CHAPTER IV

RESULTS AND DISCUSSION

4.1 Structural elucidation of the isolated compounds from the stem barks of *Croton oblongifolius* Roxb.

Table 4: **The results of separation of hexane crude extract by column chromatography.**

Compounds	Physical appearance	% wt. by wt.
1	clear crystal	0.0083
2	white solid	0.0167
3	white solid	0.0027
4	white solid	0.0050

4.1.1 Structural elucidation of compound 1

The IR spectrum of compound 1 is shown in Fig. **10** and absorption peaks were assigned as shown in Table **5**. Its important absorption bands appeared at 3200 cm^{-1} (O-H stretching vibration of alcohol), 2940, 2909, and 2868 cm^{-1} (C-H stretching vibration), 1634 cm^{-1} (C=C stretching vibration of alkene).

The ^{1}H-NMR spectrum (Fig. **11**) of compound 1 indicated that it possesses five methyl groups attaching to quaternary carbon at δ_H 0.72, 0.78, 0.85, 1.13, and 1.22 ppm, there are three olefinic protons at δ_H 4.91, 4.97, and 6.01 ppm.

Table 5: **The IR absorption band assignment of compound 1**

Wave number (cm^{-1})	Intensity	Tentative Assignment
3200	broad	O-H stretching vibration of alcohol
2971, 2930, 2873	strong	O-H stretching vibration of -CH_3, -CH_2
1634	strong	C=C stretching vibration of alkene

The ^{13}C-NMR spectrum (Fig. **12**) showed 20 lines. There were two signals of olefinic carbons appeared at 147.7 and 109.5 ppm

DEPT 90 spectrum (Fig. **13**), indicated the presence of a sp^2 methine carbons at 147.7 ppm and two saturated methine at 58.5 and 56.5 ppm. The DEPT 135 (Fig. **13**) showed eight methylene carbons at 109.5, 43.1, 42.2, 39.3, 34.8, 29.7, 19.8 and 18.6 ppm and five methyl carbons at 37.3, 33.3, 23.9, 21.2 and 15.8 ppm (Table **6**) which indicated that the carbon signals at 76.0, 73.2, 36.8 and 33.2 ppm. were quaternary carbons.

The compound 1 's molecular formular was established as $C_{20}H_{36}O_2$ on the basis of ^{1}H - ^{13}CNMR, MS data, which was confirmed by observing molecular ion at m/z 308. A degree of unsaturation of three was defined from its molecular formular ($C_{20}H_{36}O_2$), thus,this compound must consist of two rings and one double bond. From these data, the compound 1 could match to labdane diterpene skeleton, therefore the compound 1 should be labdane diterpene compound.

The comparison of ^{13}C-NMR chemical shifts of compound 1 and 13-Episclareol, [19, 21] labda-14-ene-8,13(S)-diol, [19, 20, 21] was shown in Table **6**.

Table 6: ^{13}C-NMR chemical shifts of compound 1 and Sclareol

Position	Compound 1	Sclareol
1	39.3 (t)	39.4 (t)
2	18.6 (t)	18.2 (t)
3	42.1 (t)	41.7 (t)
4	33.3 (s)	33.0 (s)
5	56.4 (d)	55.8 (d)
6	19.8 (t)	18.7 (t)
7	43.1 (t)	44.7 (t)
8	73.2 (s)	74.5 (s)
9	58.5 (d)	61.5 (d)
10	36.8 (s)	38.9 (s)
11	18.6 (t)	20.1 (t)
12	34.8 (t)	43.8 (t)
13	73.2 (s)	73.1 (s)
14	147.7 (d)	146.6 (d)
15	109.5 (t)	110.5 (t)
16	37.3 (q)	26.0 (q)
17	23.9 (q)	23.9 (q)
18	33.2 (q)	33.0 (q)
19	21.2 (q)	21.2 (q)
20	15.8 (q)	15.1 (q)

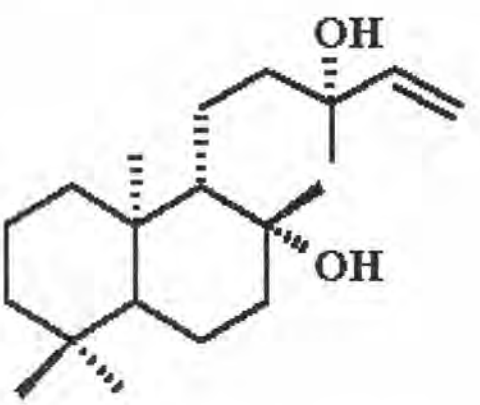


Figure 3: Structure of compound 1

4.1.2 Structure elucidation of compound 2

The IR spectrum of compound 2 was shown in Fig. **14** and the absorption peaks were assigned in Table **7**. Its important absorption bands appeared at 2940, 2909, and 2868 cm^{-1} (C-H stretching vibration), 1737 cm^{-1} (C=O stretching vibration of carbonyl group), 1204 cm^{-1} (C-O stretching vibration of ester), and 1025 cm^{-1} (C-O stretching vibration of alcohol).

Table **7: The IR absorption bands assignment of compound 2**

Wave number (cm^{-1})	Intensity	Tentative assignment
2940, 2909, 2868	medium	C-H stretching vibration of -CH_3, -CH_2
1737	strong	C=O stretching vibration of carbonyl group
1204	medium	C-O stretching vibration of ester
1025	strong	C-O stretching vibration of alcohol

This compound contains two methyls, five methylenes, ten methines and four quaternary carbon atoms. The molecule formular of compound2 was established as $C_{21}H_{26}O_6$ on the basis of ^1H - ^{13}CNMR, MS data, which was confirmed by observing molecular ion at m/z 374 in the EI mass spectrum (Fig. **15**). The furanic and ester absorptions were shown in IR spectrum. Therefore, the compound2 is a clerodane derivative which lack of hydroxyl groups and a lactone ring. In the other hand, the ^1H and ^{13}C NMR spectra (Table **8**) showed that it is a neo-furoclerodane diterpenoid with a C-20 $\rightarrow$ C-12 bridge.They revealed a -CO-OMe group corresponding to C-18 - C-21, a secondary methyl group (δ_H 0.9, 3H, *d*, J = 6.5 Hz; δ_C 16.94 q) attributable to Me-17 and surprisingly two acetal groups with carbons at δ 104.4 (C-19) and 100.7 (C-20) involving also the C-3 and C-12 in a struc-

ture moiety such as CH-O-CH-O-CH-O-CH which accounted for the C-3, C-19, C-20 and C-12 tertiary carbons. It followed that the relative configurations for all nine asymmetric centres of compound 2 was established by 2D NMR experiments (COSY and NOESY). ^{1}H-^{1}H COSY showed the $^{3}\hat{J}$ interaction between H-3β (δ 4.48) and H-4α (δ 2.83), H-12 (δ 5.31) and H_A-11 (δ 2.22), H_A-11 and H-10β (δ 2.40) and a *W* type range coupling between the equatorial protons H-2α and H-4α and between H-19 (δ 5.11) and H-10. ^{1}H-^{1}H NOESY revealed interactions between H-4α and H-19, H-19 and H-20 (δ 5.26), H-20 and Me-17α, H-12 and the equatorial proton H-1β (δ 2.30) and Me-17 and H-14 (δ 6.37). Therefore the A/B ring junction of the decalin part of compound 2 is *trans*; moreover the chemical shift and coupling of the proton H-10 proton required a *trans* arrangement of H-10 and the C-9 - C-20 bonds.

It could be concluded that compound2 exhibited the ^{13}C-NMR chemical shifts identical to Crovatin [17]. The comparation of ^{13}C-NMR chemical shifts between compound 2 and Crovatin,methyl *ent*-(18R,10β)-3, 19S:15,16:12S,20R:19,20-tetra epoxy-cleroda-13(16), 14-dien-18β-oate, was shown in Table 8.

Table 8: ^{13}C-NMR chemical shifts of compound 2 and Crovatin

Carbon	Chemical shifts (ppm)	
	Compound 2	Crovatin
1	20.2	20.2 (t)
2	26.5	26.5 (t)
3	75.7	75.8 (d)
4	54.0	54.0 (d)
5	44.3	44.3 (s)
6	30.5	30.5 (t)
7	31.5	31.5 (t)
8	37.4	37.5 (d)
9	50.3	50.4 (s)
10	38.9	38.9 (d)
11	38.6	38.6 (t)
12	74.9	75.0 (dt)
13	127.2	127.2 (s)
14	108.6	108.7 (d)
15	143.4	143.5 (d)
16	139.3	139.3 (d)
17	16.9	16.9 (q)
18	170.2	170.2
19	104.5	104.4 (d)
20	100.7	100.7 (d)
21 (OMe)	51.6	51.7 (q)

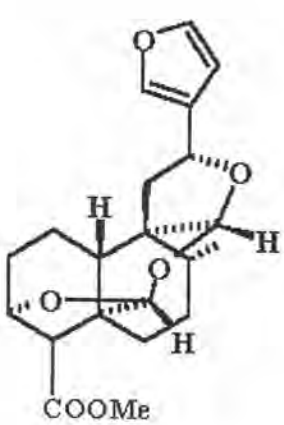


Figure 4: Structure of compound 2

4.1.3 Structure elucidation of compound 3

The IR spectrum of compound 3 (Fig. **19**) is summarized in Table **9**. Its important absorption bands appeared at 3500-3100 cm^{-1} (O-H stretching vibration of alcohol), 2955, 2906, and 2877 cm^{-1} (C-H stretching vibration), 1731 cm^{-1} (C=O stretching vibration of carbonyl group), and 1030 cm^{-1} (C-O stretching vibration of alcohol).

Table **9: The IR absorption bands assignment of compound 3**

Wave number	Intensity	Tentative assignment
3500-3100	medium	O-H stretching vibration of alcohol
2955, 2906, 2877	medium	C-H stretching vibration of -CH_3, -CH_2
1731	strong	C=O stretching vibration of carbonyl group
1030	strong	C-O stretching vibration of alcohol

The ^{1}H-NMR (Fig. **21**) spectrum of compound3 showed one methyl group attaching to quaternary carbon at δ_H 0.99 ppm and one methyl ester at δ_H 3.78 ppm, there are eight olefinic protons at δ_H 7.85, 7.85, 7.53, 7.53, 7.42, 7.36, 7.31 and 6.26 ppm.

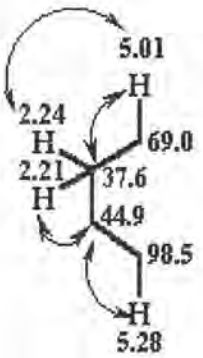
The ^{13}C-NMR spectrum (Fig. **22**) and DEPT (Fig. **23**) experiments revealed the presence of 28 nonequivalent carbons. The DEPT-90 and DEPT-135 spectrums showed the presence of ten signals of olefinic carbons appeared at δ_C 143.7, 138.7, 133.2, 129.8, 129.8, 129.6, 128.9, 128.3, 128.3 and 108.3 ppm. The signals of carbonyl group of ketone presented at δ_C 208.3 ppm. There were five sp^3 signals at δ_C 24.3, 28.8, 29.3, 35.8 and 37.6 ppm and one methyl ester at δ_C 53.9 ppm. From DEPT-90 and DEPT-135 spectrum indicated that the carbon signals at δ_C

202.3, 171.6, 165.1, 129.6, 128.3, 80.9, 46.3 and 44.9 ppm were quaternary carbons.

The molecular formula of compound 3 was assigned to be $C_{28}H_{30}O_9$, according to microanalysis and EI MS [M^+] at m/z 510 (Fig. **20**), which indicated a degree of unsaturation of fourteen. Therefore, the compound 3 must consist of six rings (two of them were furan ring and benzene ring) , five double bonds and three carbonyl groups.

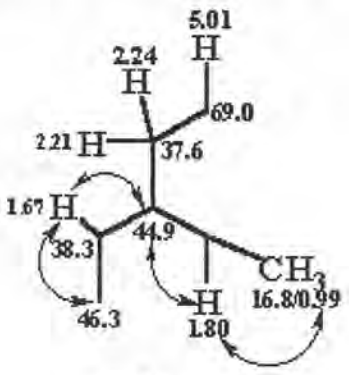
The information from 2D-NMR techniques, COSY correlations (Fig. **26**, Table **11**), HMQC correlation (Fig. **24**, Table **10**), HMBC correlation (Fig. **25**,Table **11**) were used to assist in the interpretation of the structure of compound 3. By comparison with ^{13}C-NMR spectrum (Fig. **22**,Table **8**) of crovatin defined that structure of compound 3 was quite similar to that of crovatin compound except for the C-19 position of compound 3 was broken down and attached with benzoyl group. In the other hand, carbon at the third position was carbonyl group of ketone and there was one -OH group attached to carbon at C-4 position instead of -H in crovatin compound. Two-dimentional NMR techniques were used for assisting the structure assignment. The protons directly attached to the carbons in compound 3 were assigned by HMQC spectra (Fig. **24**, Table **10**).

Crucial long-range ^{1}H-^{13}C correlations were obtained by HMBC correlations (Fig. **25**), the proton at 5.01 ppm was coupled with carbon at 37.6 ppm and the proton at 2.21 ppm and 5.28 ppm were coupled with carbon at 44.9 ppm. The COSY correlations (Fig. **26**) showed that the proton at 5.01 ppm was coupled with the proton at 2.24 ppm. (see scheme 1)



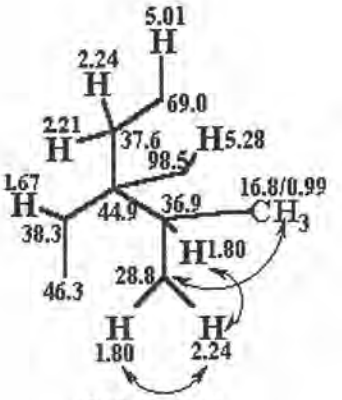
Scheme 1

The HMBC correlations showed that the proton at 1.67 ppm was coupled with the carbon at 44.9 ppm and 46.3 ppm. The proton at 1.80 ppm was coupled with carbon at 44.9 ppm and the COSY correlations showed that the proton at 1.80 ppm was coupled with the proton of methyl at 0.99 ppm. (see scheme 2)



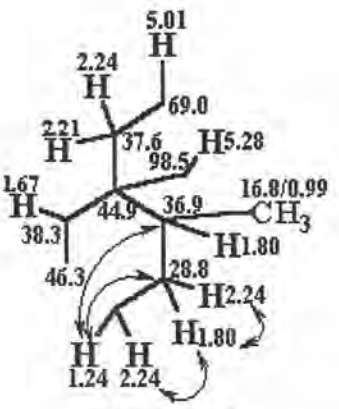
Scheme 2

According to the HMBC correlations indicated that the proton of methyl at 0.99 ppm was coupled with carbon at 28.8 ppm. From the COSY correlations showed that the proton at 1.80 ppm was coupled with the proton at 2.24 ppm. (see scheme 3)



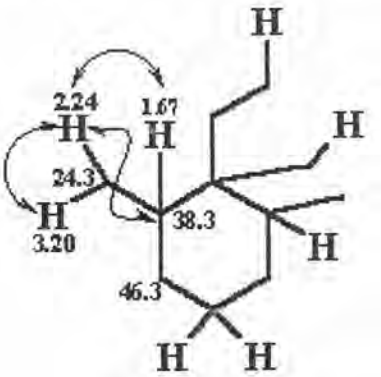
Scheme 3

Further more, the HMBC correlations indicated that the proton at 1.24 ppm was coupled to carbon at 28.8 ppm and 36.9 ppm. The COSY correlations showed that the proton at 2.24 ppm was coupled with the proton of 1.88 ppm. (see scheme 4)



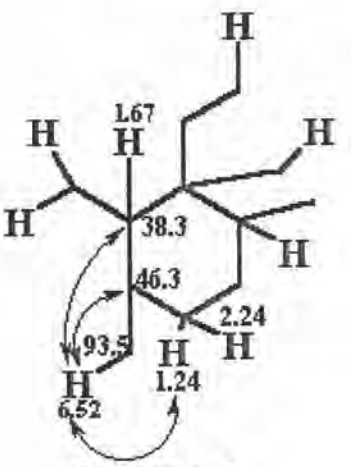
Scheme 4

By HMBC correlations, the proton at 2.24 ppm was coupled with carbon at 38.3 ppm and from COSY correlations showed that the proton at 2.24 ppm was coupled with the proton at 1.67 ppm and 3.20 ppm. (see scheme 5)



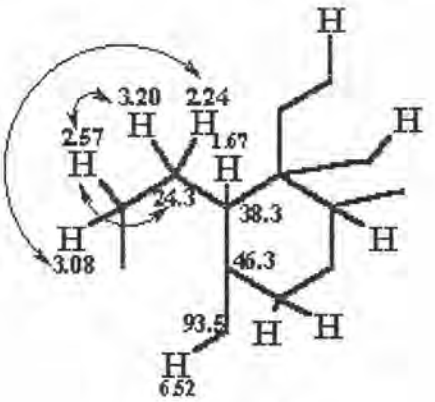
Scheme 5

According to HMBC correlations, the proton at 6.52 ppm was coupled to those carbons at 46.3 ppm and 38.3 ppm and assigned by NOESY correlations (Fig. 27), it was found the appearance of coupling between the proton at 6.52 ppm and proton at 1.24 ppm. (see scheme 6)



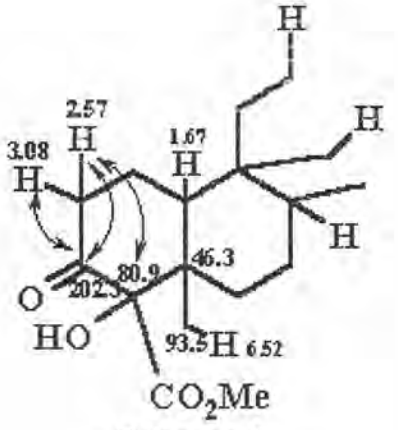
Scheme 6

The HMBC correlations indicated that the proton at 2.57 was coupled to carbon at 24.3 and the COSY correlations showed that the proton at 2.57 ppm was coupled with the proton at 3.20 ppm and the proton at 3.08 ppm was coupled with the proton at 2.24 ppm. (see scheme 7)



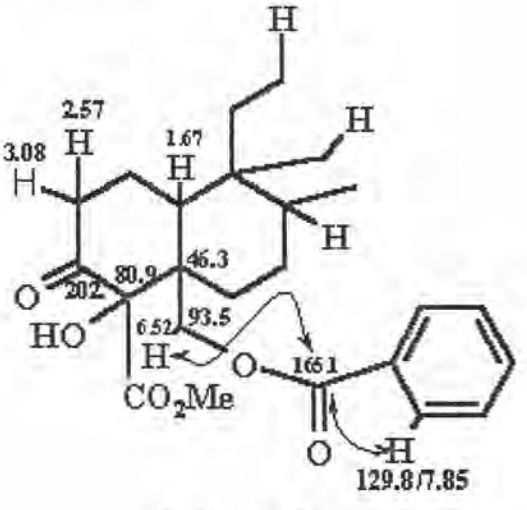
Scheme 7

The HMBC also indicated that the proton at 3.08 ppm and the proton at 2.57 ppm were coupled with carbon at 202.3 ppm. The proton at 2.57 ppm was also coupled with carbon at 80.9 ppm. From DEPT-90 and DEPT-135 experimentals showed that carbon at 202.3 and 80.9 ppm were quaternary carbons and from the chemical shieft indicated that carbon at 202.3 ppm could be carbonyl and carbon at 80.9 ppm could attach to oxygen. From HMBC correlations showed that the proton at 4.15 ppm of -OH group was coupled with carbon at 80.9 ppm and carbon at 171.6 ppm of carbonyl group of ester. That means at 80.9 ppm carbon should attach to -OH and carbonyl group of ester. Assigned by NOESY correlations, it was found the appearance of coupling betweeen the proton at 6.52 ppm and the proton at 3.78 ppm of -OMe group. (see scheme 8)



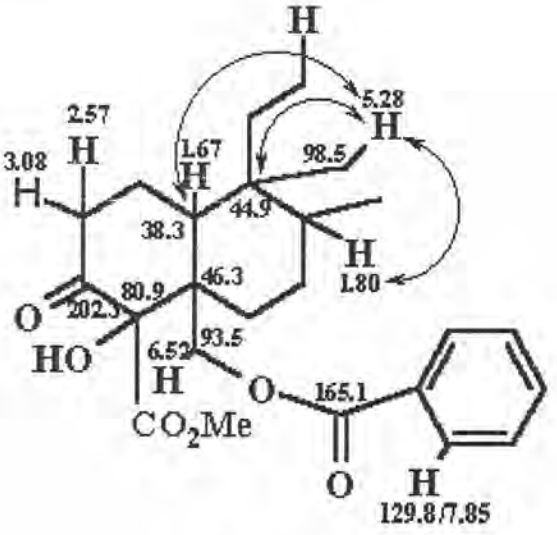
Scheme 8

From HMBC correlations, the proton at 6.52 was coupled with carbon at 165.1 ppm of carbonyl group and the proton at 7.85 ppm of benzene ring was coupled with carbon at 165.1 ppm as well. It could assign that carbon at 93.5 ppm attached to benzoyl group. (see scheme9)



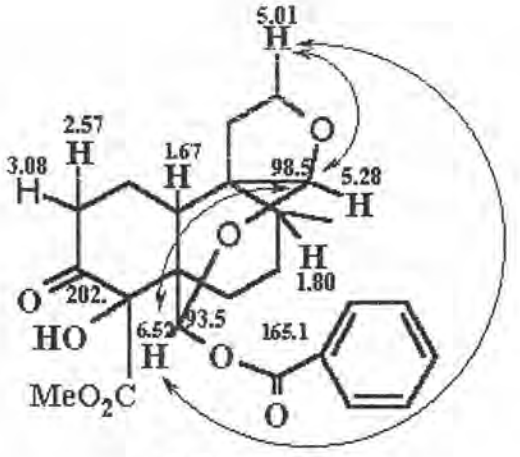
Scheme 9

HMBC correlations also showed that the proton at 5.28 ppm was coupled with carbon at 44.9 ppm and carbon at 38.3 ppm. By NOESY correlations, it showed the appearance of coupling between the proton at 5.28 ppm and the proton at 1.80 ppm. (see scheme 10)



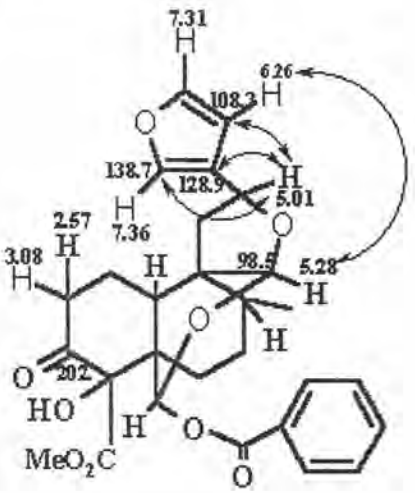
Scheme 10

From ^{13}C-NMR spectrum indicated that the carbon at 69.0 ppm, 93.5 ppm and 98.5 ppm attached to oxygen atom. By HMBC correlations showed that the proton at 5.01 ppm was coupled with carbon at 98.5 ppm and the proton at 6.52 ppm was coupled with carbon at 98.5 ppm. The NOESY correlations showed the appearance of coupling between the proton at 5.01 ppm and the proton at 6.52 ppm. (see scheme 11)



Scheme 11

The HMBC correlations also indicated that the proton at 5.01 ppm was coupled with carbon at 138.7, 128.9 and 108.3 ppm of furan ring. NOESY correlations showed the coupling between the proton at 6.26 ppm of furan ring and the proton at 5.28 ppm. (see scheme 12)



Scheme 12

Therefore, the structure of compound 3 was shown in Figure 8 The COSY correlations and long-range C-H correlations by HMBC correlations are summarized in Figure **5** and **6** respectively.

Table 10: **The HMQC spectral data of compound 3**

Position	^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (H_z)
1	24.3 (t)	2.24 (m)
		3.20 (m)
2	35.8 (t)	2.57 (ddd, J= 1.2, 5.2, 15.3)
		3.08 (ddd, J= 6.7, 13.4, 15.3)
3	202.3 (s)	-
4	80.9 (s)	-
5	46.3 (s)	-
6	29.3 (t)	1.24 (m)
		2.24 (m)
7	28.8 (t)	1.80 (m)
		2.24 (m)
8	36.9 (d)	1.80 (m)
9	44.9 (s)	-
10	38.3 (d)	4.15 (s)
11	37.6 (t)	2.21 (m)
		2.24 (m)
12	69.0 (d)	5.01 (t, J= 7.9)
13	128.9 (s)	-
14	108.3 (d)	6.26 (dd, J= 0.9, 1.8)
15	143.7 (d)	7.33 (t, J= 1.8)
16	138.7 (d)	7.31 (m)
17	16.8 (q)	0.99 (s)
18	171.6 (s)	-
19	93.5 (d)	6.52 (s)
20	98.5 (d)	5.28 (s)
21	53.9 (q)	3.78 (s)
22	165.1 (s)	-
23	129.6 (s)	-
24	129.8 (d)	7.85 (dd, J= 3.1, 10.1)
25	128.3 (d)	7.42 (t, J= 7.9)
26	133.2 (d)	7.53 (dt, J= 1.5, 7.0)
27	128.3 (d)	7.42 (t, J= 7.9)
28	129.8 (d)	7.85 (dd, J= 3.1, 10.1)

Table 11: **The HMBC and COSY spectral data of compound 3**

Position	δ_C (ppm)	δ_H (ppm)	HMBC	COSY
1	24.3 (t)	2.24 (m)	C-3, C-9, C-10	H-1 (3.20,)H-2 (3.08), H-10 (1.67)
		3.20 (m)	C-3, C-10	H-2 (2.57)
2	35.8 (t)	2.57 (ddd)	C-1, C-3, C-4, C-10	H-1 (3.20), H-2 (3.08)
		3.08 (ddd)	C-1, C-3	H-1 (2.24)
3	202.3 (s)	-	-	-
4	80.9 (s)	-	-	-
5	46.3 (s)	-	-	-
6	29.3 (t)	1.24 (m)	C-5, C-7, C-8	H-7 (1.80), H-19 (6.52)
		2.24 (m)	C-5, C-7, C-19	H-7 (1.80), H-19 (6.52)
7	28.8 (t)	1.80 (m)	C-6, C-8	H-6 (2.24), H-7 (1.80)
		2.24 (m)	C-5, C-6, C-8, C-9	H-7 (1.80), H-8 (1.80)
8	36.9 (d)	1.80 (m)	C-6, C-7, C-9, C-11	H-7 (2.24), H-17 (0.99)
9	44.9 (s)	-	-	-
10	38.3 (d)	1.67 (s)	C-2, C-5, C-9, C-11, C-19	H-1 (2.24)
11	37.6 (t)	2.21 (m)	C-9, C-10, C-12, C-13	H-11 (2.24), H-12 (5.01), H-20 (5.25)
		2.24 (m)	C-8, C-9, C-12, C-13, C-20	H-11 (2.24), H-12 (5.01)
12	69.0 (d)	5.01 (t)	C-11, C-13, c-14, C-16, C-20	H-11 (2.24)
13	128.9 (s)	-	-	-
14	108.3 (d)	6.26 (dd)	C-13, C-16	H-15 (7.33)
15	143.7 (d)	7.33 (t)	C-13, C-14, C-16	H-14 (6.26)
16	138.7 (d)	7.31 (m)	C-13, C-14, C-15	-
17	16.8 (q)	0.99 (s)	C-7, C-8, C-9	H-8 (1.80)
18	171.6 (s)	-	-	-
19	93.5 (d)	6.52 (s)	C-5, C-10, C-21	H-6 (2.24)
20	98.5 (d)	5.28 (s)	C-7, C-8, C-9, C-10, C-19	H-19 (6.52)
21	53.9 (q)	3.78 (s)	C-18	-
22	165.1 (s)	-	-	-
23	129.6 (s)	-	-	-
24	129.8 (d)	7.85 (dd)	C-21, C-24, C-26	H-25 (7.42)
25	128.3 (d)	7.42 (dt)	C-23, C-25	H-24 (7.85), H-26 (7.53)
26	133.2 (d)	7.53 (tt)	C-24	H-25 (7.42)
27	128.3 (d)	7.42 (dt)	C-23, C-27	H-26 (7.53), H-28 (7.85)
28	129.8 (d)	7.85 (dd)	C-21, C-26, C-28	H-27 (7.42)

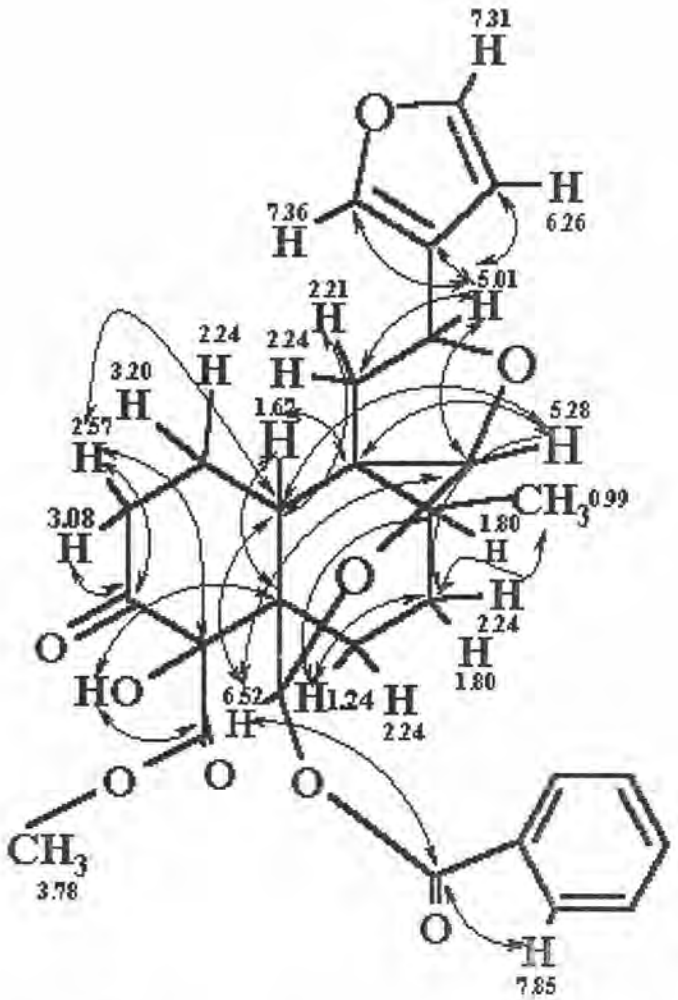


Figure 5: **The HMBC correlations of compound 3.**

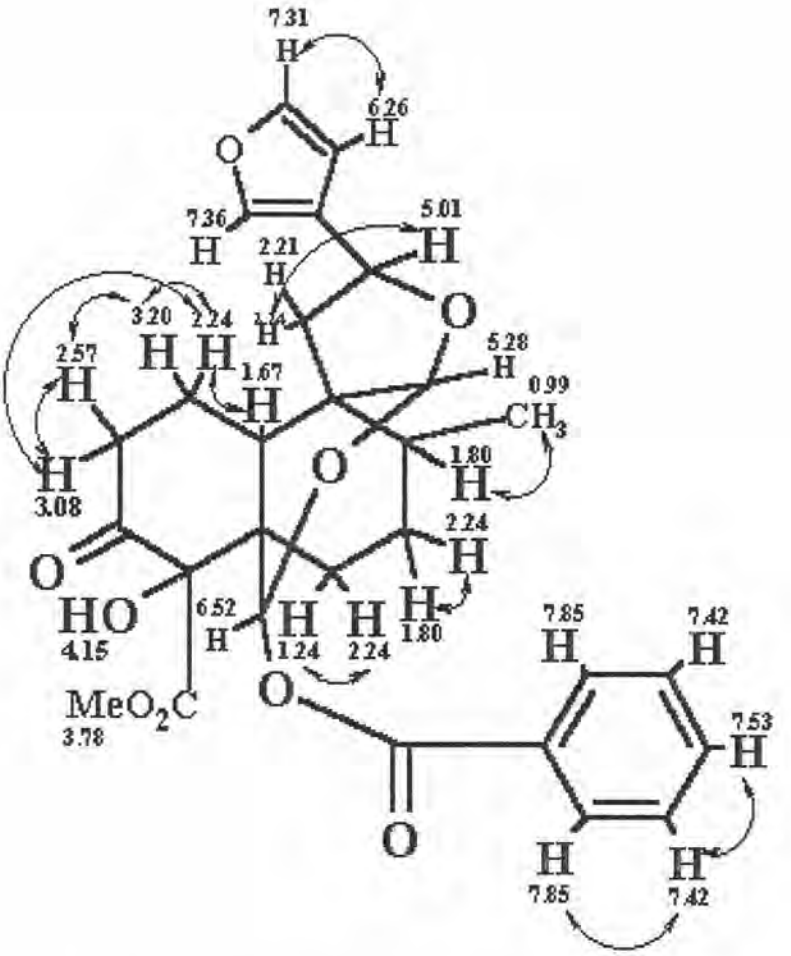


Figure 6: The COSY correlations of compound 3.

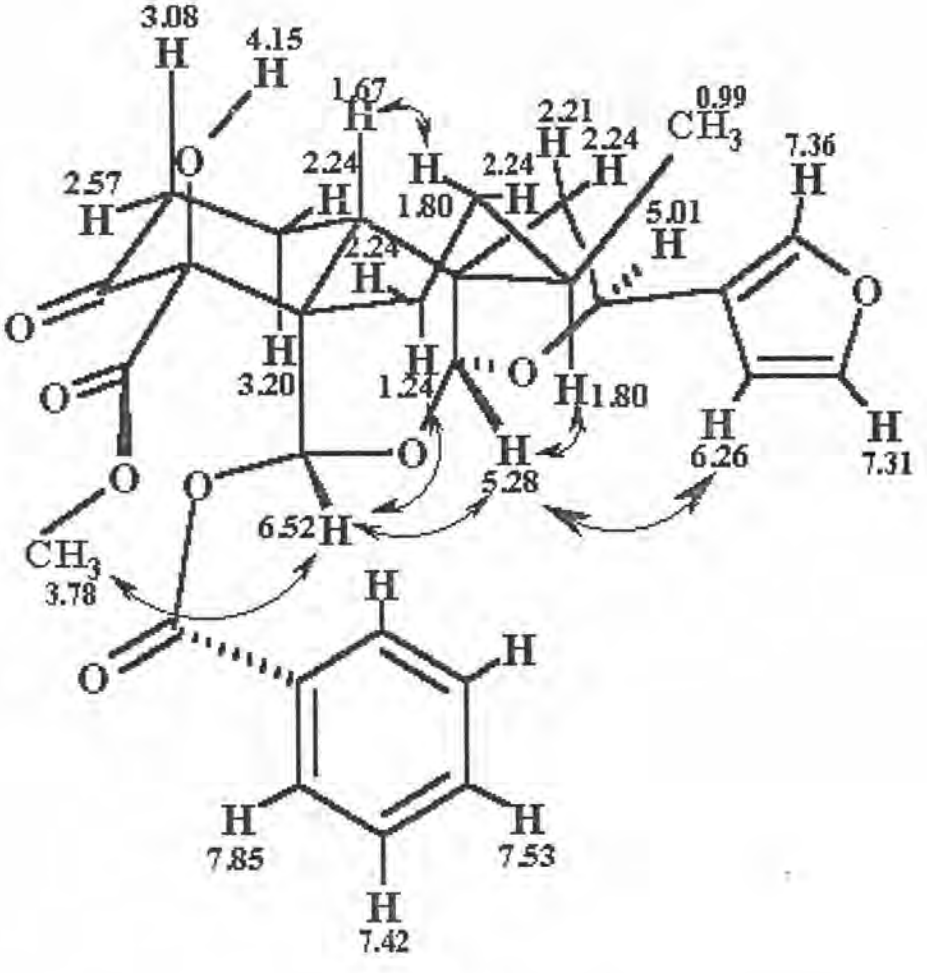


Figure 7: The NOESY correlations of compound 3.

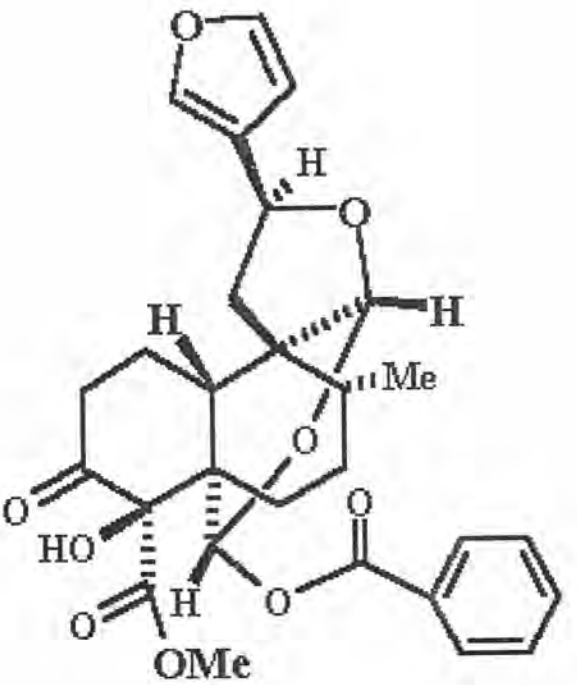


Figure 8: Structure of compound 3.

4.1.4 Structure elucidation of compound 4

The IR spectrum of compound 4 was shown in Fig. **28** and its absoption peaks were assigned in Table **12**. The important absorption bands were shown at 3405 cm^{-1} (O-H stretching vibration of alcohol), 2962, 2924, and 2863 cm^{-1} (C-H stretching vibration), 1076 cm^{-1} (C-O stretching vibration of alcohol group)

Table **12: The IR spectral bands assignment of compound 4**

Wave number (cm^{-1})	Intensity	Tentative Assignment
3405	broad	O-H stretching vibration of alcohol
2962, 2924, 2863	strong	O-H stretching vibration of -CH_3, -CH_2
1076	medium	C-O stretching vibration of alcohol group

The ^{1}H-NMR spectrum (Fig. **30**) of compound 4 showed that it possesses three methyl group (δ_H 0.77, 0.85 and 1.10 ppm), two olefinic methyl groups (δ_H 1.73 and 1.66 ppm), four olefinic protons (δ_H 6.29, 5.52, 5.02, and 5.02 ppm), and one methine protons of hydroxyl group alcohol (δ_H 3.48 ppm)

The ^{13}C-NMR (Fig. **31**), DEPT-90 and DEPT-135 spectrum (Fig. **32**) showed 20 signals. Four signals of olefinic carbons appeared at δ_C 135.5, 132.7, 141.5, and 110.6 ppm. The signal of carbons attach to hydroxyl group of alcohol should appear at δ_C 80.3, and 78.1 ppm. There were three signals of saturated methine carbons appeared at δ_C 80.3, 60.2, and 53.6. There were five signals of methylene carbons appeared at δ_C 41.6, 39.8, 27.6, 23.5, and 18.5 ppm. There were five signals of methyl carbons appeared at δ_C 33.5, 21.6, 17.9, 15.6, and 11.9 ppm, which indicated that the carbon signals at δ_C 132.7, 78.1, 39.2, and 33.2 ppm were quaternary.

The compound 4 's molecular formular was established as $C_{20}H_{34}O_2$ on the basis of ^1H - $^{13}CNMR$, MS data, which was confirmed by observing molecular ion at m/z 316 (Fig. **29**). A degree of unsaturation of four was defined from its molecular formular ($C_{20}H_{34}O_2$), thus,this compound must consist of two rings and two double bonds. From these data, the compound 4 could have a labdane diterpene skeleton, therefore the compound 4 should belabdane diterpene compound.

The comparison of ^1H-NMR chemical shifts of compound 4 and Nidorellol , (7S,12Z)-12, 14-Labdadiene-7, 8-diol, [18, 23] was shown in Table **13**.

Table 13: ^{1}H-NMR chemical shifts of compound 4 and Nidorellol

Position	Compound 4	Nidorellol
1	1.60	1.3-1.6
2	1.40	
3	1.35	
5	1.00 (dd, 2.1, 12.5)	
6ex	1.26 (dd, 12.5, 12.8)	
6eq	1.81 (ddd, 2.1, 4.6, 12.8)	1.86 (ddd, 2, 4.5, 12)
7	3.48 (dd, 4.6, 11.6)	3.52 (dd, 4.5, 12)
10	-	-
11	2.36 (dt, 6.1, 6.1, 16.2)	2.40 (ddd, 7, 7, 16)
	2.16 (dt, 6.1, 6.1, 16.2)	2.22 (ddd, 7, 7, 16)
12	5.52 (t, br, 6.7)	5.55 (t, br)
13	-	-
14	6.31 (dd, 11.0, 17.4)	6.34 (dd, 10, 17)
15	5.02 (d, 17.4)	5.06 (d, br, 17)
	4.86 (d, 11.0)	4.89 (d, br, 10)
16	1.73 (d, 1)	1.77 (d, 1)
17	1.10 (s)	1.14 (s)
18	0.77 (s)	0.80 (s)
19	0.85 (s)	0.88 (s)
20	0.81 (s)	0.84 (s)

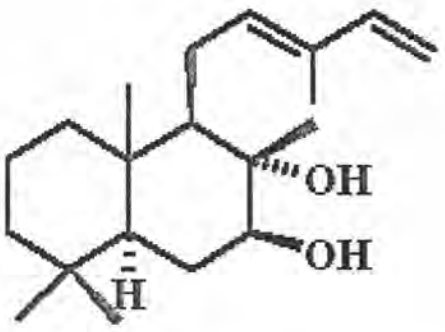


Figure 9: Structure of compound 4.

4.2 Literature reviews in cytotoxic activity of labdane diterpene compounds of *C. oblongifolius*

Previous studied in cytotoxic activity of some diterpenoid compounds from stem bark of *C. oblongifolius* against 6 human tumor cell lines; L 929 (fibroblast), HEP-G2 (hepatoma), SW 620 (colon), CHAGO (lung), KATO (gastric), and BT 474 (breast) have been assigned in Table **14.** [5]

Table **14: Cytotoxic activity against cancer cell line of isolated compound from** *C. oblongifolius*

compound	% inhibition					
	L 929 (fibroblast)	HEP-G2 (hepatoma)	SW 620 (colon)	CHAGO (lung)	KATO (gastric)	BT474 (breast)
A[a]	54	63	4	3	10	5
B[b]	18	29	92	88	90	54
C[c]	36	93	97	18	94	89
D[d]	9	14	0	0	30	0
E[e]	36	93	97	18	94	89
F[f]	18	29	4	7	94	3
G[g]	94	93	97	97	93	87
H[h]	27	43	12	41	30	9
I[i]	0	39	27	28	53	25
J[j]	0	26	42	0	35	18

[a]Neocrotocembraneic acid
[b]Neocrotocembranal
[c]crotohalimaneic acid
[d]crotohalimoneic acid
[e]Labda-7,12(*E*),14-triene-17-ol
[f]Crotocembraneic acid
[g]Labda-7,12(*E*),14-triene-17-al
[h]Labda-7,12(*E*),14-triene-17-oic acid
[i]Labda-7, 13(Z)-diene-17,12-olide-15-ol
[j](-)-20-benzyloxyhardwickiic acid

Moreover, cembrane and clerodane diterpene compounds that found in the stem bark of *C. oblongifolius* in other areas of Thailand exhibited cytotoxic activity

against tumor cell lines and anti bacterial, respectively.

4.3 Result of biological activity test

The *in vitro* activity of some compounds (10 $\mu g/ml$) from *Croton oblongifolius* Roxb. against 6 cancer cell lines such as HS 27 (fibroblast), HEP-G2 (hepatoma), SW 620 (colon), CHAGO (lung), KATO (gastric), and BT 474 (breast) were reported in Table **15**.

Table **15: Cytotoxic activity against 6 cell lines of compound 1 to compound 4 from** *C. oblongifolius* **Roxb.**

compound	% survival					
	HS 27 (fibroblast)	HEP-G2 (hepatoma)	SW 620 (colon)	CHAGO (lung)	KATO (gastric)	BT474 (breast)
Adriamycin	35	17	20	63	54	28
1	88	88	89	98	66	116
2	94	73	98	96	75	98
3	57	5	6	5	8	20
4	86	74	83	94	81	125

According to Table **15**, There was only compound 3 that exhibited strong cytotoxic activity against those 5 cancer cell lines. The compound 3 consist of alcohol , methyl ester and benzoyl group, respectively. The cytotoxicity of crovatin,methyl *ent*-(18R,10β)-3, 19S:15,16:12S,20R:19,20-tetraepoxy-cleroda-13(16), 14-dien-18β-oate, (2) and (7S,12Z)-12,14-Labdadiene-7,8-diol (4) which were tested against HS 27, KATO-3, BT 474, CHAGO, SW 620 and HEP-G2 tumor cell lines have been reported previously [4]. These were report of the cytotoxicity of Compound 1 and 3 against HS 27, KATO-3, BT 474, CHAGO, SW 620 and HEP-G2 tumor cell lines for the first time.

The comparison between compound 3 and Adriamycin could examine that compound 3 exhibit to those five tumor cell lines better than Adriamycin,further more, the compound 3 doesn't destroy human fibroblast whereas Adriamycin does.