CHAPTER IV

RESULTS AND DISCUSSION

Cytotoxic activity of hexane, ethyl acetate and methanol crude extract from the stem barks of *C. oblongifolius* against 6 tumor cell lines: HS 27(fibroblast), Hep-G2 (hepatoma), SW 620 (colon), Chago(lung), KATO (gastric) and BT 474 (breast) have been carried out and the result were summarized in Table 5.

Table 6 Screening test for cytotoxic activity of hexane, ethyl acetate and methanol crude extract of the stem barks of *C.oblongifolius* from Muang, Prachuab khiri khan province

Crude extract	%survival					
	HS 27	Kato-3	BT 474	Chago	SW620	Hep-G2
Hexane	59	9	35	7	7	9
Ethyl acetate	65	25	44	31	19	27
Methanol	51	62	77	58	33	48

From table 6, it indicated that hexane and ethyl acetate crude extract showed strong and medium cytotoxic activity against those 5 cancer cell lines, respectively. Therefore, it was very interesting to isolated compounds from hexane and ethyl acetate crude extract.

4.1 STRUCTURE ELUCIDATION OF THE ISOLATED COMPOUNDS FROM THE STEM BARKS OF *C. oblongifolius* Roxb.

4.1.1 Structure elucidation of Compound 1

Compound 1 was obtained as a white solid (122 mg). Its IR absorption bands at 3300-2400 cm^{-1} suggested the presence of carboxylic group and the strong absorption band at 2924 and 2848 cm^{-1} suggested methyl and methylene group stretching vibrations. In addition, there was the presence of carboxylic acid carbonyl group according to the strong absorption band at 1695 cm^{-1}. The absorption bands were assigned as shown in Table 7.

Table 7 The IR absorption bands assignment of compound 1

Wave number (cm-1)	Intensity	Vibration
3300-2400	broad	O-H stretching vibration of acid
2924,2848	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1695	strong	C=O stretching vibration of acid

The ^{1}H-NMR spectrum of compound 1 (Fig 19) showed signals for three tertiary methyl group at δ_H 0.88, 1.14 and 1.21 ppm.

The ^{13}C-NMR spectrum (Fig 20) showed 20 lines. One signal of carboxylic acid appeared at δ_C 184.5 ppm.

Dept 90 experiment (Fig 21) revealed that the compound had four saturated methines at δ_C 57.0, 52.8, 24.3 and 20.5 ppm.

Dept 135 experiment (Fig 21) showed three methyl carbons at δ_C 28.8, 20.5 and 12.4 ppm and eight methelene carbons at δ_C 50.3, 39.4, 39.2, 37.8, 33.1, 19.7 and

18.7 ppm. In addition, there were five quaternary carbons at δ_C 184.5, 43.7, 40.7, 38.9 and 22.4 ppm.

The molecular formula for compound 1 is $C_{20}H_{30}O_2$, which was in agreement with m/z 302 molecular ion, obtained from EIMS spectrum **(Fig 22)**. There are six double bond equivalents. Therefore, compound 1 must consisted of a pentacyclic skeleton. In addition, the signal at δ_H 0.58, J=2.4, 7.5 was typical of a cyclopropane system [33]. The IR spectrum, EIMS spectrum and NMR spectrum led to the conclusion that compound 1 belong to a trachylobane series of diterpene. Moreover the presence of a carboxylic group revealed that this compound was an acidic trachylobane diterpenoid. These results were consistent with those of trachyloban-19-oic acid [mp = 129-131 °C, $[\alpha]_D^{20}$ –73.8° ($CHCl_3$; *c*1.0)] as reported in the literature [33-37]. It was the first recorded of a trachyloban diterpene from the *C. oblongifolius* Roxb. The ^{13}C-NMR spectrum of compound 1 was compared to that of trachyloban-19-oic acid to authenticate the structure as shown in Table 7.

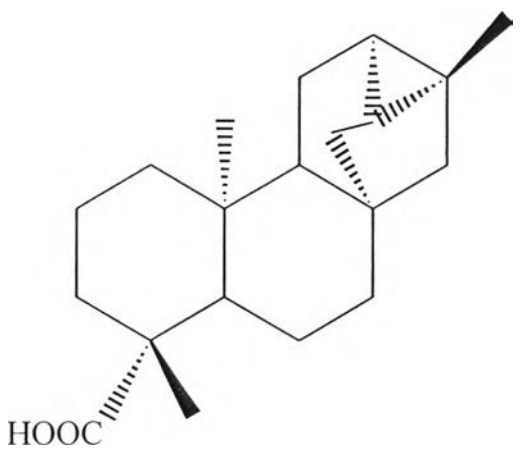


Figure 6 : Structure of Compound 1

Table 8 **Comparison of ^{13}C-NMR spectral data of Compound 1 with Trachyloban-19-oic acid**

Carbon no.	Chemical shift δ_C (ppm)	
	Compound 1	Trachyloban-19-oic acid
1	39.4(t)	39.5(t)
2	18.6(t)	18.7(t)
3	37.8(t)	37.8(t)
4	43.6(s)	43.7(s)
5	56.9(d)	57.0(d)
6	21.7(t)	21.8(t)
7	39.2(t)	39.2(t)
8	40.7(s)	40.8(s)
9	52.7(d)	52.2(d)
10	38.8(s)	38.9(s)
11	19.7(t)	19.7(t)
12	20.5(d)	20.6(d)
13	24.2(d)	24.3(d)
14	33.1(t)	33.1(t)
15	50.3(t)	50.4(t)
16	22.3(s)	22.4(s)
17	20.6(q)	20.6(q)
18	28.6(q)	28.9(q)
19	184.3(s)	184.7(s)
20	12.3(q)	12.5(q)

4.1.2 Structure elucidation of Compound 2

The IR spectrum of compound 2 (Fig 23) indicated that the carboxylic group corresponded to the broad absorption band between 3400-2400 cm^{-1} and the strong absorption band at 1694 cm^{-1} was due to the carboxylic acid carbonyl stretching vibration. The IR absorption bane of this compound is summarized in Table 9.

Table 9 The IR absorption band assignment of Compound 2

Wave number (cm^{-1})	Intensity	Vibration
3400-2400	broad	C-H stretching vibration of acid
2950	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1697	strong	C=O stretching vibration of acid
1640,1620	strong	C=C stretching vibration of alkene

The ^{1}H-NMR spectrum (Fig 24) showed that this compound possessed an isopropyl group at δ_H 0.80(3H) and 0.84(3H) ppm, two olefinic methyl group at δ_H 1.66 and 1.82 ppm, and five olefinic protons at δ_H 5.0-6.2 ppm.

The ^{13}C-NMR spectrum (Fig 25) showed 20 lines in which the carbonyl group of carboxylic acid corresponded to the signal at δ_C173.3 ppm. The eight signals of olefinic carbons appeared at δ_C 147.6, 135.1, 131.3, 130.9, 130.4, 128.8, 127.9 and 125.7 ppm.

Dept 90 experiments (Fig 26) revealed that this compound had five sp^2 methine carbons at δ_C 147.6, 131.3, 130.4, 127.9 and 125.7 ppm and also consisted of two saturated methines at δ_C 47.8 and 32.7 ppm.

Dept 135 experiments (Fig 26) indicated the presence of five methylene carbons at δ_C 38.9, 32.1, 28.9, 26.2 and 25.8 ppm and four methyl carbons at δ_C 20.9, 19.9, 19.3 and 14.4 ppm, which indicated that the carbon signals at δ_C 173.3, 135.1, 130.9 and 128.8 ppm were quaternary carbons.

The EIMS spectrum (Fig 27) suggested that the molecular formula was $C_{20}H_{30}O_2$ which agreed well with the molecular ion m/z 302. Thus it indicated DBE of 6. This compound must contain one ring in addition to the four double bonds and the carbonyl group. From these data, it was revealed that compound 2 could possess a 14-members ring diterpene skeleton as cembranoid.

The combination of spectroscopic data led to the conclusions that compound 2 could be a cembranoid diterpene compound having a carboxylic group. These results were consistent with those of poilaneic acid. Thus, compound 2 was assigned as poilaneic acid [$[\alpha]_D^{20}$ −131.8° ($CHCl_3$; *c*1.0)] which was previously isolated from *C. poilanei* [38]. The ^{13}C-NMR chemical shift of compound 2 was compared with that of poilaneic acid to confirm the structure. (Table 9)

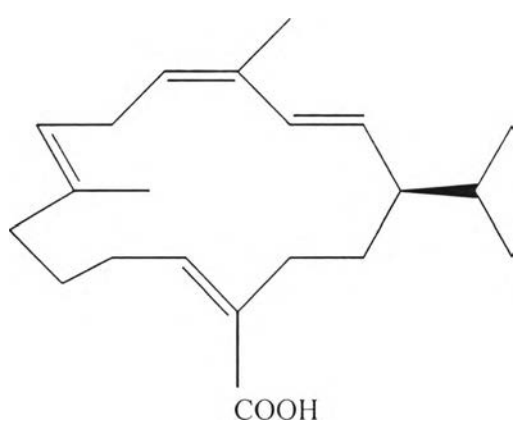


Figure 7 : Structure of Compound 2

Table 10 **^{13}C-NMR chemical shift of Poilaneic acid compared to those of Compound 2**

Carbon no.	Chemical shift δ_C (ppm)	
	Compound 2	Poilaneic acid
1	14.4(q)	14.5(q)
2	19.3(q)	19.4(q)
3	19.9(q)	19.9(q)
4	20.9(t)	20.9(t)
5	25.8(t)	25.9(t)
6	26.2(t)	26.3(t)
7	28.9(t)	29.5(t)
8	32.1(t)	32.7(t)
9	32.7(d)	32.7(d)
10	38.8(t)	38.6(t)
11	47.8(d)	47.8(d)
12	125.7(d)	125.7(d)
13	127.9(d)	128.0(d)
14	128.8(s)	128.8(s)
15	130.4(d)	130.5(d)
16	130.9(s)	131.0(s)
17	131.3(d)	131.3(d)
18	135.1(s)	135.2(s)
19	147.6(d)	147.8(d)
20	173.3(s)	173.7(s)

4.1.3 Structure elucidation of Mixture 3

The IR spectrum (Fig 28) of mixture 3 showed a broad absorption band at 3695-3040 cm^{-1} which was characteristic of a hydroxyl group. The strong absorption band at 2959 and 2937 cm^{-1} suggested the presence of C-H stretching vibration of methyl and methylene group. Besides this, the strong absorption band at 1651 cm^{-1} was due to C=C stretching of alkene. The IR absorption bands were assigned as shown in Table 11.

Table 11 The IR absorption band assignment of mixture 3

Wave number (cm-1)	Intensity	Vibration
3695-3040	broad	O-H stretching vibration of alcohol
2959,2937	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1100	strong	C-O stretching vibration of alcohol

The ^{1}H-NMR spectrum (Fig 29) showed the signal at δ_H 0.65-1.3 ppm, which indicated the signal of methyl protons and a side chain of the steroid compound and methylene. The signal of methine protons of the steroids appeared at δ_H 1.85-2.3 ppm. The chemical shift at δ_H 3.53 ppm indicated the proton attached to a carbon bearing a hydroxyl group (CH-OH). In addition, the olefinic signals at δ_H 4.90-5.10 ppm were trans-disubstituted vinyl protons and the olefinic signal at δ_H 5.34 ppm could be assigned as a trisubstituted vinyl proton.

The ^{13}C-NMR spectrum (Fig 30) revealed that this compound had 25 signals in region of δ_C 11.9-57.0 ppm were typical of sp^3 CH_3, CH_2, CH and C. The olefinic carbon signals were indicated at δ_C 121.8 ppm (CH=C) and 140.8 ppm (CH=C)

The NMR spectrum suggested that mixture 3 was a mixture of steroid compound possessing a hydroxyl group. The GC technique was used to identify this compound by comparing the chromatogram of this compound with that of the standard mixture of steroids: compesterol, stigmasterol, β-sitosterol **(Fig 32)**. The retention times of standard steroids were 17.42, 18.38 and 20.51 min, which revealed that this compound consisted of a mixtures of stigmasterol and β-sitosterol.

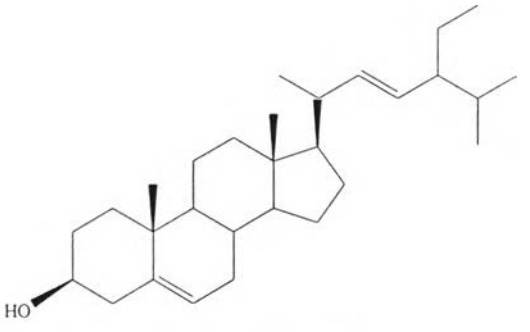
EI mass spectra (Fig 31) provided a molecular ion peak at m/z 414, which also corresponded to the molecular formula $C_{29}H_{50}O$ of β-sitosterol. The peak at m/z 412 corresponded to the molecular ion peak of stigmasterol, which was also observed. The comparison of the ^{13}C-NMR data of Mixture 3 and β-sitosterol [39] was also carried out to further confirm the structure as presented in Table 12.

Table 12 ^{13}C-NMR chemical shift of mixture 3 compared with β-sitosterol

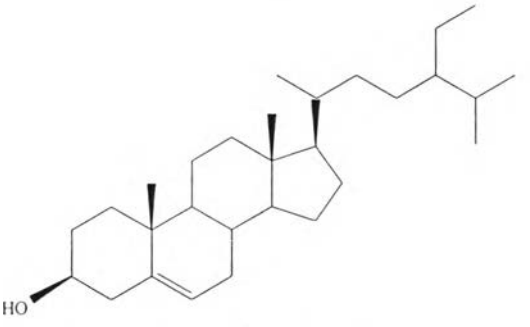
Carbon no.	Chemical shift δ_C (ppm)	
	Mixture 3	β-sitosterol
1	37.4	37.4
2	31.8	31.7
3	71.6	72.0
4	42.4	42.5
5	140.9	140.8
6	121.8	121.8
7	32.0	32.1
8	32.0	32.1
9	50.3	50.1
10	36.5	36.5
11	21.1	21.1
12	39.4	40.0
13	42.2	42.5
14	56.8	57.0
15	24.3	24.5
16	28.2	28.5
17	56.2	56.1
18	11.9	12.0
19	19.4	19.6
20	36.2	36.3

Carbon no.	Chemical shift δ_C (ppm)	
	mixture 3	β-sitosterol
21	19.1	19.2
22	34.0	34.1
23	29.3	29.5
24	50.3	50.1
25	26.2	26.2
26	18.8	18.8
27	19.8	20.1
28	21.1	23.2
29	11.9	11.9

These results suggested that mixture 3 was mixture of stigmasterol and β-sitosterol



stigmasterol



β-sitosterol

Figure 8: Structure of stigmasterol and β-sitosterol

4.1.4 Structure elucidation of Compound 4

The IR spectrum of this compound (Fig 33) showed a broad absorption band for hydroxyl group at 3408 cm^{-1}. The strong absorption band for C-H stretching vibration of methyl and methylene group at 2962, 2924 and 2863 cm^{-1} and a moderate intensity C-O stretching vibration band for alcohol group at 1076 cm^{-1}. The IR spectrum of compound 4 is summarized in Table 13.

Table 13 The IR spectrum band assignment of compound 4

Wave number (cm-1)	Intensity	Vibration
3408	broad	O-H stretching vibration of alcohol
2962,2924, 2863	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1076	medium	C-O stretching vibration of alcohol

The ^{1}H-NMR spectrum (Fig 34) of this compound showed sharp signals at δ_H 1.10, 0.78, 0.85 and 0.85 ppm, which were assigned to the methyl group .The signal at δ_H 5.52, 6.33, 5.02 and 4.87 ppm belong to olefinic protons. In addition, there was one methine proton of hydroxyl group of alcohol at δ_H 3.48 ppm.

The ^{13}C-NMR spectrum (Fig 35) of this compound indicated 20 lines. The presence of the signals at δ_C 80.3 and 78.1 ppm, which were assigned to the carbon, were attached to the hydroxyl group of alcohol. The carbon signals at δ_C 135.5, 132.7, 141.5 and 110.6 were typical of the olefinic carbons and the signals at δ_C 80.3, 60.2 and 53.6 ppm were assigned to a characteristic of saturated methine. Besides this, the presence of the signals at δ_C 132.7, 78.1, 39.2 and 33.2 ppm were typical of the quaternary carbons.

From dept 90 experiment (Fig 36) it was revealed that this compound had three signals of saturated methine carbons appearing at δ_C 80.3, 60.2 and 53.6 ppm and two sp^2 methine carbon at δ_C 141.5 and 135.5 ppm.

From dept 135 experiment (Fig 36), it was found that this compound had five signals of methylene carbons at δ_C 41.6,39.8, 27.6, 33.5 and 18.5 ppm and five signals of methyl carbons at δ_C 33.5, 21.6, 17.9, 15.6 and 11.9 ppm. Furthermore, the carbon signals at δ_C 132.7, 78.1, 39.2 and 33.6 ppm were assigned to be quaternary carbons.

The molecular formula of this compound was $C_{20}H_{34}O_2$ which was established by EIMS spectrum **(Fig 37)** [M^+](m/z 306). Its molecular formula indicated four degree of unsaturations. Therefore, compound 3 should contain two rings and two double bonds. These data indicated that this compound could be a labdane diterpene .

The combination of IR spectrum and NMR spectra led to a conclusion that compound 4 was a labdane diterpene compound having two hydroxyl groups. These results were consistent with those of Nidorellol[mp = 71-73 °C, $[\alpha]_D^{20}$ –23.8° ($CHCl_3$; *c*1.0)] [41,42]. The ^{13}C-NMR chemical shift of compound 4 was compared with Nidorellol to authenticate the structure (Table 14).

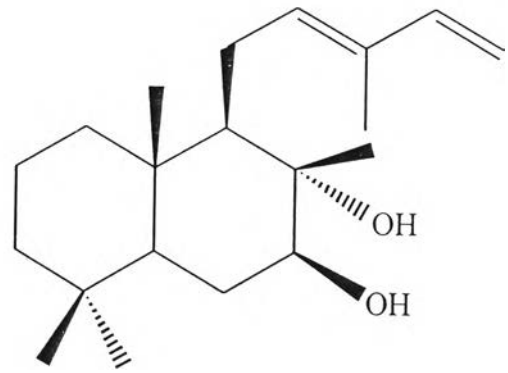


Figure 9 : Structure of Compound 4

Table 14 ^{13}C-NMR chemical shift of Compound 4 and Nidorellol

Carbon no.	Chemical shift δ_C (ppm)	
	Compound 4	Nidorellol
1	39.8(t)	39.9(t)
2	18.5(t)	18.5(t)
3	41.6(t)	41.7(t)
4	33.2(s)	33.2(s)
5	53.6(d)	53.6(d)
6	27.2(t)	27.8(t)
7	80.3(d)	80.2(d)
8	78.1(s)	78.2(s)
9	60.2(d)	60.3(d)
10	39.2(s)	39.3(s)
11	23.5(t)	22.6(t)
12	135.5(t)	135.7(t)
13	132.7(s)	132.8(s)
14	141.5(d)	141.3(d)
15	110.6(t)	110.4(t)
16	11.9(q)	11.8(q)
17	17.9(q)	17.9(q)
18	33.5(q)	33.5(q)
19	21.6(q)	21.5(q)
20	15.6(q)	15.9(q)

4.1.5 Structure elucidation of compound 5

From the IR spectrum of compound 5 (Fig 38) the presence of a broad absorption band at 3250 cm^{-1} was found to be typical for hydroxyl group and the strong absorption band at 2961 cm^{-1} was due to C-H stretching vibration of methyl and methylene group. Moreover, the strong absorption band at 1691 cm^{-1} was due to C=O stretching vibration and the C-O stretching vibration of ester was appeared at 1200 cm^{-1}. The IR absorption bands were assigned as summarized in Table 15.

Table 15 The IR absorption band assignment of compound 5

Wave number (cm^{-1})	Intensity	Vibration
3250	Broad	O-H stretching vibration of alcohol
2961	Strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1691	Strong	C=O stretching vibration of ester
1200	strong	C-O stretching vibration of ester

The ^{1}H-NMR spectral data of this compound (Fig 39) clearly showed that a 6-acetoxy group was present, which was the signal of proton of carbon attached to an ester group which appeared at δ_H 4.92 ppm and the signal of proton of the methyl group that was attached to the carbonyl group at δ_H 2.12 ppm. In addition, the chemical shift at δ_H 3.37 ppm was assigned to the methine proton attached to a carbon bearing a hydroxyl group. The signals of olefinic protons were found at δ_H 6.33, 5.52, 5.15 and 5.05 ppm.

From ^{13}C-NMR spectrum (Fig 40), Dept 90 experiment (Fig 41) and Dept 135 experiment (Fig 41) demonstrated 22 lines, which the carbonyl group of the ester group corresponded to the signal at δ_C 171.9 ppm. The presence of signals in the

downfield region at δ_C 76.7 ppm and 83.6 ppm were characteristic of the carbon attached to the hydroxyl group. The signals of methyl carbons were also observed at δ_C 22.1, 21.8, 19.5, 16.7, 11.8 and 35.9 ppm. In addition, the signals at δ_C 141.5, 135.5, 132.6 and 110.5 ppm were typical for olefinic carbons and the signals of quaternary carbons were appeared at δ_C 132.6, 76.7, 33.3 and 39.3 ppm.

The molecular formula of compound 5 was determined as $C_{22}H_{36}O_3$, which was agreed well with m/z 348 molecular ion obtained from the EIMS spectrum **(Fig 42)**. A degree of unsaturation of four was defined and should be consistent of two rings and two double bonds. From these data, it was suggested that this compound could have labdane diterpene skeleton.

As mentioned above, all of the spectroscopic data led to a combination of those carbon units to construct and to proposed the structure of compound 5 as a labdane diterpene compound having two hydroxyl group and two ester groups. These results were consistent with those of 6-acetoxy-12,14-labdadiene-7,8-diol [$[\alpha]_D^{20}$ -17.1° ($CHCl_3$; *c*1.0)] reported previously [43]. The ^{1}H-NMR chemical shift of compound 5 was compared with 6-acetoxy-12, 14-labdadiene-7, 8-diol to authenticate the structure. (Table 16)

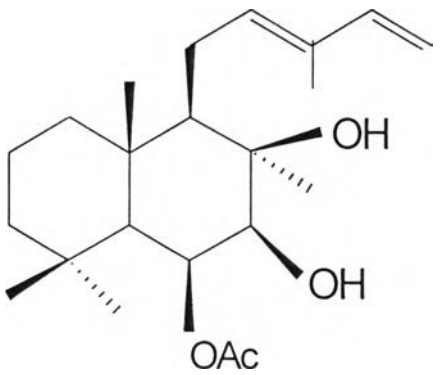


Figure 11 : Structure of compound 5

Table 16 **^{1}H-NMR chemical shift of Compound 5 and 6-acetoxy-12,14-labdadiene-7,8-diol**

position	Chemical shift δ_H (ppm)	
	Compound 5	6-acetoxy-12,14-labdadiene-7,8-diol
1	1.2-1.55	1.15-1.6
2		
3		
4	-	-
5	1.54	1.57
6	4.92(ddd)	4.85(ddd)
7	3.37(dd)	3.45(dd)
8	-	-
9	1.33(br)	1.38(br)
10	-	-
11	2.37(ddd),2.17(ddd)	2.43(ddd),2.18(ddd)
12	5.52(t)	5.56(t)
13	-	-
14	6.33(dd)	6.34(dd)
15	5.15(d),5.05(d)	5.05(d),4.90(d)
16	1.74(d)	1.77(d)
17	1.15(s)	1.17(s)
18	0.95(s)	0.79(s)
19	0.98(s)	0.86(s)
20	0.87(s)	0.87(s)
OAc	2.12(s)	2.09(s)

4.1.6 Structure elucidation of compound 6

The IR spectrum (Fig 43) revealed that this compound had a broad absorption band typical of a hydroxyl group at 3426 cm^{-1} and the strong absorption bands for C-H stretching vibration of methyl and methylene group at 2929 and 2842 cm^{-1}. There was a strong absorption band for C=C stretching of alkene at 1639 cm^{-1}. The IR spectrum of this compound is summarized in Table 17.

Table 17 The IR absorption band assignment of compound 6

Wave number (cm^{-1})	Intensity	Vibration
3426	Broad	O-H stretching vibration of alcohol
2929,2842	Strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1639	strong	C=C stretching vibration of alkene

From ^{1}H-NMR spectrum (Fig 44) of this compound indicated the presence of a chemical shift at δ_H 3.60 and 3.37 ppm which were assigned to the methine protons attached to a carbon bearing a hydroxyl group (H-6 and H-7). The olefinic proton signals were observed at δ_H 4.87, 5.04, 6.33 and 5.52 ppm.

From ^{13}C-NMR spectrum (Fig 45), Dept 90 (Fig 46) and Dept 135 (Fig 46), it was revealed that this compound had 20 carbons. The presence of five methyl carbons was also observed at δ_C 11.8, 19.4, 36.4, 22.1 and 16.9 ppm. The carbon signals in the downfield region of the spectrum at δ_C 71.7, 76.9 and 85.0 ppm were typical of the carbons attach to the hydroxyl group and the signals at δ_C 135.7, 132.6, 141.4 and 110.5 ppm were assigned to be olefinic carbons. In addition, the signals of quaternary carbons were also observed at δ_H 37.7, 76.9, 132.6 and 39.1 ppm.

The molecular formula was determined as $C_{20}H_{34}O_3$ using ^{1}H and ^{13}C NMR, which agreed well with m/z 322 molecular ion, obtained from EIMS **(Fig 47).** A degree of unsaturation of four was defined from its molecular formula ($C_{20}H_{34}O_3$). Thus, compound 6 should consist of two rings and two double bonds. From these data, it was indicated that this compound could have a labdane diterpene skeleton.

The combination of spectroscopic data (^{1}H-^{13}C NMR, COSY (Fig 48, Table 19), HMBC (Fig 49, Table 19), HMQC (Fig 50, Table 18), NOESY (Fig 51), IR spectra and mass spectra led to a combination of these carbons units to construct the structure of compound 6 as shown in Fig.13.

Table 18 **The HMQC spectra data of compound 6**

^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (Hz)
39.8 (t)	1.53 (br) ,1.38(br)
18.2(t)	1.45 (br), 1.43(br)
43.2(t)	1.35 (br), 1.28(br)
33.7(s)	-
57.3(d)	1.54 (br)
71.7(d)	3.60 (dd) (J = 9.5, 10.9)
85.0(d)	3.37 (d) (J = 9.5)
76.9(s)	-
59.4(d)	1.33 (br)
39.1(d)	-
23.7(t)	2.17 (ddd) (J =5.8,11.3,16.2), 2.37 (ddd) (J=6.1,12.8,15.9)
135.7(d)	5.52 (t) (J = 7.0, 14.0)
132.6(s)	-
141.4(d)	6.30 (dd) (J = 10.9, 17.4)
110.5(t)	5.04 (d) (J = 17.4), 4.83 (d) (J = 10.7)
11.8(q)	1.74 (s)
19.4(q)	1.15 (s)
36.4(q)	1.13 (s)
22.1(q)	0.98 (s)
16.9(q)	0.87 (s)

Table 19 The HMQC, HMBC and COSY spectra data of compound 6(Fig.14 and 15).

position	δ_C (ppm)	δ_H (ppm)	HMBC(H to C)	COSY
1	39.8 (t)	1.53 (br)	C-2,C-3,C-4,C-10,C-20	H-1 (1.38),H-2(1.45,1.43),H-20(0.88)
		1.38(br)		H-1 (1.53),H-2 (1.45,1.43)
2	18.2(t)	1.45 (br)	C-18	H-1 (1.53,1.28),H-2(1.43) ,H-3 (1.35)
		1.43(br)		H-1 (1.53,1.38) ,H-2 (1.45),H-3 (1.28)
3	43.2(t)	1.35 (br)	C-1,C-2,C-4,C-18,C-19	H-2 (1.45) ,H-3 (1.28) ,H-19(0.98)
		1.28(br)		H-2 (1.43),H-3 (1.35),H-18 (1.15)
4	33.7(s)	-	-	-
5	57.3(d)	1.54 (br)	C-1,C-3,C-6,C-7,C-18,	H-9 (1.33) ,H-6 (3.60)
			C-20,C-10	
6	71.7(d)	3.60 (dd)	C-5,C-7	H-7 (3.37) ,H-5 (1.54)
7	85.0(d)	3.37 (d)	C-5,C-6,C-8,C-9,C-17	H-6 (3.6),H-9 (1.33)
8	76.9(s)	-	-	
9	59.4(d)	1.33 (br)	C-6,C-7,C-11,C-12,	H-5 (1.54) , H-7(3.37) ,H-11 (2.17,2.37)
			C-17,C-20	,H-12 (5.52) ,H-17(1.13)
10	39.1(d)	-	-	
11	23.7(t)	2.17 (dt)	C-9,C-8,C-10,C-13	H-9 (1.33), H-11 (2.37) ,H-12 (5.52)
		2.37 (d)		H-9 (1.33) ,H-11 (2.17) ,H-12(5.52)
12	135.7(d)	5.52 (t)	C-9,C-11,C-14,C-16	H-9 (1.33),H-11(2.17,2.37),H-1(6.33)
13	132.6(s)	-	-	
14	141.4(d)	6.30 (dd)	C-13,C-12,C-16	H-12 (5.52) ,H-15 (5.04)
15	110.5(t)	5.04 (d)	C-13,C-14	H-16 (1.74)
		4.83 (d)	C-13	H-14 (6.33)
16	11.8(q)	1.74 (s)	C-12,C-13,C-16	H-15 (5.04)
17	19.4(q)	1.15 (s)	C-7,C-9	H-9 (1.33)
18	36.4(q)	1.13 (s)	C-4,C-5,C-3,C-19	H-3 (1.28),H-19(0.98)
19	22.1(q)	0.98 (s)	C-3,C-4,C-5,C-18	H-3 (1.35) ,H-18 (1.13), H-20 (0.88)
20	16.9(q)	0.87 (s)	C-1,C-5,C-9,C-10	H-1 (1.53) ,H-19 (0.98)

[a] Carbon type as determined by DEPT experiment spectra :s=singlet, d=doublet, t=triplet, q=quartet.

From all of the above spectral data, this compound was consistent with those of crotomachlin or 12,14-labdadiene-6, 7,8-triol [mp = 81-83 °C, $[\alpha]_D^{20}$ −14.2° ($CHCl_3$; *c*1.0)] previously reported in the literature [44-46]. In comparison of the ^{13}C-NMR chemical shift of compound 6 with those of crotomachlin, it was suggested that compound 6 was indeed crotomachlin. (Table 20)

Table 20 **Comparison of ^{13}C-NMR chemical shift of compound 6 with Crotomachlin**

Carbon no.	Chemical shift δ_C (ppm)	
	Compound 6	Crotomachlin
1	39.8 (t)	40.5(t)
2	18.2(t)	18.4(t)
3	43.2(t)	43.7(t)
4	33.7(s)	33.8(s)
5	57.3(d)	57.1(d)
6	71.7(d)	72.5(d)
7	85.0(d)	84.5(d)
8	76.9(s)	76.6(s)
9	59.4(d)	60.6(d)
10	39.1(d)	38.5(d)
11	23.7(t)	23.7(t)
12	135.7(d)	136.7(d)
13	132.6(s)	132.4(s)
14	141.4(d)	141.7(d)
15	110.5(t)	110.2(t)
16	11.8(q)	12.0(q)
17	19.4(q)	19.4(q)
18	36.4(q)	35.8(q)
19	22.1(q)	23.0(q)
20	16.9(q)	17.2(q)

The NOESY correlation (Fig 12) of crotomachlin[44] showed that the ^{1}H-^{1}H correlation was observed between the protons at H-6 (3.60) and H-7 (3.37), H-9 (1.33) and proton of methyl (H-17), H-6 (3.60) and proton of methyl (H-17), and H-6 (3.60) and H-9 (1.33). Therefore, relative stereochemistry of Compound 6 agreed well with that of crotomachlin (Fig 16). Thus, it was the first recorded of trihydroxyl labdane diterpene from *C. oblongifolius*

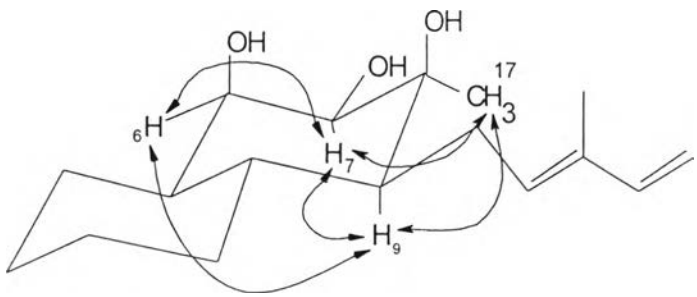


Figure 12 : The NOESY correlation of crotomachlin

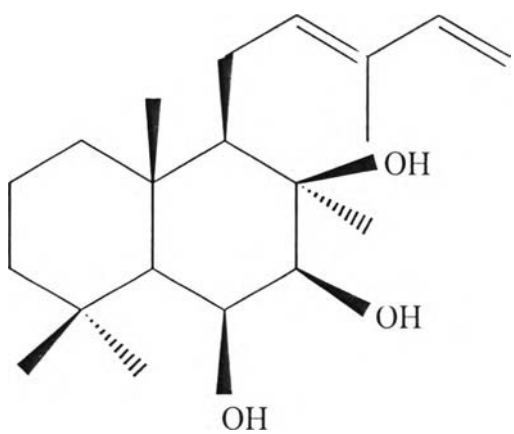


Figure 13: The structure of compound 6

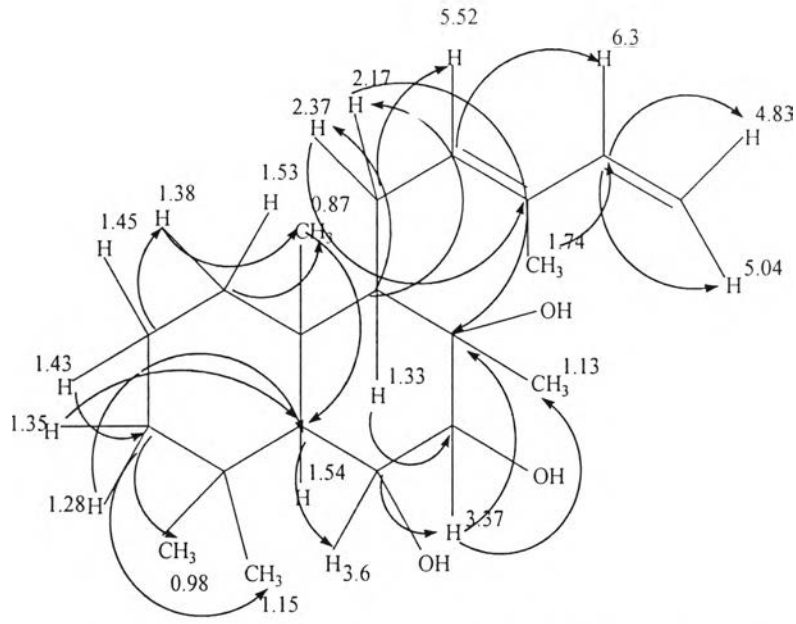


Figure 14 : The HMBC correlation of compound 6

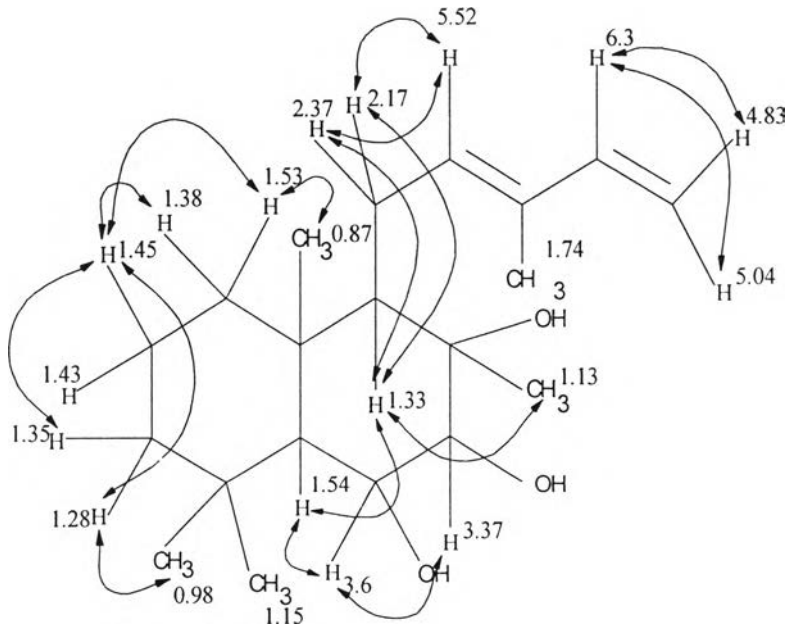


Figure 15 : The COSY correlation of compound 6

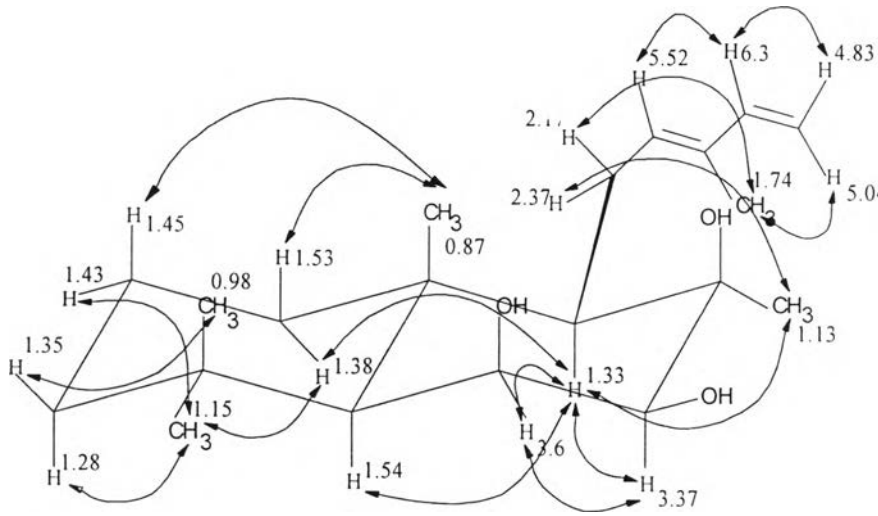


Figure 16 : The NOESY correlation of compound 6

4.1.7 Structure elucidation of Compound 4a

The IR spectrum of compound 4a (Fig 52) showed an absorption band at 3457 cm^{-1} , which was typical of a hydroxyl group and the strong absorption band at 2929 cm^{-1} and 2868 cm^{-1} were due to C-H stretching of methyl and methylene group. Also, the strong absorption band at 1726 cm^{-1} was characteristic of C=O stretching vibration band of ester. The medium absorption bands of C=O stretching of alkene appeared at 1654 cm^{-1} and 1639 cm^{-1} and a strong absorption band at 1278 cm^{-1} suggested the presence of C-O stretching vibration of ester. The IR absorption bands were assigned as shown in Table 21.

Table 21 The IR absorption band assignment of compound 4a

Wave number (cm^{-1})	Intensity	Vibration
3457	broad	O-H stretching vibration of alcohol
2929,2868	strong	C-H stretching vibration of $-CH_3$, $-CH_2-$
1726	strong	C=O stretching vibration of ester
1654,1659	medium	C=C stretching vibration of alkene
1278	strong	C-O stretching vibration of ester

From EIMS spectrum (Fig 55) of compound 4a it was suggested that the molecular formula was $C_{22}H_{36}O_3$, which was agreed well with the molecular ion at m/z 348. The ion at m/z 330 and 288 were formed by the loss of H_2O and an acetic acid group, respectively.

The ^{13}C-NMR spectrum (Fig 54) was consistent with those of compound 4 except for the presence of an ester group at δ_C 171.3 ppm which was typical of a carbonyl of ester group. The presence of a methyl group was reveals at δ_C 21.3 ppm.

The ^{1}H-NMR spectrum (Fig 53) was similar to that of compound 4. The clear difference was the moving up field of a proton at carbon attached to the ester group (δ_H 2.09 ppm).

From all this spectroscopic data it was concluded that compound 4a was consistent to that of 7-acetyoxy-12,14-labdadiene-8-ol[$[\alpha]_D^{20}$ –36.1° ($CHCl_3$; *c*1.0)], which was previously isolated from *Koanophyllon conglobatum* [43]. The ^{1}H-NMR chemical shift of this compound 4a was compared with that of 7-acetoxy-12,14-labdadiene-8-ol to authenticate the structure (Table 22).

Table 22 ^{13}C-NMR chemical shift of compound **4a** compared with 7-acetoxy-12,14-labdadiene-8-ol

position	Chemical shift δ_H (ppm)	
	Compound 4a	7-acetoxy-12,14-labdadiene-8-ol
1	39.5(t)	39.8(t)
2	18.3(t)	18.5(t)
3	41.5(t)	41.6(t)
4	33.1(s)	33.2(s)
5	53.2(d)	53.6(d)
6	26.2(t)	27.2(t)
7	81.3(d)	80.3(d)
8	75.9(s)	75.1(s)
9	60.9(d)	60.6(d)
10	39.5(s)	39.2(s)
11	23.4(t)	23.5(t)
12	135.9(t)	135.5(t)
13	132.2(s)	132.7(s)
14	141.2(d)	141.5(d)
15	110.1(t)	110.6(t)
16	11.8(q)	11.9(q)
17	17.8(q)	17.9(q)
18	33.3(q)	33.5(q)
19	21.8(q)	21.6(q)
20	15.5(q)	15.6(q)
21	171.5(s)	171.3(s)
22	21.3(q)	21.5(s)

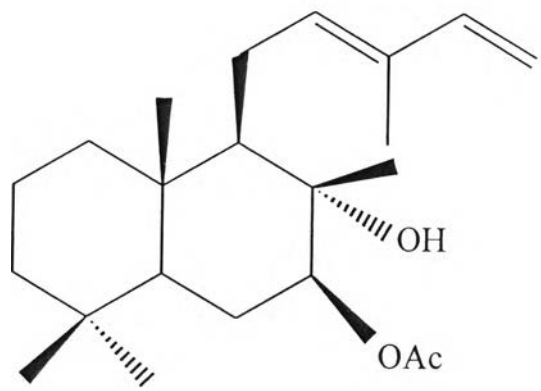


Figure 17: Structure of compound 4a

4.2 Result of Biological activity test

Following the preliminary cytotoxic screening test against 6 cell lines namely HS 27 (fibroblast), Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), Kato-3 (gastric) and BT 474 (breast), the hexane crude extract of stem barks showed high activity (Table 23).

The *in vitro* activity of some compounds (10 ug/ml) from *C. oblongifolius* Roxb. against 6 cancer cell lines are reported in Table 23.

Table 23 Cytotoxic activity against 6 cell lines of compounds from *C. oblongifolius* and Doxorubicin hydrochloride

compound	% survival					
	HS 27 (fibroblast)	Kato-3 (gastric)	BT 474 (breast)	Chago (lung)	SW 620 (colon)	Hep-G2 (hepatoma)
1	85	74	72	78	50	58
2	76	95	98	98	83	64
4	18	8	10	4	5	4
5	37	30	75	45	25	38
6	35	9	27	24	4	8
4a	73	38	88	59	34	37
Doxorubicin *	35	54	28	63	20	17
Crude hexane	59	9	35	7	7	9

Doxorubicin*: Doxorubocin hydrochloride was used as positive control.

From Table 23, it indicated that compound 4 and compound 6 showed strong cytotoxic activity against 5 cancer cell lines. These compounds contained two alcohols and three alcohol groups, respectively. From previous studies, compound 4 was tested against Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), Kato-3 (gastric) and BT 474 (breast) tumer cell lines [5] and compound 6 was tested against antilipoxygenase activity [45]. Moreover, the cytotoxic activity of compound 5 and compound 4a showed moderate activity against Hep-G2 (hepatoma), SW 620 (colon), Chago (long) and Kato-3 (gastric) cancer cell lines. Moreover, they were tested against cancer cell lines for the first time. These compounds contained ester and hydroxyl group. From pervious studies, compound 5 was tested against antilipoxygenase[45] and compound 4a was tested against antibacterial[43] . Finally, compound 1 and compound 2 showed weak cytotoxic activity against Hep-G2 (hepatoma) and SW 620 (colon) cancer cell lines. The bioassay against P-388 of compound 2 (poilaneic acid) has been reported previously [38].