6	37.1
8	147.8	147.5
9	57.2	49.9
10	38.8	39.1
11	21.9	22.1
12	131.8	131.3
13	131.3	131.8
14	133.8	133.9
15	113.3	113.2
16	19.7	19.7
17	108.3	108.2
18	184.9	179.0
19	16.4	16.7
20	14.7	14.7
OMe	-	51.7

Therefore, compound COY4 was identified as *ent*-8(17),12*E*,14-labdatrien-18-oic acid [136].



[136]

2. Structure Determination of Compound COY11

Compound COY11 was obtained as white needle-like crystals (0.06 g) and had a melting point of 83-86°C.

The IR spectrum of compound COY11 (Figure 19) was concluded as follows.

Table 5. The IR absorption band assignments of compound COY11

Wave number (cm ⁻¹)	Tentative assignment
1513, 885	furan ring
1646	C=C

According to the FT-IR spectrum, the peaks at 1513 and 885 cm⁻¹ suggested the presence of a furan ring system and the peak at 1646 revealed a double bond (C=C).

The molecular formula of compound COY11 was assigned to be C₂₀H₃₀O corresponded with EI-MS [M⁺] (m/z: 286) (Figure 18) which indicated 6 DBE. The presence of a furan moiety was supported by ion at m/z 95.

The ^1H -NMR spectrum (Figures 20-22) of compound COY11 showed three methyl groups attached to two quaternary carbons (0.87, 0.81 and 0.75 ppm) and one olefinic methyl group (1.95 ppm) and four olefinic protons (7.16, 6.10, 4.74 and 4.55 ppm).

The ^{13}C -NMR spectrum (Figure 23) indicated 20 signals. Six signals of olefinic carbons appeared at 150.9, 148.7, 139.3, 113.0, 112.8 and 106.8 ppm.

The DEPT-90 spectrum (Figure 24) showed the presence of two sp^2 methine carbons at 139.3 and 112.8 ppm and two saturated methine carbons at 55.4 and 54.4 ppm.

The DEPT-135 spectrum (Figure 25) revealed seven methylene carbons at 106.8, 42.1, 38.9, 38.1, 24.2, 21.5 and 19.4 ppm and four methyl carbons at 33.6, 21.8, 14.2 and 10.1 ppm which indicated that the carbon signals at 150.9, 148.7, 113.0, 39.5 and 33.6 ppm were quaternary carbons.

The spectroscopic data from 2D-NMR techniques, including COSY (Figures 31-33, Table 6), HMQC (Figures 26-27, Table 6) and HMBC (Figures 28-30, Table 6) were used to assist the elucidation of the chemical structure of compound COY11.

Table 6. The HMQC, HMBC and COSY spectral data of compound COY11

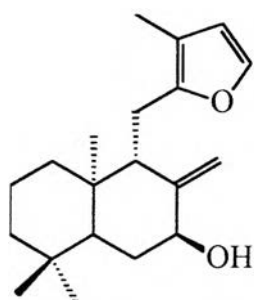
position	δ_C^a	δ_H	HMBC (H to C)	COSY
1	38.9 t	1.14 dt ($J = 3.9, 12.8$) 1.75 dtd ($J = 1.8, 5.5, 14.3$)	C-20	H-2 (1.57) H-2 (1.57)
2	19.4 t	1.48 pd ($J = 3.7, 14.3$) 1.57 tq ($J = 3.4, 13.6$)		H-1 (1.75), H-3 (1.39)
3	42.1 t	1.20 dt ($J = 4.3, 14.0$) 1.39 dtd ($J = 1.5, 3.2, 13.1$)	C-4, C-18	H-3 (1.39), H-2 (1.57) H-3 (1.20), H-2 (1.57)
4	33.6 s	-	-	-
5	55.4 d	1.18 dd ($J = 2.6, 12.7$)	C-4, C-6, C-9, C-18, C-19, C-20	H-6 (1.32)
6	24.2 t	1.32 dq ($J = 4.3, 2.8$) 1.71 pd ($J = 2.4, 12.5$)	C-7	H-7 (2.33), H-6 (1.71) H-6 (1.32)
7	38.1 t	2.00 dt ($J = 5.2, 13.1$) 2.33 ddd ($J = 2.4, 4.2, 12.9$)	C-6, C-8, C-17 C-8, C-9, C-17	H-6 (1.32), H-6 (1.71) H-6 (1.32), H-6 (1.71), H-7 (2.00)
8	148.7 s	-	-	-
9	54.4 d	2.28 dd ($J = 3.5, 10.4$)	C-8	H-11 (2.6), H-11 (2.71)
10	39.5 s	-	-	-
11	21.5 d	2.60 dd ($J = 10.4, 15.3$) 2.71 dd ($J = 3.4, 14.9$)	C-8, C-9, C-12 C-8, C-9, C-12	H-9 (2.28) H-9 (2.28)
12	150.9 s	-	-	-
13	113.0 s	-	-	-
14	112.8 d	6.10 d ($J = 1.8$)	C-12, C-15	H-15 (7.16)
15	139.3 d	7.16 d ($J = 1.8$)	C-12, C-14	H-14 (6.10)
16	10.1 q	1.95 s	C-12, C-13, C-14	
17	106.8	4.55 q ($J = 1.5$) 4.74 q ($J = 1.5$)	C-7, C-8, C-9 C-7, C-9	
18	21.8 q	0.81 s	C-3, C-4, C-5, C-19	
19	33.6 q	0.87 s	C-3, C-4, C-5, C-18	
20	14.2 q	0.75 s	C-1, C-5, C-9, C-10	

^a Carbon type as determined by DEPT experiments

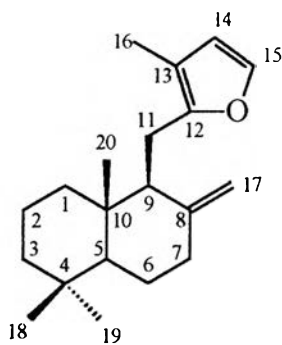
According to its spectroscopic data, the structure of compound COY11 could be assigned. Most of proton signals of compound COY11 were nearly to 7 β -hydroxypumiloxide (Bohlmann *et al.*,1982) as shown.

Table 7. Proton chemical shift assignments of compound COY11 and 7 β -hydroxypumiloxide [137]

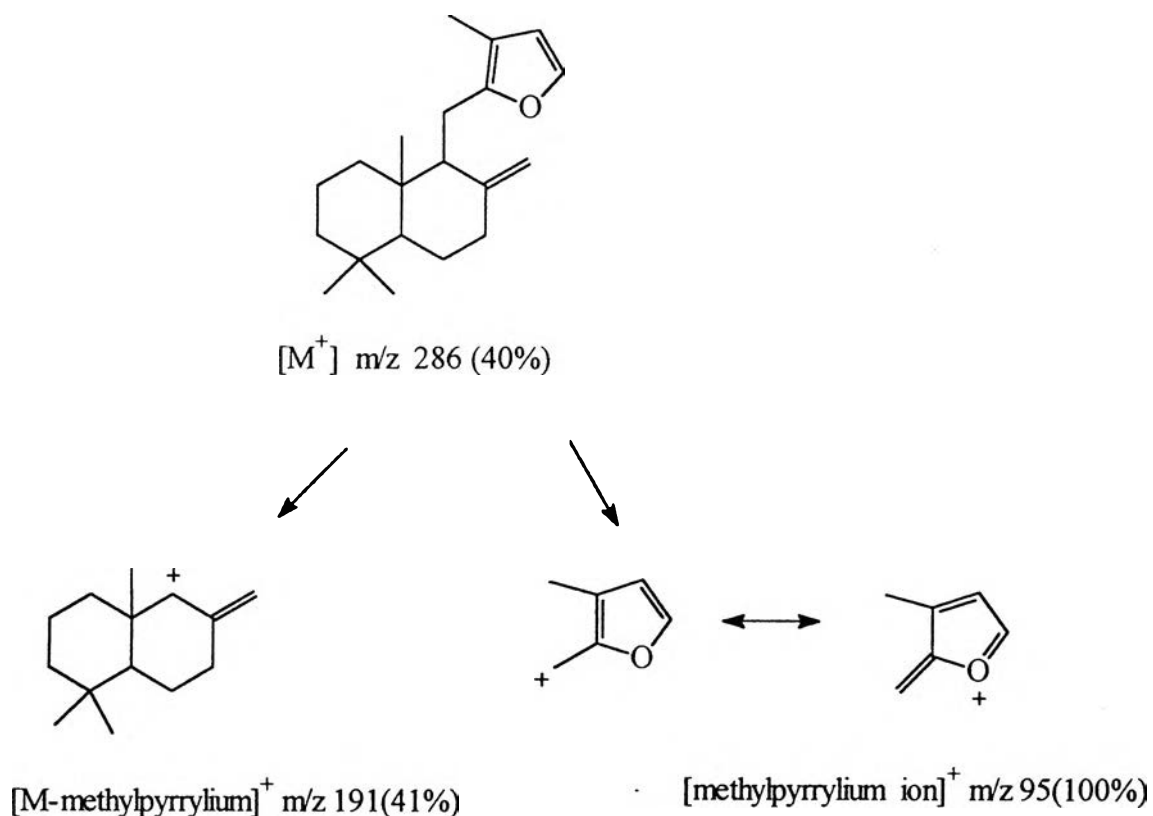
Position	Compound COY11	7 β -hydroxypumiloxide
1	1.15 dt, 1.75 dtd	} Overlapping multiplets
2	1.48 pd, 1.57 tq	
3	1.20 dt, 1.39 dtd	
4	-	
5	1.18 dd	
6	1.32 dq, 1.71pd	
7	2.0 dt, 2.33 ddd	4.33 dd (H-7 α)
8	-	-
9	2.28 br dd	2.83 br d
10	-	-
11	2.6 dd, 2.7 dd	2.77 dd, 2.58 dd
12	-	-
13	-	-
14	6.10 d	6.10 d
15	7.16 d	7.17 d
16	1.95 s	1.97 s
17	4.55 q, 4.74 q	4.97 br s, 4.69 br s
18	0.81 s	0.89 s
19	0.87 s	0.82 s
20	0.75 s	0.74 s

[137] 7 β -hydroxypumiloxide

It is reasonable to assume that compound COY11 belongs to the normal-labdane series, unlike *ent*-12,15-epoxy-8(17),12,14-labdatriene or pumiloxide, on the basis of the different signs of their specific rotations. Thus, compound COY11 was elucidated as 12,15-epoxy-8(17),12,14-labdatriene as shown below.



[138] 12,15-epoxy-8(17),12,14-labdatriene



Scheme 3. Proposed mass fragmentation of compound COY11

Therefore, labdane and *ent*-labdane were found in this study. In other cases, the presence of normal and *ent*-series of diterpenoids in one species were found such as a normal labdane zamoranic acid and a rearranged *ent*-labdane valparolone in *Halimium viscosum* (De Mendonca, *et al.*, 1997), a normal clerodane sonderianial and an *ent*-clerodane sonderianin in *Croton sonderianus* (Craveiro, 1982 and McChesney, 1989).

3. Structure Determination of Compound COY10

Compound COY10 was obtained as white needle-like crystals (0.49g) and had a melting point of 177-179°C.

The IR spectrum of compound COY10 (Figure 35) indicated the presence of a carboxylic group according to the broad absorption band between 3600-3200 cm^{-1} and a strong absorption band at 1694 cm^{-1} due to the carboxylic acid carbonyl stretching.

Table 8. The IR absorption band assignments of compound COY10

Wave number (cm^{-1})	Tentative assignment
3600-3200	O-H stretching
1694	C=O stretching
1264	C-O stretching
1468	O-H bending
1660, 870	C=CH ₂

The molecular formula of compound COY10 was proposed to be $\text{C}_{20}\text{H}_{30}\text{O}_2$ based on EI-MS [M^+] (m/z 302) (Figure 34) which indicated the DBE of 6. The presence of carboxylic acid moiety was confirmed by the ^{13}C resonance at δ_{C} 183.8 ppm.

The ^1H -NMR spectrum (Figures 36-37) of compound COY10 showed two methyl groups attached to two quaternary carbons (1.22 and 0.93 ppm) and two olefinic protons (4.78 and 4.72 ppm).

The ^{13}C -NMR spectrum (Figure 38) showed 20 lines. The most downfield signal at δ_{C} 183.8 ppm supported the presence of carboxylic moiety. Two signals of olefinic carbons appeared at 102.9 and 155.9 ppm.

The DEPT-90 spectrum (Figure 39), indicated the presence of two saturated methine carbons at 57.1, 55.1 and 44.2 ppm.

The DEPT-135 spectrum (Figure 40), showed nine methylene carbons at 48.9, 41.3, 40.7, 39.7, 37.8, 33.1, 21.8, 19.1 and 18.4 ppm and two methyl carbons at 28.9 and 15.6 ppm indicating that the carbon signals at 155.9, 43.8, 43.7 and 39.7 ppm were quaternary carbons.

The data from 2D-NMR techniques, including COSY (Figures 46-48, Table 9), HMQC (Figures 41-42, Table 9) and HMBC (Figures 43-45, Table 9) were employed to assist the interpretation of the chemical structure of compound COY10.

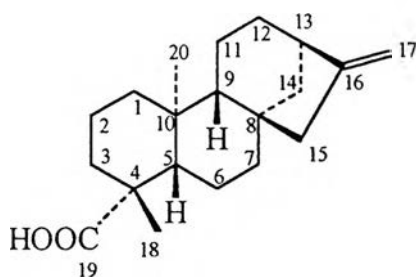
Table 9. The HMQC, HMBC and COSY spectral data of compound COY10

position	δ_C^a	δ_H	HMBC (H to C)	COSY
1	40.7 t	0.8 (overlap) 1.86 (overlap)	C-9, C-10, C-20	H-1 (1.86) H-1 (0.8)
2	19.1 t	(2H, overlap)		
3	37.8 t	0.98 (overlap) 2.14 br d ($J = 14.9$)	C-2, C-4, C-18	
4	43.7 s	-	-	-
5	57.1 d	1.06 (overlap)	C-4, C-10, C-18, C-19	H-6 (1.88)
6	18.4 t	1.60 (overlap) 1.88 (overlap)	C-6, C-8	H-7 (1.5) H-5 (1.06)
7	41.3 t	1.50 (2H, overlap)	C-8, C-14	H-6 (1.60)
8	43.8 s	-	-	-
9	55.1 d	1.04 (overlap)	C-8, C-11, C-12	H-11 (1.81)
10	33.7 s	-	-	-
11	21.8 t	0.93 (overlap) 1.31 (overlap)	C-13	H-9 (1.04), H-12 (1.45)
12	33.1 t	1.45 (overlap) 1.60 (overlap)	C-11	H-11(1.81), H-12 (1.60) H-12 (1.45)
13	44.2 d	2.62 dd ($J = 3.9, 4.3$)	C-8, C-15, C-16	H-12 (1.45), H-14 (1.11)
14	39.7 t	1.11 dd ($J = 4.9, 10.1$) 0.97 dd ($J = 1.8, 11.3$)	C-8, C-9, C-12 C-12, C-15, C-16	H-14 (1.97), H-13 (2.62) H-14 (1.11)
15	48.9 t	1.03 s	C-8, C-9, C-16	
16	155.9 s	-	-	-
17	102.9 t	4.72 br s 4.78 br s	C-13, C-15, C-16 C-13, C-15	
18	28.9 q	1.22 s	C-3, C-4, C-5, C-19	
19	183.8 s	-	-	-
20	15.6 q	0.93 s	C-1, C-5, C-9, C-10	

^a Carbon type as determined by DEPT experiments

The optical rotation of compound COY10 indicated that an *ent*-kaurane was more likely, if the rotation was compared with kaurenoic acid.

When compared with the previous reported *ent*-kaur-16-en-19-oic acid (Hutchison *et al.*,1984), the carbon (as shown in Table 10) chemical shifts were similar. Therefore, compound COY10 was identified as *ent*-kaur-16-en-19-oic acid or kaurenoic acid.



[139] *ent*-kaur-16-en-19-oic acid

Table 10. Carbon chemical shift assignments of compound COY10 and *ent*-kaur-16-en-19-oic acid

Carbon	Chemical shift (δ) ppm	
	Compound COY10	<i>ent</i> -kaur-16-en-19-oic acid
1	40.7	40.7
2	19.1	19.1
3	37.8	37.8
4	43.7	43.8
5	57.1	57.1
6	21.8	21.8
7	41.3	41.3
8	43.8	44.2
9	55.1	55.1
10	39.7	39.7
11	18.4	18.4
12	33.1	33.1
13	44.2	43.9
14	39.7	39.7
15	48.9	49.0
16	155.9	155.9
17	102.9	103.0
18	28.9	29.0
19	183.8	184.6
20	15.6	15.6

4. Structure Determination of Isolate COY6

Figure 50 showed the IR spectrum of isolate COY6 and the absorption bands were assigned as shown in the table below.

Table 11. The IR absorption band assignments of isolate COY6

Wave number (cm ⁻¹)	Tentative assignment
3600-3100	O-H stretching
2936	C-H stretching
1464	C-H bending of -CH ₂ -, and asymmetric C-H bending of -CH ₃
1381	symmetric C-H bending of -CH ₃
1060	C-O bending
970	C-H out of plane bending

The ¹H-NMR spectrum (Figure 51) exhibited the signals of protons attached to sp³ carbons between 0.6-2.35 ppm. The signal at 3.5 ppm. was a signal of a proton attached to a carbon bearing hydroxyl group. Two signals of olefinic protons were observed at 5.10 and 5.35 ppm.

Isolate COY6 should be steroid because of the similarity of its proton spectrum to those of the previously reported steroids (Surachetpan, 1996).

Then, this compound was analyzed by GC technique. By means of comparing its retention time with standard steroids, isolate COY6 was shown to be a mixture of campesterol, stigmasterol and β-sitosterol. Table 12 shows the retention times of each component of isolate COY6.

Table 12. The retention time of isolate COY6

Compound	Retention time (min.)	% Relative Amount
campesterol	18.99	6.31
stigmasterol	20.21	37.69
β -sitosterol	22.93	55.99

The mass spectrum of isolate COY6 (Figure 49) showed the molecular ion peaks at m/z 414, 412 and 400 which corresponded to the molecular ion peaks of β -sitosterol ($C_{29}H_{50}O$) [18], stigmasterol ($C_{29}H_{48}O$) [33] and campesterol ($C_{29}H_{48}O$) [89] respectively.

Thus, isolate COY6 was assigned to be a mixture of the steroids: β -sitosterol, stigmasterol and campesterol.

5. Structure Determination of Isolate COY8

According to Figure 54, the IR absorption band assignment was shown as follow.

Table 13. The IR absorption band assignments of isolate COY8

Wave number (cm^{-1})	Tentative assignment
3600-3000	O-H stretching
2938-2866	C-H stretching
1463	C-H bending of $-CH_2-$, and asymmetric C-H bending of $-CH_3$
1379	symmetric C-H bending of $-CH_3$
1027	C-O bending

The $^1\text{H-NMR}$ spectrum (Figure 55) showed signals in olefinic region, possibly due to other unsaturated impurity.

This isolate was identified by means of GC analysis compared with authentic long chain alcohols (Figures 56-57).

Comparison to gas chromatogram of standard long chain alcohols and using calibration curve of log retention time with number of carbon atoms (Figure 58, Table 14) revealed that isolate COY8 was a mixture of C_{19} - C_{26} long chain alcohols as followed.

	Relative amount (%)
$\text{C}_{19}\text{H}_{39}\text{OH}$	2.31
$\text{C}_{20}\text{H}_{41}\text{OH}$	6.01
$\text{C}_{21}\text{H}_{43}\text{OH}$	10.74
$\text{C}_{22}\text{H}_{45}\text{OH}$	41.47
$\text{C}_{23}\text{H}_{47}\text{OH}$	12.55
$\text{C}_{24}\text{H}_{49}\text{OH}$	10.44
$\text{C}_{25}\text{H}_{51}\text{OH}$	9.06
$\text{C}_{26}\text{H}_{53}\text{OH}$	7.41

Table 14. The relationship between retention time and the number of carbon atoms of the authentic long chain alcohols and isolate COY8

Chemical compound	Retention time (Rt) (minute)	log Rt	No. of carbon atoms
tetradecanol	0.92	-0.0362	14
hexadecanol	1.24	0.0934	16
octadecanol	1.81	0.2577	18
eicosanol	2.83	0.4518	20
docosanol	4.54	0.6570	22
COY8	2.18	0.3385	19
	2.73	0.4362	20
	3.44	0.5366	21
	4.57	0.6599	22
	5.60	0.7482	23
	7.19	0.8567	24
	9.30	0.9685	25
	12.08	1.0821	26

Therefore, isolate COY8 was identified as the mixture of C₁₉₋₂₆ long chain alcohols.