

CHAPTER III

RESULTS AND DISCUSSION

3.1 Isolation of crude extract of *Croton oblongifolius* Roxb.

3.1.1 Separation of hexane crude extract

The hexane crude extract was obtained as a yellowish green oil (75 g) after evaporation. The crude extract (75 g) was fractionated by Silica gel column chromatography using Merck's silica gel Art. 7734.1000 (70-230 mesh ASTM) as adsorbent. The column was eluted with hexane-chloroform gradient in a stepwise fashion. The separation of hexane crude extract gave compounds 1-3 shown in Table 2.

3.1.2 Separation of ethylacetate crude extract

Concentrated ethylacetate crude extract (5 g) was measured by NMR, the result was the same as the hexane crude extract, showing that many compounds in the ethylacetate crude extract were identical to hexane crude extract.

3.1.3 Separation of methanol crude extract

The remaining extraction from ethylacetate resulted in the methanol crude extract (0.5 g). It was still identical to the hexane crude extract by comparing the spectral data.

Table 2. The results of separation of hexane extract crude by column chromatography.

Compounds	Physical appearance	wt.	% wt. by wt.
1	Transparent oil	4.2	5.6
2	Transparent oil	2.5	3.3
3	Transparent oil	0.3	0.4

3.2 Characterization of isolated compounds

3.2.1 Properties of Compound 1

Compound 1 is a viscous transparent oil (4.2 g, 5.60% yield from hexane crude and 0.53% yield from starting material), $[\alpha]_D^{25} -85.5^\circ$ (CHCl_3 , c 1.0). The R_f value was 0.30 in 100% chloroform (SiO_2).

FT-IR spectrum (KBr), λ_{max} (cm^{-1}): 2400-3600 (br), 2971, 2930 and 2873 (m), 1685(s), 1634(m), and 1460 and 1396(m) and 1381(m) and 1280(m). (Fig. 10)

$^1\text{H-NMR}$ spectrum (CDCl_3 , 200 MHz) δ (ppm) 7.33(1H, s), 7.19(1H, s), 6.85 (1H, s), 6.26(1H, s), 2.16-2.50(6H, m), 1.40-1.73(8H, m), 1.26(3H, s), 0.84(3H, d), 0.76(3H, s). (Fig. 11)

$^{13}\text{C-NMR}$ spectrum (CDCl_3 , 50 MHz) δ (ppm) 172.8(s), 142.6(d), 141.5(s), 140.2(d), 138.3(d), 125.4(s), 110.9(d), 46.7(d), 38.8(s), 38.6(t), 37.6(s), 36.3(d), 35.8 (t), 27.5(t), 20.5(q), 18.4(q), 18.2(t), 17.5(t), 16.0(q). (Fig. 12)

EI MS spectrum m/z : 316 [M^+] 299, 283, 221, 203, 105 and 96 (Fig. 14).

3.2.2 Properties of Compound 2

Compound 2 is a transparent oil (2.5 g, 3.3%), $[\alpha]_D^{27} +100^\circ$ (CHCl_3 , c 13.3), R_f : 0.65 (10% ethyl acetate in hexane), UV (EtOH) λ_{max} 234 sh ($\log \epsilon$ 4.21).

FT-IR spectrum (KBr) (Fig. 15) ν_{max} (cm^{-1}) : 3426(br), 2965(s), 2827(s), 1706 (s), 1623(m), 1455(m), 1383(s), 780(w).

$^1\text{H-NMR}$ spectrum (CDCl_3 , 500 MHz) (Fig. 16) δ (ppm) : 5.78(1H, s), 5.42 (1H, ddd, $J=2.44, 2.44, 4.89$), 2.71(1H, ddd, $J=5.49, 14.65, 14.65$), 2.24(1H, ddd, $J=14.04, 3.96, 3.96$), 2.19(1H, m), 2.13(1H, ddd, $J=3.36, 5.19, 13.43$), 1.85(1H, m), 1.62(1H, dd, $J=4.88, 11.9$), 1.46(3H, ddd, $J=3.97, 13.73, 13.73$), 1.10(3H, s), 1.03(3H, s), 0.99(3H, s), 0.98(3H, d, $J=4.58$), 0.98(3H, d, $J=4.58$).

$^{13}\text{C-NMR}$ spectrum (CDCl_3 , 125 MHz) (Fig. 17) δ (ppm) : 216.6(s), 145.6(d), 135.2(s), 122.0(d), 120.0(d), 51.4(d), 50.0(d), 47.5(s), 38.0(t), 34.8(d), 34.7(t), 34.6(s), 27.3(t), 25.0(q), 24.1(t), 22.6(t), 22.1(q), 21.3(q), 20.8(q), 13.4(q).

m/z (EI) (rel int.) (Fig. 19) : 286[M⁺](100), 269(44), 243(22), 227(31), 197(17), 185(16), 157(18), 135(34), 105(44), 91(34).

3.2.3 Properties of Compound **3**

Compound **3** is a viscous transparent oil (0.3 g, 0.4%), $[\alpha]_D^{25} +4.31^\circ$ (CHCl₃, c 1.0), UV (EtOH) λ_{\max} 241.5 sh (log ϵ 3.29).

FT-IR spectrum (KBr) (Fig. 24) ν_{\max} (cm⁻¹) : 3078(br), 2930(s), 2863(s), 1706(s), 1655(s), 1634(s), 1450(s), 1414(s).

¹H-NMR spectrum (CDCl₃, 200 MHz) (Fig. 25) δ (ppm) : 6.00(1H, m), 5.02(1H, m), 5.00(1H, m), 4.85(2H, m), 4.65(2H, m), 4.56(1H, m), 2.82(1H, dd), 2.40(1H, m), 2.30(1H, m), 2.17(2H, m), 1.95(1H, m), 1.73(3H, s), 1.72(2H, m), 1.70(1H, m), 1.64(2H, m), 1.56(1H, m), 1.42(1H, m), 1.26(3H, m), 1.24(1H, m), 0.82(3H, s).

¹³C-NMR spectrum (CDCl₃, 50 MHz) (Fig. 26) δ (ppm) : 180.8(s), 151.8(s), 147.4(s), 137.3(d), 116.1(t), 113.6(t), 106.7(t), 54.6(d), 50.7(d), 41.0(d), 40.3(d), 38.9(s), 32.1(t), 31.4(t), 31.3(t), 28.1(t), 27.5(t), 27.3(s), 23.8(q), 16.7(q).

m/z (EI) (rel int.) (Fig. 28) : 302[M⁺](68), 287(40), 259(56), 201(80), 145(72), 105(84), 91(100).

3.2.4 Properties of Compound **2a**

Compound **2** is an orange-red crystalline (670 mg, 67% yield), m.p. 115 °C, R_f 0.45 (10% ethyl acetate in hexane).

FT-IR spectrum (neat) (Fig. 29) ν_{\max} (cm⁻¹) : 3324(s), 2960(s), 1621(s), 1511(s), 1337(s), 758(s).

¹H-NMR spectrum (CDCl₃, 200 MHz) (Fig. 30) δ (ppm) : 11.22(1H, s), 9.09(1H, m), 8.26(1H, m), 7.93(1H, d), 5.78(1H, s), 5.44(1H, m), 2.68(1H, m), 2.41(1H, m), 2.17(1H, m), 2.13(1H, m), 1.82(1H, m), 1.62(1H, m), 1.31(1H, m), 1.27(3H, s), 1.24(3H, s), 1.03(3H, s), 1.00(3H, s), 0.98(3H, d).

¹³C-NMR spectrum (CDCl₃, 50 MHz) (Fig. 31) δ (ppm) : 167.5(s), 145.7(s), 145.6(s), 137.3(s), 135.1(s), 129.8(d), 128.8(s), 123.4(d), 122.1(d), 120.6(d), 116.4(d),

51.6(d), 50.0(d), 42.7(s), 37.6(t), 35.0(d), 34.9(s), 27.3(t), 27.2(q), 24.4(t), 23.7(q), 22.7(t), 21.4(q), 20.8(q), 20.6(t), 13.4(q).

m/z (EI) (rel int.) (Fig. 33) : 466[M^+](100), 305(16), 199(38), 157(47), 131(78), 105(78).

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

3.3.1 Structure elucidation of Compound 1

The IR spectrum of Compound 1 is shown in Fig. 10 and the absorption peaks were assigned as shown in Table 3. Its IR spectrum showed important absorption bands at 2400-3600 cm^{-1} (O-H stretching vibration of alcohol), 2971, 1930 and 1873 cm^{-1} (C-H stretching vibration), 1685 cm^{-1} (C=O stretching vibration of carbonyl group) and 1634 cm^{-1} (C=C stretching vibration of alkene).

Table 3. The IR absorption bands assignment of Compound 1

Wave number (cm^{-1})	Intensity	Tentative Assignment
2400-3600	Broad	O-H stretching vibration of acid
2971, 2930, 2873	Strong	C-H stretching vibration of -CH ₃ , -CH ₂
1685	Strong	C=O stretching vibration of carbonyl group
1634	Medium	C=C stretching vibration of alkene

The $^1\text{H-NMR}$ spectrum (Fig. 11) of Compound 1 indicated that it possesses three methyl groups (δ 0.76, 0.84 and 1.26 ppm), three olefinic protons of furanoid group (δ 7.33, 7.19 and 6.26 ppm) and one vinylic proton (δ 6.85 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 13) showed 120 signals. Six signals of olefinic carbons appeared at δ 142.6, 141.5, 140.2, 138.3, 125.4

and 110.9 ppm. The signal at 172.8 ppm should be the carbonyl of carboxylic acid. There were thirteen sp^3 carbon signals at δ 46.7(d), 38.8(s), 38.6(t), 37.6(s), 36.3(d), 35.8(t), 27.5(t), 25.3(t), 20.5(q), 18.48(q), 18.2(t), 17.5(t) and 16.0(q) ppm.

Its molecular formula was established as $C_{20}H_{28}O_3$, which was confirmed by observing the molecular ion at m/z 316 (Fig. 13). The molecular formula, $C_{20}H_{28}O_3$, of Compound 1 defined a degree of unsaturation of seven, therefore, Compound 1 must consist of one ring of furan (DBE = 3) in addition to one double bond, two rings and one carbonyl group of carboxylic acid.

Compound 1 exhibited the ^{13}C -NMR chemical shifts identical to hardwickiic acid [19]. A comparison of the ^{13}C -NMR chemical shifts of Compound 1 and hardwickiic acid is shown in Table 4. These data indicated that Compound 1 was hardwickiic acid [5].

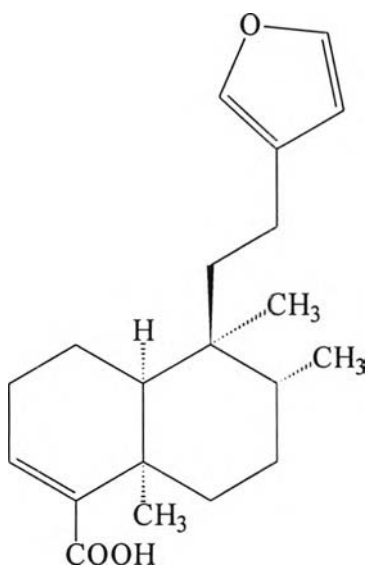


Fig. 3 The structure of Compound 1

Table 4. ^{13}C -NMR chemical shifts of Compound 1 and Hardwickiic acid [5]

Carbon	Chemical shifts (ppm)	
	Compound <u>1</u>	Hardwickiic acid
1	17.5t	17.5t
2	27.5t	27.5t
3	140.2d	140.2d
4	141.5s	141.5s
5	37.6s	37.6s
6	35.8t	35.8t
7	27.5t	27.3t
8	36.3d	36.3d
9	38.8s	38.8s
10	46.7d	46.7d
11	38.6t	38.6t
12	18.2t	18.2t
13	125.4s	125.5s
14	110.9d	110.9d
15	142.6d	142.7d
16	138.3d	138.3d
17	16.0q	16.0q
18	172.8s	172.8s
19	20.5q	20.5q
20	18.2q	18.3q

3.2.2 Structure Elucidation of Compound 2

The IR spectrum of Compound 2 (Fig. 15) is summarized in Table 5.

Table 5. The IR absorption bands assignment of Compound 2

Wave number (cm ⁻¹)	Intensity	Tentative Assignment
2965	Strong	C-H stretching vibration of -CH ₂ , -CH ₃
1706	Strong	C=O stretching vibration
1623	Medium	C=C stretching vibration
1455, 1383	Medium	-CH ₂ , -CH ₃ bending

The ¹H-NMR spectrum (Fig. 16, Table 7) of Compound 2 showed three methyl groups attaching to quaternary carbons (0.99, 1.03 and 1.10 ppm), two similar methyl groups (0.98 and 0.98 ppm) and two olefinic protons (5.42 and 5.78 ppm).

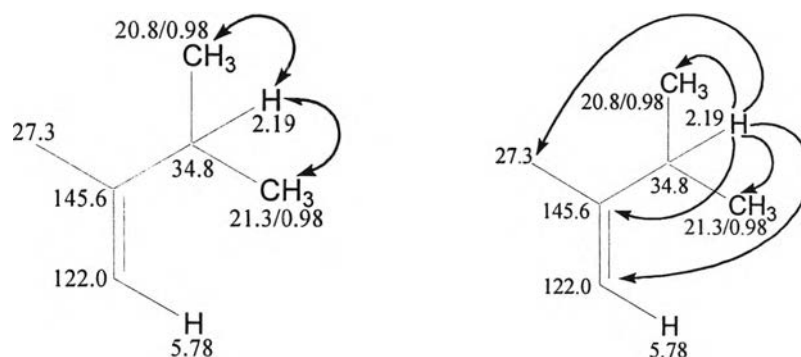
The ¹³C-NMR spectrum (Fig. 17, Table 6) showed 20 lines. Four signals of olefinic carbons appeared at 145.6, 135.2, 122.0 and 120.6 ppm.

DEPT 90 experiments (Fig. 18), indicated the presence of two sp² methine carbons at 120.6 and 122.0 ppm and three saturated methines at 34.8, 50.0 and 51.4 ppm. The DEPT-135 spectrum (Fig. 18) showed five methylene carbons at 22.6, 24.1, 27.3, 34.7 and 38.0 ppm and five methyl carbons at 13.4, 20.8, 21.3, 22.1 and 25.0 ppm (Table 6), which indicated that the carbon signals at 34.6, 47.5, 135.2, 145.6 and 216.6 were quaternary.

Compound 2 showed a molecular ion with *m/z* 286 (C₂₀H₃₀O), which indicated DBE of 6. The information from 2D-NMR techniques, COSY correlations (Fig. 19, Table 8), HMQC correlations (Fig. 20, Table 8), HMBC correlations (Fig. 21, Table 8) were used to assist in the interpretation of the structure of Compound 2.

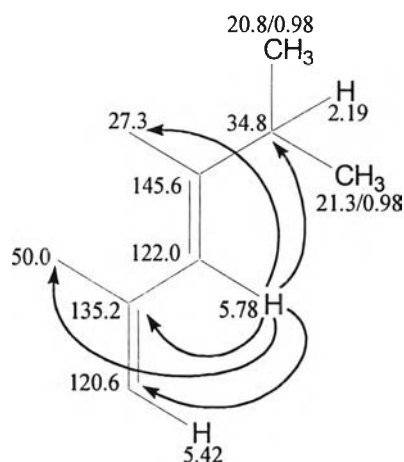
Two-dimensional NMR techniques were used for assisting the structure assignment. The protons directly attached to the carbons in Compound 2 were assigned by HMBC spectra (Fig. 21, Table 8).

Crucial long-range ^1H - ^{13}C correlations were obtained by HMBC correlations (Fig 21). The proton at 2.19 ppm was coupled with methyl carbon at 20.8, 21.3 ppm and was coupled with the carbon at 145.6, 27.3 and 122.0 ppm HMQC spectrum (Fig 20) showing that the carbon at 122.0 ppm was connected with the proton at 5.78 ppm and the proton at 219 ppm was connected with the carbon at 34.8 ppm. According to COSY spectrum (Fig 19) the proton at 2.19 ppm was coupled with the proton of both methyls (0.98 ppm) (see Scheme 2).



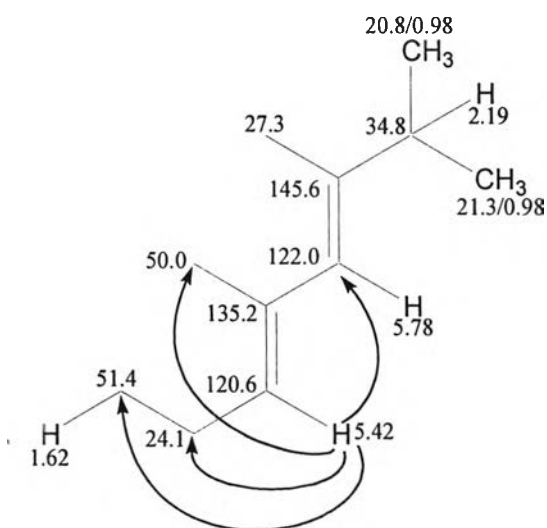
Scheme 2

HMBC spectrum showed that the proton at 5.78 ppm was coupled with carbon at 34.8, 27.3, 135.2, 50.0 and 120.6 ppm. According to HMQC spectrum, the carbon at 120.6 ppm was connected with the proton at 5.42 ppm, the proton at 5.78 ppm was a singlet peak showing that the quaternary carbon was the next carbon (see Scheme 3).



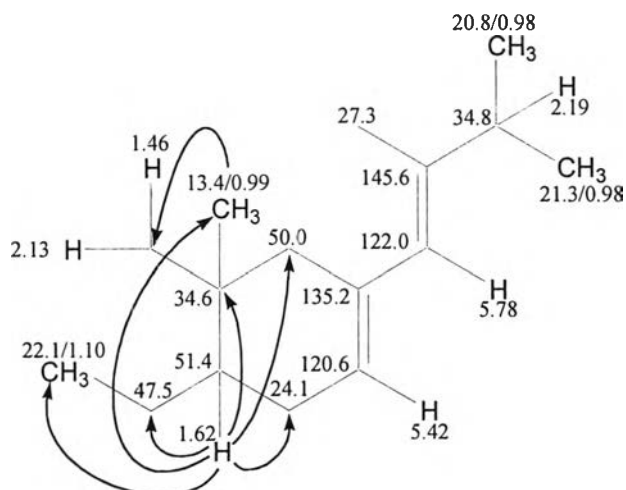
Scheme 3

HMBC spectrum showed that the proton at 5.42 ppm was coupled with carbon at 122.0, 50.0, 24.1 and 51.4 ppm. According to HMQC spectrum, the carbon at 51.4 ppm was connected with the proton at 1.62 ppm (see Scheme 4).



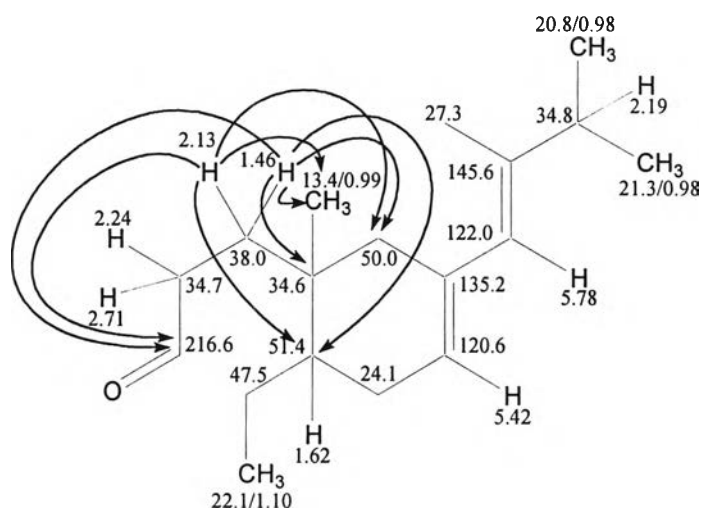
Scheme 4

HMBC spectrum showed that the proton at 1.62 ppm was coupled with carbon at 24.1, 50.0, 34.6, 47.5, 22.1 ppm and the methyl carbon at 13.4 ppm and the proton of methyl (0.99 ppm.) was coupled with the carbon at 38.0 ppm. According to the HMQC spectrum, the carbon at 38.0 ppm was connected with proton at 1.46 ppm and 2.13 ppm (see Scheme 5).



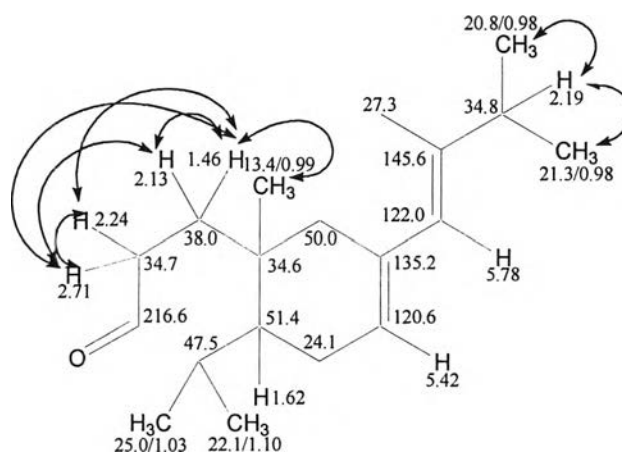
Scheme 5

HMBC spectrum showed that the proton at 1.46 ppm was coupled with carbon at 34.6, 51.4, 50.0, 216.6 ppm and methyl carbon at 13.4 ppm and the proton at 2.13 ppm was coupled with carbon at 51.4, 50.0, 216.6 and 13.4 ppm. HMQC spectrum showed that the carbon at 34.7 ppm was connected with proton at 2.71 ppm and 2.24 ppm. Furthermore, COSY spectrum showed that the proton at 1.46 ppm was coupled with the proton at 0.99, 2.71, 2.24 and 2.13 ppm. The proton at 2.13 ppm was coupled with proton at 2.71 and 1.46 ppm (see Scheme 6).



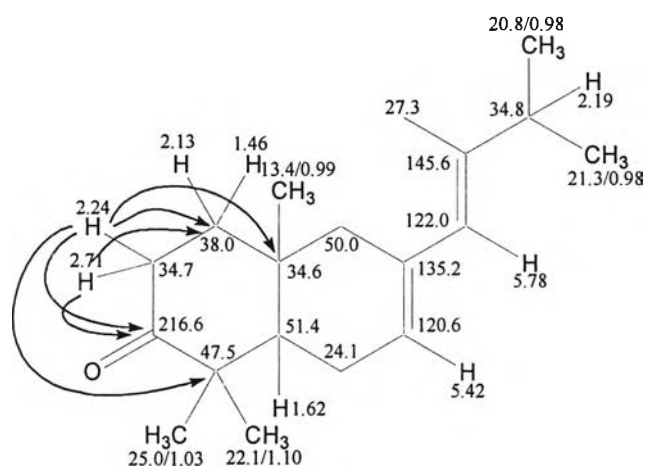
Scheme 6

COSY spectrum showed that the proton at 1.46 ppm was coupled with proton at 2.13, 2.24, 2.71 and 0.99 ppm. The proton at 2.13 ppm was coupled with proton at 1.46 and 2.24 ppm (see Scheme 7).



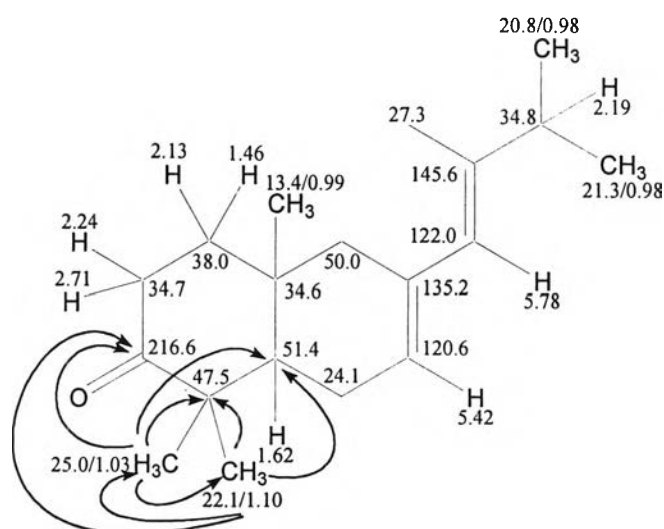
Scheme 7

HMBC spectrum showed that the proton at 2.24 ppm was coupled with carbon at 34.6, 38.0, 47.5 and 216.6 ppm. The proton at 2.71 ppm was coupled with the carbon at 38.0 and 216.6 ppm (see Scheme 8).



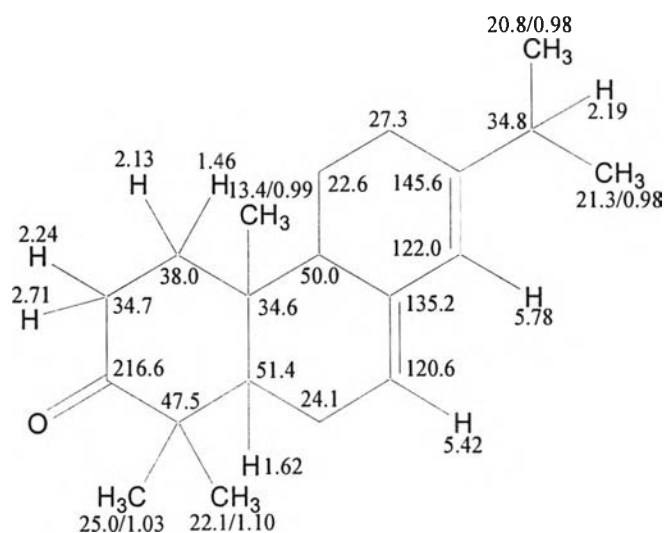
Scheme 8

HMBC spectrum showed that the proton of methyl (1.10 ppm.) was coupled with carbon at 51.4, 47.5, 216.6 and methyl carbon at 25.0 ppm. And the proton of methyl (1.03 ppm.) was coupled with carbon at 22.1, 216.6, 51.4 and 47.5 ppm (see Scheme 9)



Scheme 9

From the terminating carbon at 27.3 and 50.0 ppm, it was most suitable to add the last carbon at 22.6 ppm (see Scheme 10).



Scheme 10

Thus, Compound 2 was identical to the structure as shown in Figure 4. Compound 2 exhibited the ^{13}C -NMR chemical shifts identical to abieta-7, 13-dien-3-one [20]. A comparison of the ^{13}C -NMR chemical shifts of Compound 2 and abieta-7, 13-dien-3-one was shown in Table 3. These data indicated that Compound 2 was abieta-7, 13-dien-3-one. The COSY correlations, long-range C-H correlations by HMBC and NOESY spectrum are summarized in Fig 5, Fig 6 and Fig 7.

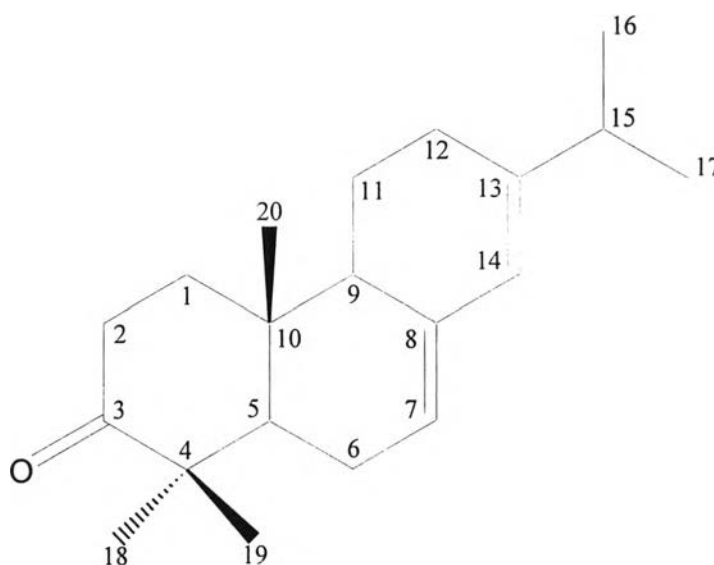


Fig. 4 The structure of Compound 2

Table 6 ^{13}C -NMR chemical shifts of Compound 2 and Abieta-7, 13-dien-3-one [20]

Carbon	Chemical shifts (ppm)	
	Compound <u>2</u>	Abieta-7, 13-dien-3-one,
1	38.0t	38.1t
2	34.7t	34.9t
3	216.6s	216.3s
4	47.5s	47.6s
5	51.4d	51.6d
6	24.1t	24.2t
7	120.6d	120.6d
8	135.2s	135.4s
9	50.0d	50.1d
10	34.6s	34.9s
11	22.6t	22.8t
12	27.3t	27.4t
13	145.6s	145.6s
14	122.0d	122.3d
15	34.8d	34.9d
16	20.8q	20.9q
17	21.3q	21.4q
18	22.1q	22.1q
19	25.0q	25.1q
20	13.4q	13.5q

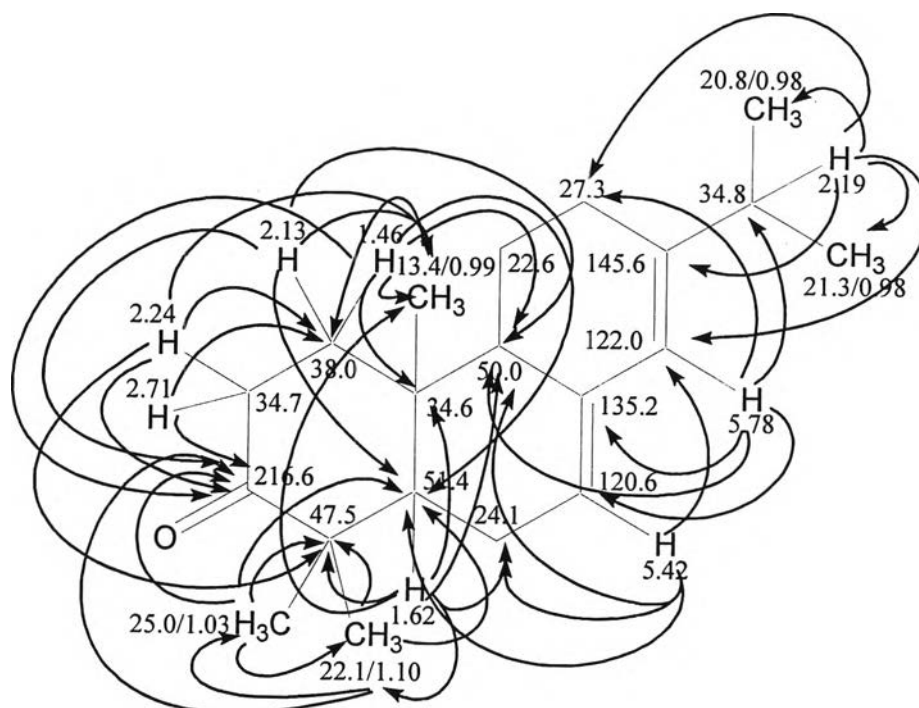


Fig. 5 The HMBC correlations of Compound 2

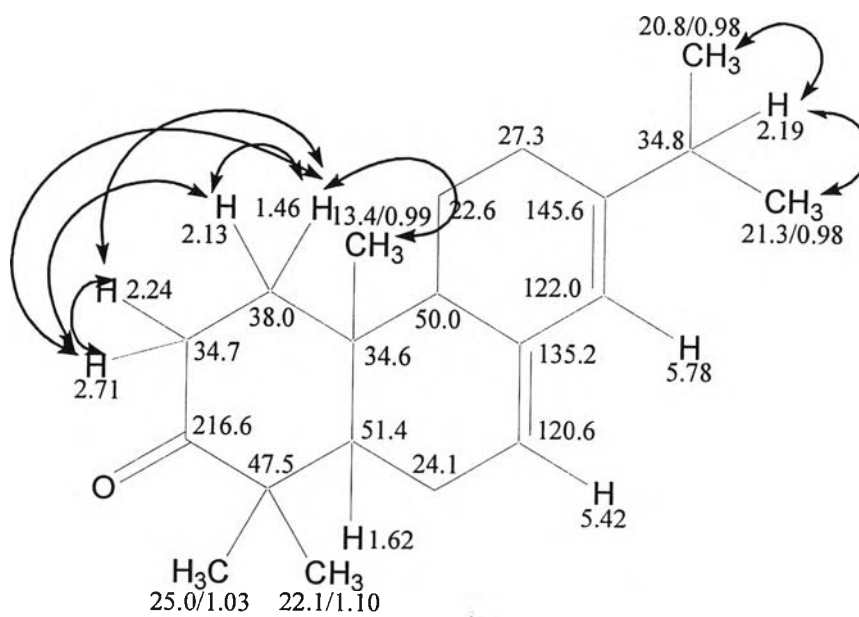


Fig. 6 The COSY correlations of Compound 2

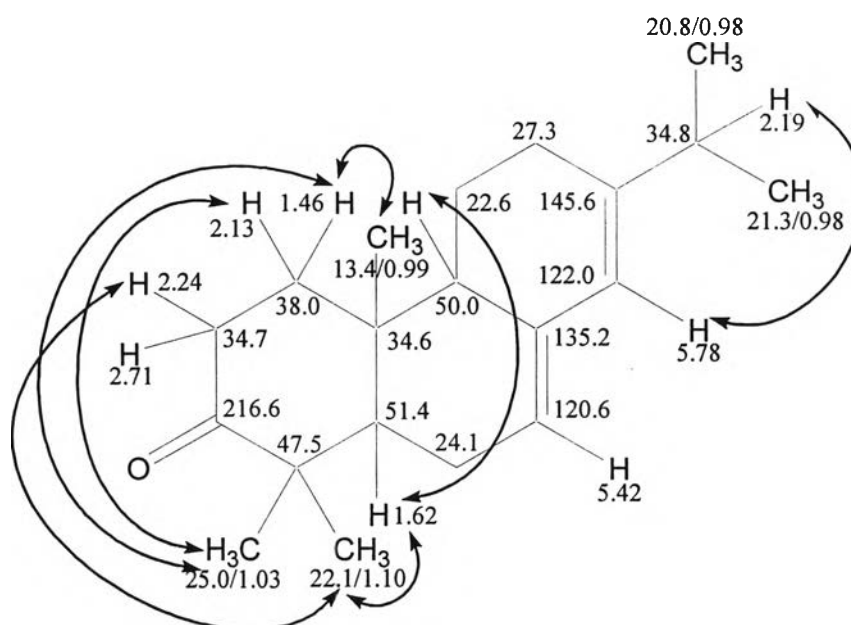


Fig. 7 The NOESY correlations of Compound 2

Table 7. The HMQC spectral data of Compound 2

¹³ C-NMR (ppm)	¹ H-NMR (ppm), coupling constant (Hz)
38.0t	1.46ddd(J=3.97, 13.73, 13.73) 2.13ddd(J=3.36, 5.19, 13.43)
34.7t	2.24ddd(J=14.04, 3.96, 3.96) 2.71ddd(J=5.49, 14.65, 14.65)
216.6s	-
47.5s	-
51.4d	1.62dd(J=4.88,11.9)
24.1t	-
120.6d	5.42ddd(J=2.44, 2.44, 4.89)
135.2s	-
50.0d	1.85m
34.6s	-
22.6t	-
27.3t	-
145.6s	-
122.0d	5.78s
34.8d	2.19m
20.8q	0.98d(J=4.58)
21.3q	0.98d(J=4.58)
22.1q	1.10s
25.0q	1.03s
13.4q	0.99s

Table 8 The HMQC, HMBC and COSY spectral data of Compound 2

Position	δ_C^a	δ_H	HMBC (H to C)	COSY
1	38.0t	1.46	C-3, C-5, C-9, C-10, C-20	H-1(2.13), H-2(2.24), H-2(2.71), H-20(0.99)
		2.13	C-3, C-9, C-20	H-1(1.46), H-2(2.71)
2	34.7t	2.24	C-1, C-3, C-4, C-10	H-1(1.46), H-2(2.71)
		2.71	C-1, C-3	H-1(1.46), H-1(2.13), H-2(2.71)
3	216.6s	1.50	-	-
4	47.5s	1.50	-	-
5	51.4d	1.20	-	-
6	24.1t	1.48	-	-
7	120.6d	-	-	-
8	135.2s	1.31	C-5, C-6, C-9, C-14	-
9	50.0d	2.09	-	-
10	34.6s	2.37	C-4, C6, C10, C19, C20	-
11	22.6t	7.33	-	-
12	27.3t	-	-	-
13	145.6s	2.25	-	-
14	122.0d	-	C-7, C-8, C-9, C-12, C-15	-
15	34.8d	1.55	C-13, C-13, C-14, C-16, C- 17	H-16(0.98), H-17(0.98)
16	20.8q	1.78	-	H-15(2.19)
17	21.3q	4.60	-	H-15(2.19)
18	22.1q	-	C-3, C-4, C-19	-
19	25.0q	5.59	C-3, C-4, C-5, C-18	-
20	13.4q	1.64	C-4	-

^aCarbon type as determined by DEPT experiments spectra : s = singlet, d = doublet,

t = triplet, q = quartet.

3.2.3 Structure elucidation of Compound 3

The IR spectrum of Compound 3 is shown in Fig.24 and the absorption peaks were assigned as shown in Table 9. Its IR spectrum showed important absorption bands at 3078 cm^{-1} (O-H stretching vibration of carboxyl group), 2930 and 2863 cm^{-1} (C-H stretching vibration), 1706 cm^{-1} (C=O stretching vibration of carbonyl group) and 1655 cm^{-1} (C=C stretching vibration of alkene).

Table 9 The IR absorption bands assignment of Compound 3

Wave number (cm^{-1})	Intensity	Tentative Assignment
3078	Broad	O-H stretching vibration of acid
2930, 2873	Strong	C-H stretching vibration of -CH ₃ , -CH ₂
1706	Strong	C=O stretching vibration of carbonyl group
1655	Medium	C=C stretching vibration of alkene

The ^1H -NMR spectrum (Fig.25) of Compound 3 indicated that it possesses two methyl groups (δ 0.82 and 1.73 ppm), seven olefinic protons (δ 4.56, 4.65, 4.65, 4.85, 5.00, 5.02 and 6.00 ppm)

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig.27) showed 20 signals. Six signals of olefinic carbons appeared at δ 106.7, 113.6, 116.1, 137.3, 147.4 and 151.8 ppm. The signal at 180.8 ppm should be the carbonyl of carboxylic acid. There were thirteen sp^3 carbon signals at δ 16.7(q), 23.8(q), 27.3(t), 27.5(t), 28.1(t), 31.3(t), 31.4(t), 32.1(t), 38.9(s), 40.3(d), 41.0(d), 50.7(d) and 54.6(d) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}_2$, which was confirmed by observing the molecular ion at m/z 302 (Fig.28). The molecular formula, $\text{C}_{20}\text{H}_{30}\text{O}_2$, of Compound 3 defined a degree of unsaturation of six, therefore, Compound 3 must have three double bonds, two rings and one carbonyl group of carboxylic acid.

Compound 3 exhibited the ^{13}C -NMR chemical shifts identical to cleistantha-4, 13(17), 15-triene-3-oic acid [15]. A comparison of the ^{13}C -NMR chemical shifts of Compound 3 and cleistantha-4, 13(17), 15-triene-3-oic acid is shown in Table 10. These data indicated that Compound 3 was cleistantha-4, 13(17), 15-triene-3-oic acid.

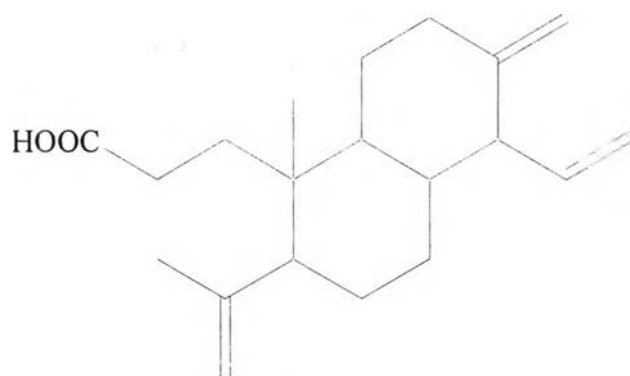


Fig. 8 The structure of Compound 3

Table 10 ^{13}C -NMR chemical shifts of Compound 3 and cleistantha-4, 13(17), 15-triene-3-oic acid [15]

Carbon	Chemical shifts (ppm)	
	Compound <u>3</u>	cleistantha-4, 13(17), 15-triene-3-oic acid
1	32.1t	32.1t
2	28.1t	28.1t
3	180.8s	180.8s
4	147.4s	147.4s
5	50.7d	50.7d
6	27.5t	27.5t
7	31.4t	31.4t
8	40.3d	40.3d
9	41.0d	41.0d
10	38.9s	38.9s
11	27.3t	27.3t
12	31.3t	31.3t
13	151.8s	151.8s
14	54.6d	54.6d
15	137.3d	137.3d
16	116.1t	116.1t
17	106.7t	106.7t
18	113.6t	113.6t
19	23.8q	23.8q
20	16.7q	16.7q

3.3 Derivative of Compound 2.

3.3.1 Coupling of Compound 2 with 2,4-dinitrophenylhydrazine

Compound 2 was coupled with 2,4-dinitrophenylhydrazine in methanol to give 2,4-dinitrophenylhydrazone of Compound 2, Compound 2a, as a crystalline. The IR spectrum of Compound 2a is shown in Fig.29 and the absorption peaks were assigned as shown in Table 11. Its IR spectrum showed important absorption bands at 3324 cm^{-1} (N-H stretching), 2960 cm^{-1} (C-H stretching vibration), 1621 cm^{-1} (C=N stretching vibration) and 1511 cm^{-1} (C=C stretching vibration of olefin).

Table 11 The IR absorption bands assignment of Compound 2a

Wave number (cm^{-1})	Intensity	Tentative assignment
3324	Strong	N-H stretching vibration
2960	Strong	C-H stretching vibration of -CH ₃ , -CH ₂
1621	Strong	C=N stretching vibration
1511	Medium	C=C stretching vibration of olefin

The $^1\text{H-NMR}$ spectrum (Fig.30) of Compound 2a showed that it possesses five methyl groups of proton (δ_{H} 0.98, 0.98, 0.99, 1.03 and 1.10 ppm), two amine protons (δ_{H} 8.26 and 7.92 ppm) and two olefinic protons (δ_{H} 5.79 and 5.44 ppm).

The $^{13}\text{C-NMR}$, DEPT-90 and DEPT-135 spectrum (Fig. 32) showed the carbon at 216.6 ppm (C=O) was changed to 167.5 ppm (C=N). It was down scaled and the other carbon was identical to Compound 2 (Table 12). Its molecular formula was established as $\text{C}_{26}\text{H}_{34}\text{N}_4\text{O}_4$, which was confirmed by observing molecular ion at m/z 466 (Fig. 33).

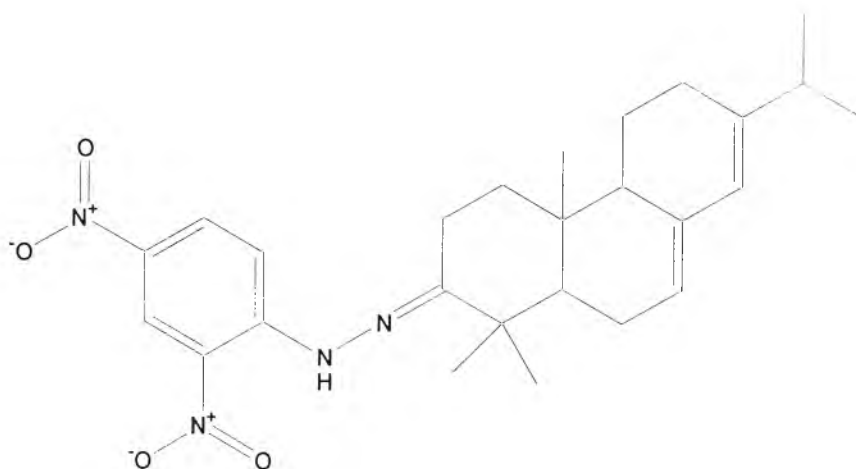


Fig. 9 The structure of Compound 2a

Table 12 ^{13}C -NMR spectral data of Compounds 2 and derivatives 2a

Position	Compound <u>2</u>	Compound <u>2a</u>
C-1	38.0t	37.6t
C-2	34.7t	20.6t
C-3	216.6s	167.5s
C-4	47.5s	42.7s
C-5	51.4d	51.6d
C-6	24.1t	24.4t
C-7	120.6d	120.6d
C-8	135.2s	135.1s
C-9	50.0d	50.0d
C-10	34.6s	34.9s
C-11	22.6t	22.7t
C-12	27.3t	27.3t
C-13	145.6s	145.6s
C-14	122.0d	122.1d
C-15	34.8d	35.0d
C-16	20.8q	20.8q
C-17	21.3q	21.4q
C-18	22.1q	23.7q
C-19	25.0q	27.2q
C-20	13.4q	13.4q
C-21	-	145.7s
C-22	-	137.3s
C-23	-	128.8s
C-24	-	116.4d
C-25	-	123.4d
C-26	-	129.8d