

CHAPTER IV

RESULTS AND DISCUSSIONS

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

4.1.1 Structure elucidation of compound 1

The IR spectrum of compound 1 is shown in Fig. 26 and the absorption peaks were assigned as in Table 2. Its IR spectrum showed important absorption bands at 2400 – 3500 cm^{-1} (O-H stretching vibration of carboxylic acid), 2960 and 2925 cm^{-1} (C-H stretching vibration), 1685 cm^{-1} (C=O stretching vibration of carbonyl group), and 1644 cm^{-1} (C=C stretching vibration of olefin).

Table 2. The IR absorption band assignment of compound 1

Wave number (cm^{-1})	Intensity	Tentative assignment
2400 – 3500	Broad	O-H stretching vibration of carboxylic acid
2960 , 2925	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1685	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin

The ^1H -NMR spectrum (Fig. 27, Table 3) of compound 1 indicated that it possesses an isopropyl group (δ 1.04 ppm), two vinylic methyl groups (δ 1.54 and 1.73 ppm) and four olefinic protons (δ 5.10, 5.90, 6.01, and 6.03 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 28, Table 3) showed 19 signals. Eight signals of olefinic carbons appeared at δ 146.9, 146.3, 135.2, 134.0, 130.9, 125.7, 121.6, and 118.7 ppm. The signal at 174.1 ppm should be the carboxyl group of acid. There were sp^3 eleven carbon signals at δ 39.2 (t), 38.6 (t), 33.8 (d), 33.6 (t), 28.7 (t), 26.4 (t), 25.1 (t), 22.1 (2xq), 17.0 (q), and 15.8 (q) ppm.

Its molecular formula was established as $C_{20}H_{30}O_2$, which was confirmed by observing molecular ion at m/z 302 (Fig. 32). High resolution mass spectroscopy at 25,000 resolution gave m/z 302.212, which corresponded to $C_{20}H_{20}O_2$ (calcd. m/z 302.225). The molecular formula, $C_{20}H_{30}O_2$, of compound 1 defined a degree of unsaturation of six. Therefore, the compound must consist of one ring in addition to the four double bonds and a carboxyl group. This data indicated that compound 1 could be a novel cembranoid possessing a 14-membered ring diterpene skeleton.

The 1H - ^{13}C long-range correlation, as determined from the HMBC spectrum, allowed the connectivity of the structure fragments around each methyl group to be deduced. Crucial 1H - ^{13}C long-range correlation included the protons of the C-19 methyl group (H_3 -19, δ 1.54) to C-8 (δ 134.0), C-7 (δ 125.7), and C-9 (δ 38.6); the protons of the C-18 methyl groups (H_3 -18, δ 1.73) to C-4 (δ 135.2), C-3 (δ 121.6), and C-5 (δ 39.2); the isopropyl methyl protons at δ 1.04 (H_3 -16, H_3 -17) to C-15 (δ 33.8) and C-1 (δ 146.9); and the isopropyl methine proton at δ 2.34 (H_1 -15) to C-16 and C-17 (δ 22.1), C-1 (δ 146.9), C-2 (δ 118.7), and C-14 (δ 28.7).

The position of the C-20 carboxyl group was established by a ^{13}C - 1H three bonds correlation with H-11 and H-13. These partial structures were joined together from information obtained from the long-range ^{13}C - 1H correlation of vinylic or methylene protons and methylene carbons of each fragment and *vice versa*. These included the CH_2 proton at δ 2.20 (H-6) to C-5 (δ 39.2) and C-7 (δ 125.7), the CH_2 protons at δ 2.70 (H-10) to C-9 (δ 38.6), C-8 (δ 134.0), C-11 (δ 146.3), and C-12 (δ 130.9); and the CH_2 protons at δ 2.41 (H-13) to C-14 (δ 28.7), (Fig 4).

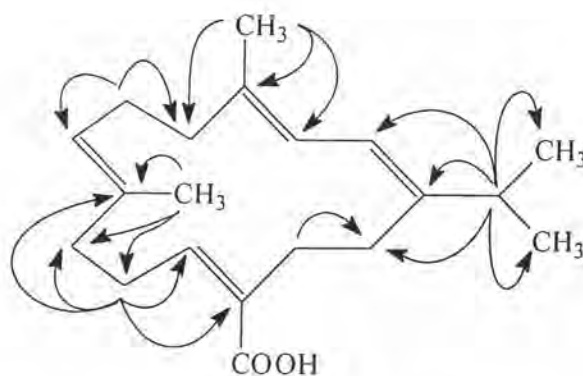


Fig. 4 The HMBC correlation of compound 1

A COSY experiment also established a correlation between the two vinylic protons at δ 5.90 (H-3) and δ 6.03 (H-2). This allowed the assignment of the complete structure of compound 1 as shown in Fig. 5.

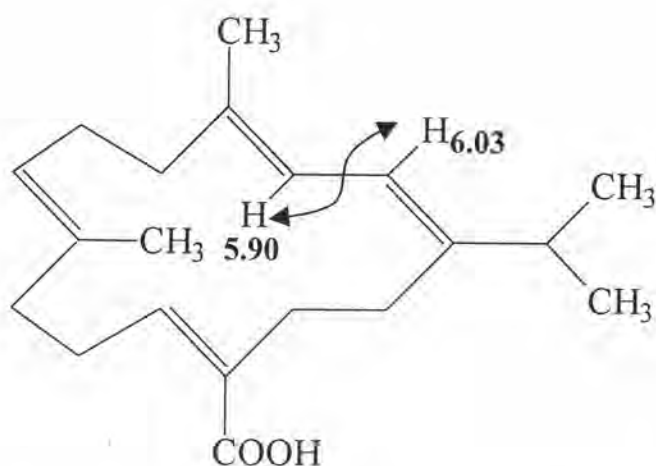


Fig. 5 The COSY correlation of compound 1

The configuration of all double bonds were determined from a NOESY experiment on compound 1. The lowfield doublet at δ 6.03 (H-2) showed NOESY cross peaks with the isopropyl protons (H₃-16, H₃-17 at δ 1.04 and H-15 at δ 2.34) and the methyl group attached to C-4 (H-18, δ 1.73), but not with H-3 (δ 5.90). This

indicated that Δ^1 the isopropyl group was on the same side as H-2, and the Δ^3 conjugated olefin also has the *E* configuration. The latter was supported by the UV spectrum, which exhibited a λ_{\max} at 246 nm, in good agreement with the value reported for isoneocembrene A [(1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8,12-trimethylcyclo-tetradeca-1,3,7,11-tetraene]. [27] The configuration of the Δ^3 olefin was also shown to be *E* by the presence of a cross peak between H-3 and H-5 (δ 2.15), which indicated that the two groups were on the same side of the olefin. Likewise, the *E* configuration of another olefin at Δ^7 could be deduced by the presence of a cross peak between H-7 and H-9 (δ 2.15). The ^{13}C -NMR chemical shifts of methyl groups *trans* to vinylic proton such as those reported in isoneocembrene A.[64,65]

The presence of a NOESY cross peak between the vinylic H-11 (δ 6.02) and H-13/H-14 (δ 2.41) made it possible to unambiguously identify the configuration of the Δ^{11} olefin as *Z*. Furthermore, the H-11 proton resonance fell in the region expected for a vinylic proton *trans* to a carboxyl group in a trisubstituted olefin (calcd. for $\Delta_{\text{H trans}} = 6.19$, found δ 6.01).[66] Thus, compound 1 was assigned as (1*E*,3*E*,7*E*,11*Z*)-1-isopropyl-4,8-dimethylcyclo-tetradeca-1,3,7,11-tetraene-12-carboxylic acid or crotocebraneic acid.

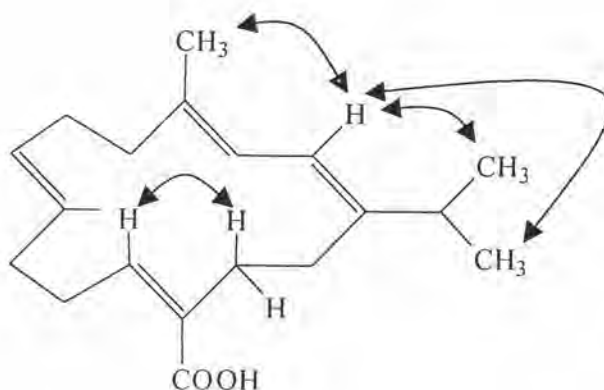


Fig. 6 The NOESY correlation of compound 1

Table 3 ^1H -, ^{13}C -NMR and 2D long range ^1H - ^{13}C correlation in the HMBC spectrum data of compound **1**

Position	^1H -NMR	^{13}C -NMR	Correlated hydrogen
1	-	146.9 s	H-3, H-14, H-15, H-16,17
2	6.03 (1H, d, $J = 11.0$ Hz)	118.7 d	H-14, H-15
3	5.90 (1H, dd, $J = 11.0, 0.9$ Hz)	121.6 d	H-2, H-18
4	-	135.2 s	H-2, H-5, H-6, H-18
5	2.15 (2H, m)	39.2 t	H-3, H-6, H-18
6	2.20 (2H, m)	25.1 t	H-5, H-7
7	5.10 (1H, dt, $J = 6.4, 1.2$ Hz)	125.7 d	H-6, H-9, H-19
8	-	134.0 s	H-9, H-10, H-19
9	2.15 (2H, m)	38.6 t	H-7, H-10, H-11, H-19
10	2.70 (2H, m)	26.4 t	H-9
11	6.01 (1H, t, $J = 6.5$ Hz)	146.3 d	H-9, H-10
12	-	130.9 s	H-10, H-13
13	2.41 (2H, m)	33.6 t	H-14
14	2.41 (2H, m)	28.7 t	H-2, H-13, H-15
15	2.34 (1H, m)	33.8 d	H-2, H-16,17
16,17	1.04 (6H, d, $J = 6.7$ Hz)	2x22.1 q	H-15
18	1.73 (3H, d, $J = 0.9$ Hz)	17.0 q	H-3
19	1.54 (3H, bs, s)	15.8 q	H-7, H-9
20-COOH	-	174.1 s	H-11, H-13

4.1.2 Structure elucidation of compound 2

The IR spectrum of compound 2 is shown in Fig. 33 and the absorption peaks were assigned as in Table 4. Its IR spectrum showed important absorption bands at 2400 – 3500 cm^{-1} (O-H stretching vibration of carboxylic acid), 2955, 2930, and 2868 cm^{-1} (C-H stretching vibration), 1690 cm^{-1} (C=O stretching vibration of carbonyl group), and 1644 cm^{-1} (C=C stretching vibration of olefin).

Table 4. The IR absorption band assignment of compound 2

Wave number (cm^{-1})	Intensity	Tentative assignment
2400 – 3500	Broad	O-H stretching vibration of carboxylic acid
2955, 2930, 2868	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1690	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin

The $^1\text{H-NMR}$ spectrum (Fig. 34, Table 5) of compound 2 showed the proton signals of an isopropyl group (δ 1.05 ppm), two vinylic methyl groups (δ 1.68 and 1.71 ppm) and four olefinic protons (δ 5.14, 5.91, 6.01, and 6.89 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 35, Table 5) showed 19 signals. Eight signals of olefinic carbons appeared at δ 146.5, 145.7, 135.6, 134.8, 132.1, 127.8, 120.0, and 118.6 ppm. The signal at 173.5 ppm should be the carboxyl group of carboxylic acid. There were sp^3 eleven carbon signals at δ 38.5 (t), 37.7 (t), 34.6 (d), 30.5 (t), 29.1 (t), 26.7 (t), 24.7 (t), 22.1 (2xq), 18.0 (q), and 17.4 (q) ppm.

The EI mass spectrum (Fig 40) showed the molecular ion peak at m/z 302. If it is assumed that this compound contains only carbons, protons and oxygens, then the molecular formula of $\text{C}_{20}\text{H}_{30}\text{O}_2$ (calcd. $m/z = 302.225$; High-resolution mass spectroscopy) can be established. The molecular formula, $\text{C}_{20}\text{H}_{20}\text{O}_2$, of this compound indicated six degrees of unsaturation; therefore, compound 2 must consist of one ring in addition to the four double bonds and one carbonyl group. This data

indicated that compound 2 could be a novel a novel cembranoid possessing a 14-membered ring diterpene skeleton.

The spectral data of compound 2 are similar to that of the compound 1; crotocebraneic acid. The structure elucidation of compound 2 was confirmed by ^1H - ^{13}C -NMR information including HMQC, HMBC and COSY experiments in Table 9. The ^{13}C -NMR chemical shifts of the C-18 and C-19 methyl groups (δ 18.0 and 17.4 ppm) again suggested the configuration of the double bond to be *E*. However, marked differences between their ^1H and ^{13}C -NMR spectra were found in the region near the carboxylic acid. Therefore, compound 1 and 2 were assigned as geometrical isomers around Δ^{11} conjugated to the carboxylic acid group. Because this functionality in compound 1 was assigned as *Z*; therefore, compound 2 was proposed as having Δ^{11} in the *E* configuration, from the chemical shift of H-11 at δ 6.89 (calcd δ_{H} , *cis* = 6.83). [66] Thus, compound 2 was assigned as (1*E*,3*E*,7*E*,11*E*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxylic acid or neocrotocebraneic acid.

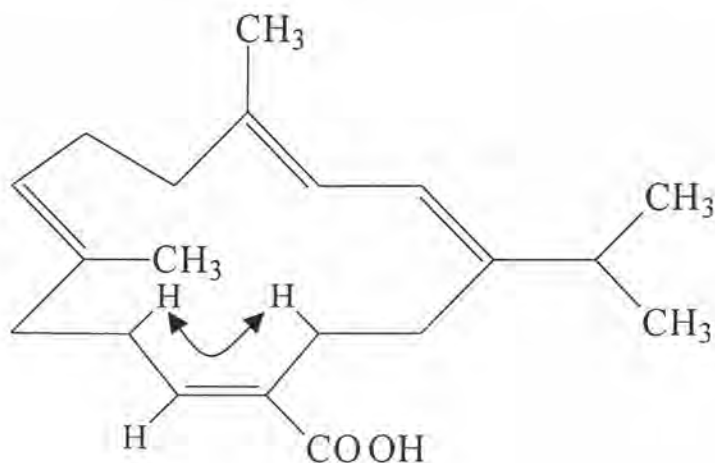


Fig. 7 The Structure of compound 2

Table 5 ^1H -, ^{13}C -NMR and 2D long range ^1H - ^{13}C correlation in the HMBC spectrum data of compound 2

Position	^1H -NMR	^{13}C -NMR	Correlated hydrogen
1	-	146.5 s	H-2, H-3, H-14, H-15, H-16,17
2	6.01 (1H, d, $J = 11.0$ Hz)	118.6 d	H-14, H-15
3	5.91 (1H, dd, $J = 11.0$ Hz)	120.0 d	H-2, H-18
4	-	135.6 s	H-2, H-5, H-6, H-18
5	2.15 (2H, m)	37.7 t	H-3, H-6, H-18
6	2.23 (2H, m)	24.7 t	H-5, H-7
7	5.14 (1H, dt, $J = 8.0, 2.2$ Hz)	127.8 d	H-6, H-9, H-19
8	-	134.8 s	H-9, H-10, H-19
9	2.20 (2H, m)	38.5 t	H-7, H-10, H-19
10	2.38 (2H, m)	30.5 t	H-11
11	6.89 (1H, t, $J = 8.0$ Hz)	145.7 d	H-9, H-10
12	-	132.1 s	H-10, H-11, H-13, H-14
13	2.36 (2H, m)	26.7 t	H-14
14	2.26 (2H, m)	29.1 t	H-2, H-13
15	2.39 (1H, m)	34.6 d	H-2, H-14, H-16,17
16,17	1.05 (6H, d, $J = 7.0$ Hz)	2x22.1 q	H-15
18	1.71 (3H, s)	18.0 q	H-3
19	1.68 (3H, s)	17.4 q	H-7
20-COOH	-	173.5 s	H-11, H-13

4.1.3 Structure elucidation of compound 3

The IR spectrum of compound 3 is shown in Fig. 41 and the absorption peaks were assigned as in Table 6. Its IR spectrum showed important absorption bands at 2960, 2925, and 2873 cm^{-1} (C-H stretching vibration), 2710 (C-H stretching vibration of the aldehyde functional group), 1690 cm^{-1} (C=O stretching vibration of α, β -unsaturated carbonyl group), and 1639 cm^{-1} (C=C stretching vibration of olefin).

Table 6. The IR absorption band assignment of compound 3

Wave number (cm^{-1})	Intensity	Tentative assignment
2960, 2925, 2873	Medium	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
2710	Weak	C-H stretching vibration of aldehyde
1690	Strong	C=O stretching vibration of carbonyl group
1639	Weak	C=C stretching vibration of alkene

The ^1H -NMR spectrum (Fig. 42, Table 7) of compound 3 showed an isopropyl group (δ 1.04 ppm), two vinylic methyl groups (δ 1.67 and 1.68 ppm) and four olefinic protons (δ 5.13, 5.84, 5.96, and 6.39 ppm), and one aldehyde proton (δ 9.28 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 43, Table 7) indicated the presence 19 nonequivalent carbons, of which eleven are sp^3 and eight are sp^2 hybridized carbons, together with a carbonyl carbon of aldehyde functional group. The molecular formula, $\text{C}_{20}\text{H}_{30}\text{O}$ (Fig. 48), of this compound indicated six degrees of unsaturation, therefore, compound 3 must consist of one ring in addition to the four double bonds and one carbonyl group. The spectral data of compound 3 are similar to compound 2; necrotocembraneic acid, except that this compound consists of an aldehyde group instead of a carboxylic acid.

The structure elucidation of compound 3 was confirmed by ^1H - ^{13}C -NMR information including HMQC, HMBC and COSY experiments in Table 7. NOESY experiment on compound 3 suggested the configuration of all double bonds. The

downfield doublet at δ 5.96 ppm (H-2) indicated NOESY cross peaks with the isopropyl protons (H₃-16, H₃-17 at δ 1.04 and H-15 at δ 2.40), but not with H-3 (δ 5.84). This showed that Δ^1 has the *E* configuration (the isopropyl group was on the same side as H-2). The configuration of Δ^1 olefin was also assigned to be *E* configuration by the presence of a cross peak between H-3 and H-5 (δ 2.12), which suggested that two methyl groups were on the same side of olefin. Likewise, the configuration of Δ^7 olefin could be determined to be *E* configuration by the presence of a cross peak between H-7 and H-9 (δ 2.24). The ¹³C-NMR chemical shift of C-18 and C-19 methyl groups (δ 17.2 and 18.0 respectively) were similar to the chemical shifts of the methyl groups *trans* to vinylic protons such as those reported in isoneocembrene A.[64,65]

The presence of a NOESY cross peak between the vinylic H-11 (δ 6.39) and H-20 of aldehyde proton (δ 9.28), but not with H-13 (δ 2.32), made it possible to identify the configuration of Δ^7 olefin as *E* configuration. Furthermore, the H-11 proton resonance excises in the region expected for a vinylic proton *cis* to aldehyde group in trisubstituted olefin (calcd. for δ *cis* = 6019, found 6.39).[66] Thus, compound 3 was assigned as (1*E*,3*E*,7*E*,11*Z*)-1-isopropyl-4,8-dimethylcyclotetradeca-1,3,7,11-tetraene-12-carboxaldehyde or neocrotocembranal.

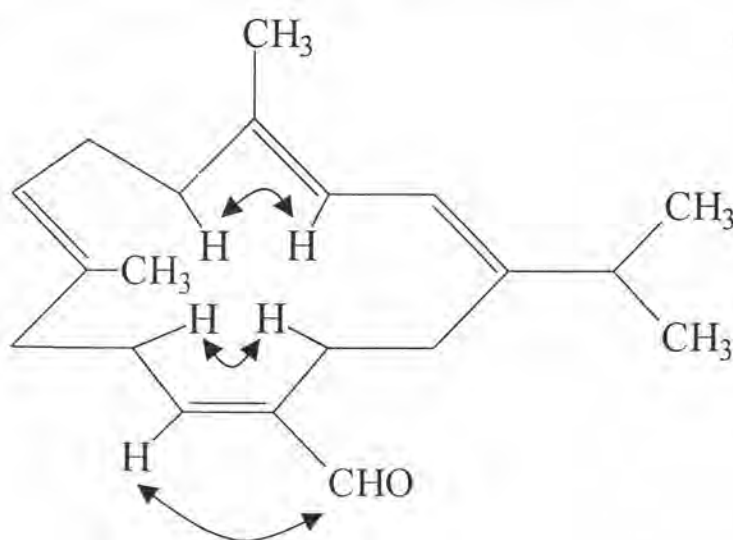


Fig. 8 The structure of compound 3

Table 7 ^1H , ^{13}C -NMR and 2D long range ^1H - ^{13}C correlation in the HMBC spectrum data of compound 3

Position	^1H -NMR	^{13}C -NMR	Correlated hydrogen
1	-	146.2 s	H-2, H-3, H-13, H-14, H-16,17
2	5.96 (1H, d, $J = 11.0$ Hz)	118.4 d	H-14, H-15
3	5.84 (1H, dd, $J = 1.4, 11.0$ Hz)	119.8 d	H-2, H-18
4	-	135.6 s	H-2, H-5, H-6, H-18
5	2.12 (2H, m)	37.4 t	H-3, H-6, H-7, H-8, H-18
6	2.22 (2H, m)	24.8 t	H-5, H-7, H-8
7	5.13 (1H, t, $J = 6.4$ Hz)	128.0 d	H-5, H-6, H-9, H-11, H-19
8	-	134.0 s	H-9, H-10, H-19
9	2.24 (2H, m)	38.5 t	H-7, H-8, H-10, H-11, H-19
10	2.50 (2H, m)	30.0 t	H-9, H-11, H-20
11	6.39 (1H, t, $J = 7.8$ Hz)	155.0 d	H-9, H-10, H-13
12	-	143.9 s	H-10, H-13, H-14, H-20
13	2.32 (2H, m)	24.1 t	H-11, H-14, H-20
14	2.20 (2H, m)	28.7 t	H-2, H-13, H-15
15	2.40 (1H, heptet, $J = 6.7$ Hz)	34.0 d	H-2, H-14, H-16,17
16,17	1.04 (6H, d, $J = 6.7$ Hz)	2x22.0 q	H-15
18	1.67 (3H, s)	18.0 q	H-3
19	1.68 (3H, s)	17.2 q	H-7, H-8, H-9
20-CHO	9.28 (1H, s)	194.7 s	H-11, H-13

4.1.4 Structure Elucidation of compound 4

The IR spectrum of compound 4 is shown in Fig. 49 and the absorption peaks were assigned as in Table 8. Its IR spectrum showed important absorption bands 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 8. The IR absorption band assignment of compound 4

Wave number (cm^{-1})	Intensity	Tentative assignment
2940, 2909, 2868	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
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

Compound 4 contains two methyls, five methylenes, ten methines and four quaternary carbon atoms. Compound 4 had the $[\text{M}]^+$ ion at m/z 374 in the EI mass spectrum (Fig. 56) corresponding to the molecular formula $\text{C}_{21}\text{H}_{26}\text{O}_6$. The IR spectrum showed the furanic and ester absorptions. Thus compound 4 is a clerodane derivative lacking hydroxyl groups and a lactone ring. Furthermore, the ^1H and ^{13}C NMR spectra (Table 9) showed that it is a neo-furoclerodane diterpenoid with a C-20 \rightarrow C-12 bridge. They revealed a $-\text{CO}-\text{OMe}$ 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 structure moiety such as $\text{CH}-\text{O}-\text{CH}-\text{O}-\text{CH}-\text{O}-\text{CH}$ which accounted for the C-3, C-19, C-20 and C-12 tertiary carbons. It followed that the relative configurations for C-3, C-19 and C-9 must be consistent with this system. The relative configuration for all nine asymmetric centers of compound 4 was established by 2D NMR experiments (COSY and NOESY). $^1\text{H}-^1\text{H}$ COSY showed the 3J interaction between $\text{H}-3\beta$ (δ 4.48) and $\text{H}-4\alpha$ (δ 2.83), $\text{H}-12$ (δ 5.31) and H_A-11 (δ 2.22), H_A-11 and $\text{H}-10\beta$ (δ 2.40) and a *W* type long range coupling between the

equatorial protons H-2 α and H-4 α and between H-19 (δ 5.11) and H-10. ^1H - ^1H NOESY revealed interactions between H-4 α and H-19, H-19 and H-20 (δ 5.26), H-20 and Me -17 α , H-12 and the equatorial proton H-1 β (δ 2.30) and Me-17 and H-14 (δ 6.37). Thus the A/B ring junction of the decalin part of compound 4 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 compound 4 exhibited the ^{13}C -NMR chemical shifts identical to Crovatin.[67] The ^{13}C -NMR chemical shifts of compound 4 and Crovatin could be compared as in Table 9.

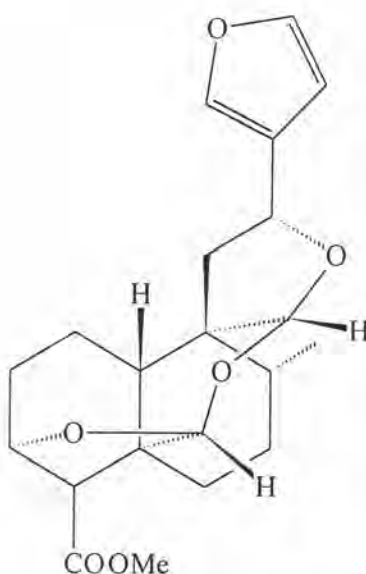


Fig. 9 The structure of compound 4

Table 9 ^{13}C -NMR chemical shifts of compound 4 and Crovatin

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

4.1.5 Structure elucidation of compound 5

The IR spectrum of compound 5 is shown in Fig. 57 and the absorption peaks were assigned as in Table 10. Its IR spectrum showed important absorption bands at 2400 – 3500 cm^{-1} (O-H stretching vibration of carboxylic acid), 2960 and 2925 cm^{-1} (C-H stretching vibration), 1685 cm^{-1} (C=O stretching vibration of carbonyl group), and 1644 cm^{-1} (C=C stretching vibration of olefin).

Table 10 The IR absorption band assignment of compound 5

Wave number (cm^{-1})	Intensity	Tentative assignment
2400 – 3500	Broad	O-H stretching vibration of carboxylic acid
2960 , 2925	Medium	C-H stretching vibration of CH_3 - , - CH_2 -
1685	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin

The $^1\text{H-NMR}$ spectrum (Fig. 58) of compound 5 indicated that it possesses an isopropyl methyl group (δ 0.80 and 0.82 ppm), two vinylic methyl groups (δ 1.65 and 1.80 ppm) and five olefinic protons (δ 5.15, 5.21, 5.56, and 6.05 (2H) ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 59, Table 11) showed 20 signals. Eight signals of olefinic carbons appeared at δ 147.8, 135.1, 131.2, 130.9, 130.5, 128.9, 128.0, and 125.7 ppm. The signal at 173.7 ppm should be the carboxyl group of acid. There were sp^3 eleven carbon signals at δ 47.9 (d), 38.6 (t), 32.8 (d), 32.1 (t), 29.5 (t), 26.2 (t), 25.9 (t), 20.9 (q), 20.0 (q), 19.3 (q), 14.4 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}_2$, which was confirmed by observing molecular ion at m/z 302 (Fig. 60). The molecular formula, $\text{C}_{20}\text{H}_{30}\text{O}_2$, of compound 5 defined a degree of unsaturation of six, therefore, compounds must consist of one ring in addition to the four double bonds and a carboxyl group. These data indicated that compound 5 could be a cembranoid possessing a 14-membered ring diterpene skeleton. These data indicated that compound 5 could be identical to Poilaneic acid which was found in *Croton poilanei*. [32]

It could be concluded that compound 5 exhibited the ^{13}C -NMR chemical shifts identical to Poilaneic acid. The ^{13}C -NMR chemical shifts of compound 5 and Poilaneic acid could be compared as in Table 11.

Table 11 ^{13}C -NMR chemical shifts of compound 5 and Poilaneic acid

	^{13}C -NMR Chemical shifts (ppm)	
	Compound <u>5</u>	Poilaneic acid
1	14.4 q	14.5
2	19.3 q	19.4
3	20.0 q	20.0
4	20.9 q	21.0
5	25.9 t	25.9
6	26.2 t	26.3
7	29.5 t	29.5
8	32.1 t	32.2
9	32.8 d	32.8
10	38.6 t	38.6
11	47.9 d	48.0
12	125.7 d	125.7
13	128.0 s	128.0
14	128.9 d	128.9
15	130.5 d	130.5
16	130.9 s	131.0
17	131.2 d	131.3
18	135.1 s	135.2
19	147.8 d	147.8
20	173.7 s	173.7

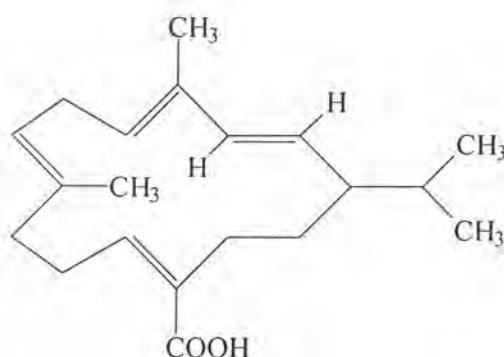


Fig. 10 The structure of compound 5

4.1.6 Structure Elucidation of compound 6

The IR spectrum of compound 6 is shown in Fig. 61 and the absorption peaks were assigned as in Table 12. Its IR spectrum showed important absorption bands at 3350 cm^{-1} (O-H stretching vibration of alcohol), 2935, 2879, and 2858 cm^{-1} (C-H stretching vibration), 1670 cm^{-1} (C=C stretching vibration of alkene), and 1020 cm^{-1} (C-O stretching vibration of primary alcohol).

Table 12. The IR absorption band assignment of compound 6

Wave number (cm^{-1})	Intensity	Tentative assignment
3350	Broad	O-H stretching vibration of alcohol
2935, 2879, 2858	Strong	C-H stretching vibration of CH_3 - , - CH_2 -
1670	Weak	C=C stretching vibration of alkene
1020	Medium	C-O stretching vibration of primary alcohol

The $^1\text{H-NMR}$ spectrum (Fig. 62, Table 13) of compound 6 indicated that it possesses three methyl groups (δ 0.74, 0.78 and 1.01 ppm), two olefinic methyl groups (δ 1.65 and 1.66 ppm), two olefinic protons (δ 5.24 and 5.38 ppm), and one methylene protons of allylic alcohol (δ 5.24 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 63, Table 13) showed 20 signals. Four signals of olefinic carbons appeared at δ 140.9, 139.8, 123.1, and

122.8 ppm. The signal at 59.4 ppm should be the methylene carbon of allylic alcohol. There were sp^3 fifteen carbon signals at δ 44.6 (d), 40.0 (s), 37.7 (t), 37.3 (d), 36.8 (s), 36.4 (t), 33.0 (q), 32.7 (t), 28.7 (t), 24.0 (t), 19.7 (q), 17.7 (t), 17.2 (q), 16.5 (q).

Its molecular formula was established as $C_{20}H_{34}O$, which was confirmed by observing molecular ion at m/z 290 (Fig. 68). The molecular formula, $C_{20}H_{34}O$, of compound 6 defined a degree of unsaturation of four, therefore, compound 6 must consist of two ring and two double bonds. These data indicated that compound 6 could be labdane or clerodane diterpene skeleton.

Crucial 1H - ^{13}C long-range correlation (HMBC) included the allylic protons of the C-15 methylene group (H_2 -15, δ 4.11) to C-13 (δ 140.9) and C-14 (δ 122.8); the protons of the C-16 methyl groups (H_3 -16, δ 1.66) to C-12 (δ 32.9) and C-13 (δ 140.9); the protons of C-17 methyl group (H_3 -17, δ 0.74) to C-7 (δ 28.7), C-8 (δ 37.3) and C-9 (δ 40.0); the proton of C-18 methyl group (H_3 -18, δ 0.78) to C-9 (δ 40.0), C-10 (δ 44.6) and C-11 (δ 36.4) the proton of C-19 methyl group (H_3 -19, δ 1.01) to C-4 (δ 139.8), C-5 (δ 36.8), C-6 (δ 37.7) and C-10 (δ 44.0) and proton of C-20 methyl group (H_3 -20, δ 1.65) to C-3 (δ 127.1) and C-4 (δ 139.8).

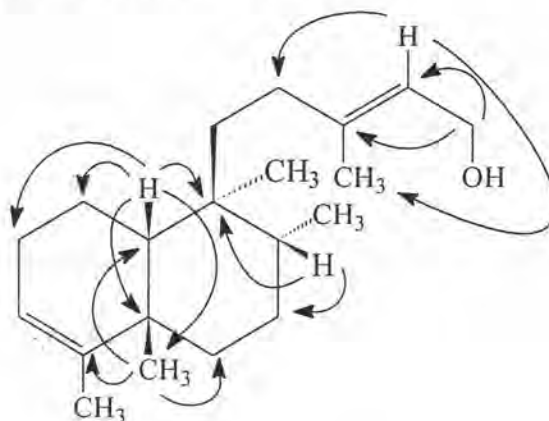


Fig. 11 The HMBC correlation of compound 6

A COSY experiment also established a correlation between the two vinylic protons at δ 5.24 (H-3) and δ 5.38 (H-14). This allowed the assignment of the complete structure of compound 6 as shown in Fig. 12.

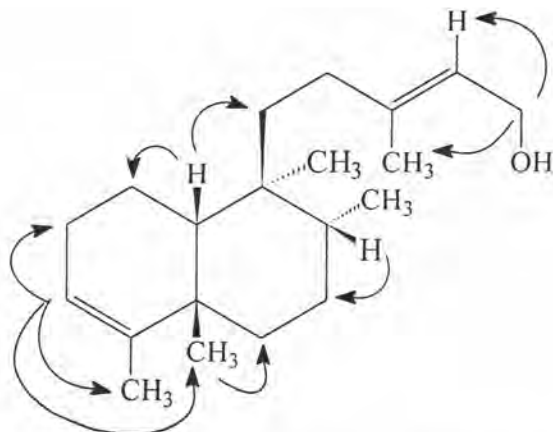


Fig. 12 The COSY correlation of compound 6

The presence of a NOESY cross peak between the protons of C-19 methyl group to the proton of C-10 methine group (δ 1.33). This allowed the assignment of the complete structure of compound 6 as shown in Fig. 13.

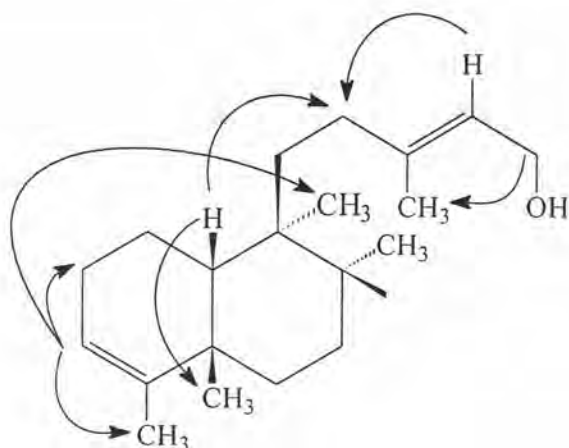


Fig. 13 The NOESY correlation of compound 6

It could be concluded that compound 6 exhibited the $^1\text{H-NMR}$ chemical shifts identical to Isokolavenol.[68] The $^1\text{H-NMR}$ chemical shifts of compound 6 and Isokolavenol could be compared as in Table 13.

Table 13 ^1H -NMR spectrum data of compound 6 and Isokolavenol

Position	Compound <u>6</u>		Isokolavenol
	^{13}C -NMR	^1H -NMR	^1H -NMR
1	17.7 t	1.96 (1H, m)	NR*
		1.78 (1H, m)	NR
2	24.0 t	1.94 (1H, m)	NR
		2.08 (1H, m)	NR
3	123.1 d	5.24 (1H, s, br)	5.20 (s, br)
4	129.8 s	-	.
5	36.8 s	-	
6	37.7 t	1.04 (1H, m)	NR
		1.96 (1H, m)	NR
7	28.7 t	1.15 (1H, m)	NR
		1.22 (1H, m)	NR
8	37.3 d	1.40 (1H, m)	NR
9	40.0 s	-	
10	44.6 d	1.33 (1H, dd, br, 1.5, 5.5)	NR
11	36.4 t	1.29 (1H, m)	NR
		1.54 (1H, m)	NR
12	32.7 t	1.84 (2H, dd, br, 8.5, 9.0)	NR
13	122.8 s	-	
14	140.9 d	5.38 (1H, t, br, 7 Hz)	5.42 (t, br)
15	59.4 t	4.11 (2H, d, 7 Hz)	4.17 (d, 7 Hz)
16	16.5 q	1.66 (3H, s, br)	1.68 (s, br)
17	15.9 q	0.74 (3H, d, 6.7 Hz)	0.79 (d, 7 Hz)
18	17.2 q	0.78 (3H, s)	0.82 (s)
19	33.0 q	1.01 (3H, s)	1.05 (s)
20	19.7 q	1.65 (3H, br)	1.69 (s, br)

* NR = None reported

4.1.7 Structure Elucidation of compound 7

The IR spectrum of compound 7 is shown in Fig. 69 and the absorption peaks were assigned as in Table 14. Its IR spectrum showed important absorption bands at 2800-3600 cm^{-1} (O-H stretching vibration of alcohol), 2966, 2940, and 2873 cm^{-1} (C-H stretching vibration), 1701 cm^{-1} (C=O stretching vibration of carbonyl group).

Table 14 The IR absorption band assignment of compound 7

Wave number (cm^{-1})	Intensity	Tentative assignment
2800-3600	Broad	O-H stretching vibration of acid
2966, 2940, 2873	Strong	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1701	Strong	C=O stretching vibration of carbonyl group

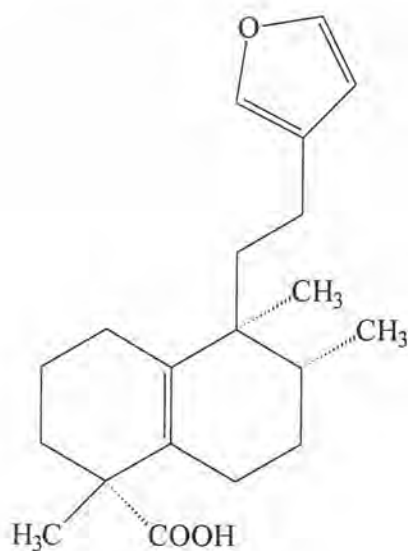
The $^1\text{H-NMR}$ spectrum (Fig. 70) of compound 7 indicated that it possesses three methyl groups (δ 0.87, 0.88 and 1.27 ppm), three olefinic protons of furanoid group (δ 7.32, 7.18 and 6.24 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 71) showed 19 signals. Six signals of olefinic carbons appeared at δ 142.7, 138.4, 136.3, 131.3, 125.7 and 111.0 ppm. The signal at 184.4 ppm should be the carbonyl of carboxylic acid. There were sp^3 thirteen carbon signals at δ 47.5 (t), 41.1 (s), 36.5 (t), 36.4 (t), 33.6 (d), 27.7 (t), 26.9 (t), 25.1 (t), 24.3 (q), 20.8 (q), 20.0 (t), 19.5 (t), 16.1 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{28}\text{O}_3$, which was confirmed by observing molecular ion at m/z 316 (Fig. 76). The molecular formula, $\text{C}_{20}\text{H}_{28}\text{O}_3$, of compound 7 defined a degree of unsaturation of seven, therefore, compound 7 must consist of one ring of furan (DBE = 3) in addition to one double bond, two ring and one carbonyl group of carboxylic acid. These data indicated that compound 7 could not match to be labdane or clerodane diterpene skeleton, thus, compound 7 should be halimane group which was a new group in *Croton oblongifolius* Roxb. The method of structure elucidation of compound 7 was assigned following to compound 8 and compound 9 below.

Table 15 ^1H , ^{13}C -NMR spectrum data of compound 7

Position	^1H -NMR	^{13}C -NMR
1	1.84 (1H, m) 2.10 (1H, m)	25.1 t
2	1.64 (2H, m)	20.0 t
3	1.63 (1H, m) 2.04 (1H, m)	36.4 t
4	-	47.5 s
5	-	131.3 s
6	1.82 (1H, m) 2.10 (1H, m)	27.7 t
7	1.46 (2H, m)	26.9 t
8	1.70 (1H, m)	33.6 d
9	-	41.1 s
10	-	136.3 s
11	1.48 (1H, m) 1.63 (1H, m)	36.5 t
12	2.00 (1H, m) 2.32 (1H, m)	19.5 t
13	-	125.7 s
14	6.24 (1H, m, br)	111.0 d
15	7.32 (1H, t, 1.5 Hz)	142.7 d
16	7.18 (1H, t, 1.5 Hz)	138.4 d
17	0.87 (3H, d, 7.0 Hz)	16.1 q
18	0.88 (3H, s)	20.8 q
19	1.27 (3H, s)	24.3 q
20-COOH	-	184.0 q

**Fig. 14** The structure of compound 7

4.1.8 Structure Elucidation of compound 8

The IR spectrum of compound 8 is shown in Fig. 77 and the absorption peaks were assigned as in Table 16. Its IR spectrum showed important absorption bands at 2850-3600 cm^{-1} (O-H stretching vibration of alcohol), 2966, 2935, and 2873 cm^{-1} (C-H stretching vibration), 1721 and 1696 cm^{-1} (C=O stretching vibration of carbonyl group) and 1270 cm^{-1} (C-O stretching vibration of ester).

Table 16 The IR absorption band assignment of compound 8

Wave number (cm^{-1})	Intensity	Tentative assignment
2850-3600	Broad	O-H stretching vibration of acid
2966, 2935, 2873	Medium	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1721, 1696	Strong	C=O stretching vibration of carbonyl group
1270	Strong	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 78) of compound 8 indicated that it possesses three methyl groups (δ 0.84, 0.88 and 0.92 ppm), three olefinic protons of furanoid group (δ 8.00, 7.96 and 6.19 ppm) and five olefinic proton of benzene ring (δ 7.33 – 7.56 and 6.43 ppm)

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 79) showed 25 signals. Ten signals of olefinic carbons appeared at δ 143.2 (d), 139.8 (d), 136.2 (s), 133.0 (d), 130.4 (s), 130.2 (s), 129.6 (2xd), 128.4 (2xd), 126.5 (s) and 108.9 (d) ppm. The signal at 184.0 ppm should be the carbonyl of carboxylic acid. There were sp^3 thirteen carbon signals at δ 47.2 (s), 40.7 (s), 40.6 (t), 35.8 (t), 33.8 (d), 27.5 (d), 26.8 (d), 25.7 (d), 23.3 (q), 21.6 (q), 20.0 (t), 16.1 (q) ppm and the signal at δ 65.8 ppm should be methine carbon of alcohol or ester group.

From $^1\text{H-}$ and $^{13}\text{C-NMR}$ pattern of compound 8 were similar to compound 9, but compound 8 had a fragment of benzoyl group attach to C-12 instead of carbonyl group of C-12 in compound 9. Thus, compound 8 should be a benzoyl derivative of compound 9.

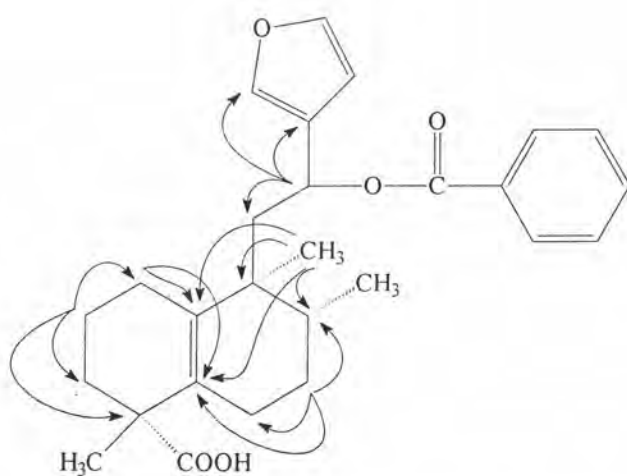


Fig. 15 The HMBC correlation of compound 8

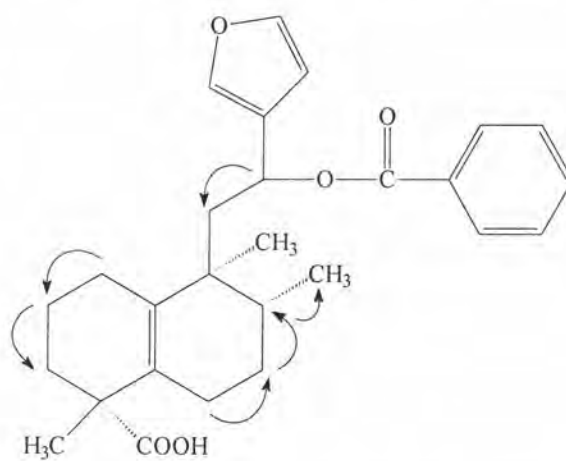


Fig. 16 The COSY correlation of compound 8

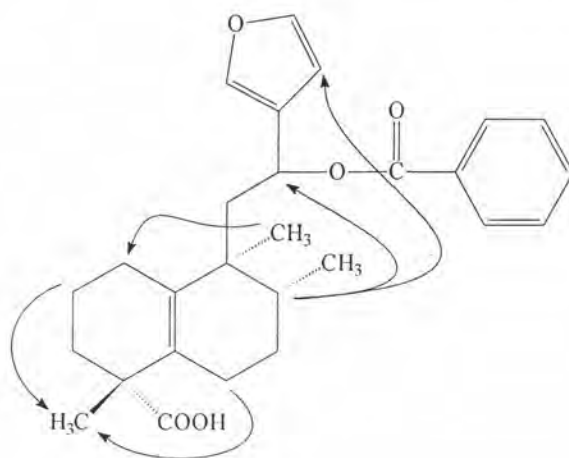


Fig. 17 The NOESY correlation of compound 8

Table 17 ^1H , ^{13}C -NMR and 2D long range ^1H - ^1H correlation in the NOSEY spectrum data of compound **8**

Position	^1H -NMR	^{13}C -NMR	Correlated hydrogen
1	2.10 (2H, m)	25.6 t	H-3
2	1.52 (2H, m)	20.0 t	H-18
3	1.08 (1H, ddd, 4.6, 10.7, 14.7 Hz)	35.8 t	H-2, H-19
	1.77 (1H, m)		
4	-	47.2 s	
5	-	130.4 s	
6	1.70 (1H, m)	27.5 t	
	1.96 (1H, m)		
7	1.44 (2H, m)	26.8 t	H-18
8	1.84 (1H, m)	33.8 d	H-12, H-17
9	-	40.6 s	
10	-	136.2 s	
11	1.91 (1H, dd, 4.0, 15.6 Hz)	40.7 t	H-12, H-18
	2.40 (1H, dd, 8.8, 15.6 Hz)		
12	6.19 (1H, dd, 4.00, 8.8 Hz)	65.8 d	H-8, H-11, H-14, H-15, H-16
13	-	126.6 s	
14	6.43 (1H, dd, 0.9, 1.8 Hz)	108.9 d	H-12, H-17
15	7.34 (1H, dd, 1.8, 1.8 Hz)	143.2 d	H-12, H-18
16	7.42 (1H, m)	139.9 d	H-12, H-14
17	0.85 (3H, d, 6.7 Hz)	16.1 q	H-8, H-14, H-16
18	0.92 (3H, s)	21.6 q	H-1, H-7, H-11, H-15
19	0.85 (3H, s)	23.3 q	H-3
20	-	183.7 s	

4.1.9 Structure Elucidation of compound 9

The IR spectrum of compound 9 is shown in Fig. 85 and the absorption peaks were assigned as in Table 18. Its IR spectrum showed important absorption bands at 2850-3600 cm^{-1} (O-H stretching vibration of alcohol), 2966, 2935, and 2884 cm^{-1} (C-H stretching vibration), 1726 and 1690 cm^{-1} (C=O stretching vibration of carbonyl group).

Table 18 The IR absorption band assignment of compound 9

Wave number (cm^{-1})	Intensity	Tentative assignment
2850-3600	Broad	O-H stretching vibration of acid
2966, 2935, 2884	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2-$
1726 , 1690	Strong	C=O stretching vibration of carbonyl group

The $^1\text{H-NMR}$ spectrum (Fig. 86) of compound 9 indicated that it possesses three methyl groups (δ 0.86, 0.97 and 1.29 ppm), three olefinic protons of furanoid group (δ 8.00, 7.38 and 6.72 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 87) showed 20 signals. Six signals of olefinic carbons appeared at δ 146.7, 144.1, 135.5, 129.5, 129.2 and 108.6 ppm. The signal at 184.8 ppm should be the carbonyl of carboxylic acid and at 194.0 ppm should be the carbonyl of ketone group. There were sp^3 twelve carbon signals at δ 47.4 (s), 46.6 (t), 41.4 (s), 36.1 (t), 34.6 (d), 26.8 (t), 26.2 (t), 25.9 (t), 23.6 (q), 21.0 (q), 19.9 (t), 16.4 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{26}\text{O}_4$, which was confirmed by observing molecular ion at m/z 330 (Fig. 92). The molecular formula, $\text{C}_{20}\text{H}_{26}\text{O}_4$, of compound 9 defined a degree of unsaturation of eight, therefore, compound must consist of one ring of furan (DBE = 3) in addition to one double bond, two ring, one carbonyl group of carboxylic acid and one carbonyl of ketone. These data indicated that compound 9 could not match to be labdane or clerodane diterpene skeleton, thus,

compound 9 should be halimane group, which was a new group in *Croton oblongifolius* Roxb.

Crucial ^1H - ^{13}C long-range correlation (HMBC) included the allylic protons of the protons of the C-17 methyl groups (H_3 -17, δ 0.86) to C-7 (δ 26.8), C-8 (δ 34.6) and C-9 (δ 41.4); the protons of C-18 methyl group (H_3 -21.0, δ 0.96) to C-8 (δ 34.6), C-9 (δ 41.4), C-10 (δ 135.5) and C-11 (δ 46.6); the proton of C-19 methyl group (H_3 -19, δ 1.30) to C-3 (δ 36.1), C-4 (δ 47.4) and C-5 (δ 129.6).

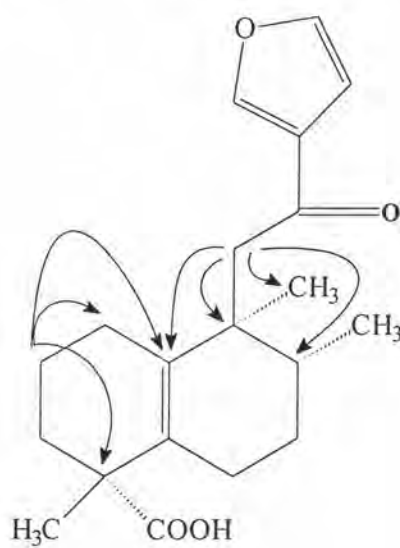


Fig. 18 The HMBC correlation of compound 9

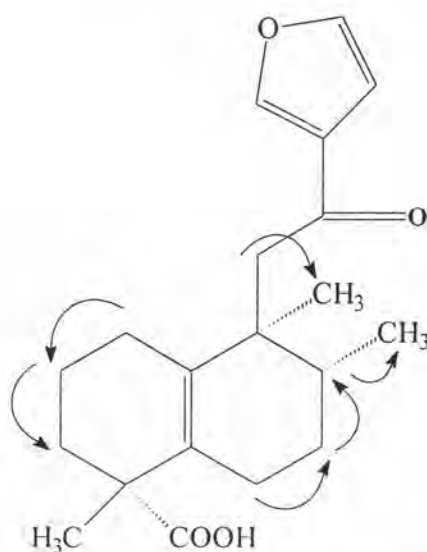


Fig. 19 The COSY correlation of compound 9

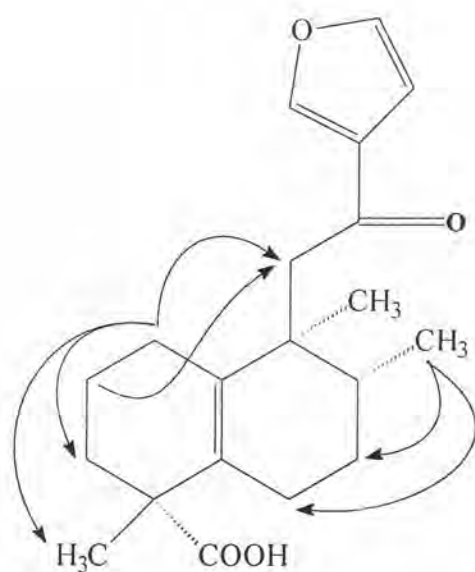


Fig. 20 The NOESY correlation of compound **9**

However, X-ray crystallographic analysis (Fig. 21) of compound **9** obtained by crystallization from CHCl_3 -MeOH established its relative stereochemistry as being identical to that which has been elucidated by spectral data. X-ray crystallographic data are reported in the appendix.

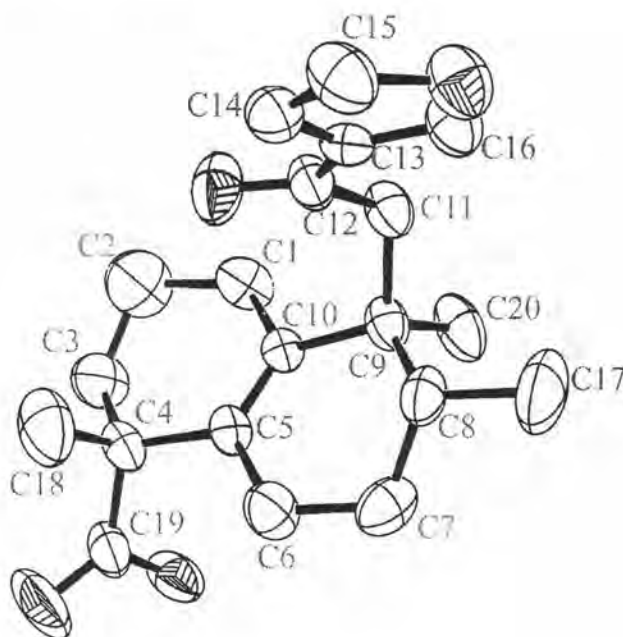


Fig. 21 ORTEP drawing of compound **9**

Table 19 ^1H , ^{13}C -NMR and 2D long range ^1H - ^1H correlation in the NOESY spectrum data of compound **9**

Position	^1H -NMR	^{13}C -NMR	Correlated hydrogen
1	1.88 (1H, m) 2.00 (1H, m)	25.9 t	H-3,H-11
2	1.50 (1H, m) 1.62 (1H, m)	19.9 t	H-3,H-11,H-14,H-15,H-16, H-19
3	1.53 (1H, m) 2.00 (1H, m)	36.1 t	H-1,H-2,H-19
4	-	47.4 s	
5	-	129.6 s	
6	1.84 (1H, m) 2.10 (1H, m)	26.2 t	
7	1.44 (1H, m) 1.53 (1H, m)	26.8 t	
8	1.96 (1H, m)	34.6 d	
9	-	41.4 s	
10	-	135.5 s	
11	2.76 (1H, d, 15.8 Hz) 2.92 (1H, d, 15.8 Hz)	46.6 t	H-2,H-7,H-14,H-15,H-16,H-17,H-18,H-19
12	-	194.0 s	
13	-	129.2 s	
14	6.72 (1H, dd, 0.9, 1.8 Hz)	108.6 d	H-1,H-2,H-11,H-15,H-16,H-17,H-18,H-19
15	7.96 (1H, t-like, 0.9, 0.9 Hz)	146.7 d	H-1,H-2,H-11,H-15,H-16,H-17,H-18,H-19
16	7.39 (1H, t, 1.8 Hz)	144.1 d	H-1,H-2,H-11,H-15,H-16,H-17,H-18,H-19
17	0.86 (3H, d, 7.0 Hz)	16.4 q	H-6,H-7,H-8,H-11,H-14,H-15,H-16,H-18,H-19
18	0.97 (3H, s)	21.0 q	H-2,H-7,H-11,H-14,H-15,H-16,H-17,H-19
19	1.29 (3H, s)	23.6 q	H-3,H-11,H-14,H-15,H-16,H-17,H-18
20	-	184.1 s	

4.1.10 Structure Elucidation of compound 10

The IR spectrum of compound 10 was shown in Fig. 93 and the absorption peaks were assigned in Table 20. Its IR spectrum showed important absorption bands at 2400-3600 cm^{-1} (O-H stretching vibration of alcohol), 2971, 2930, and 2873 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 20 The IR absorption band assignment of compound 10

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 -, $-\text{CH}_2$ -
1685	Strong	C=O stretching vibration of carbonyl group
1634	Medium	C=C stretching vibration of alkene

The $^1\text{H-NMR}$ spectrum (Fig. 94) of compound 10 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. 95) 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 sp^3 thirteen 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), 16.0 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{28}\text{O}_3$, which was confirmed by observing molecular ion at m/z 316 (Fig. 96). The molecular formula, $\text{C}_{20}\text{H}_{28}\text{O}_3$, of compound 10 defined a degree of unsaturation of seven, therefore, compound 10 must consist of one ring of furan (DBE = 3) in addition to one double bond, two ring and one carbonyl group of carboxylic acid. These data indicated that compound 10 could be identical to hardwickiic acid.

It could be concluded that compound 10 exhibited the ^{13}C -NMR chemical shifts identical to hardwickiic acid.[69] The ^{13}C -NMR chemical shifts of compound 10 and hardwickiic acid could be compared as in Table 21.

Table 21 ^{13}C -NMR chemical shifts of compound 10 and Hardwickiic acid

Carbon	Chemical shifts (ppm)	
	Compound <u>10</u>	Hardwickiic acid
1	17.5t	17.5t
2	27.5 t	27.5 t
3	140.2 d	140.2 d
4	141.5 s	141.5 s
5	37.6 s	37.6 s
6	35.8 t	35.8 t
7	27.3 t	27.3 t
8	36.3 d	36.3 d
9	38.8 s	38.8 s
10	46.7 d	46.7 d
11	38.6 t	38.6 t
12	18.2 t	18.2 t
13	125.5 s	125.5 s
14	110.9 d	110.9 d
15	142.7 d	142.7 d
16	138.3 d	138.3 d
17	16.0 q	16.0 q
18	172.8 s	172.8 s
19	20.5 q	20.5 q
20	18.36 q	18.36 q

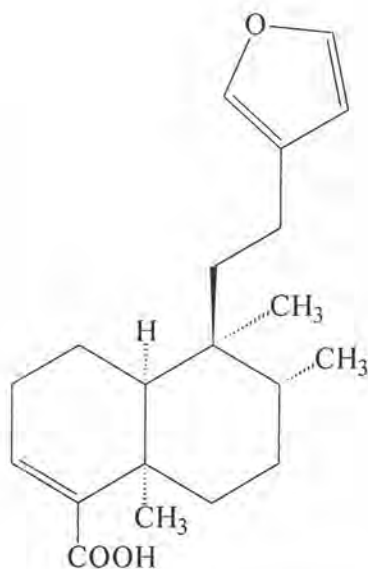


Fig. 22 The structure of compound 10

4.1.11 Structure Elucidation of compound 11

The IR spectrum of compound 11 was shown in Fig. 97 and the absorption peaks were assigned in Table 22. Its IR spectrum showed important absorption bands at 3421 cm^{-1} (O-H stretching vibration of alcohol), 2950 , 2925 , and 2879 cm^{-1} (C-H stretching vibration), 1090 cm^{-1} (C-O stretching vibration of alcohol group).

Table 22 The IR absorption band assignment of compound 11

Wave number (cm^{-1})	Intensity	Tentative assignment
3421	Broad	O-H stretching vibration of acid
2966, 2940, 2873	Strong	C-H stretching vibration of CH_3 - , - CH_2 -
1090	Medium	C-O stretching vibration of alcohol group

The $^1\text{H-NMR}$ spectrum (Fig. 98) of compound 11 indicated that it possesses three methyl groups (δ 0.77, 0.85 and 1.10 ppm), two olefinic methyl groups (δ 1.73 and 1.66 ppm), four olefinic protons (δ 6.29, 5.52, 5.02 and 5.02 ppm), and one methine proton of hydroxyl group alcohol (δ 3.48 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 99) showed 20 signals. Four signals of olefinic carbons appeared at δ 141.5, 135.7, 132.5 and 110.4 ppm. The signal at 80.2 and 78.1 ppm should be the carbons attached to the hydroxyl group of alcohol. There were sp^3 fourteen carbon signals at δ 60.2 (d), 53.5 (d), 41.6 (t), 39.7 (t), 39.2 (s), 33.4 (t), 33.1 (s), 27.8 (t), 23.5 (t), 21.5 (q), 18.4 (t), 17.8 (q), 15.5 (q), 11.8 (q). ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{34}\text{O}_2$, which was confirmed by observing molecular ion at m/z 316 (Fig. 104). The molecular formula, $\text{C}_{20}\text{H}_{34}\text{O}_2$, of compound 11 defined a degree of unsaturation of four, therefore, compound 11 must consist of two ring in addition to two double bonds. These data indicated that compound 11 could match to be labdane diterpene skeleton, thus, compound 11 should be a labdane skeleton.

The ^1H -NMR chemical shifts of compound 11 and Nidorellol [70] could be compared as in Table 23.

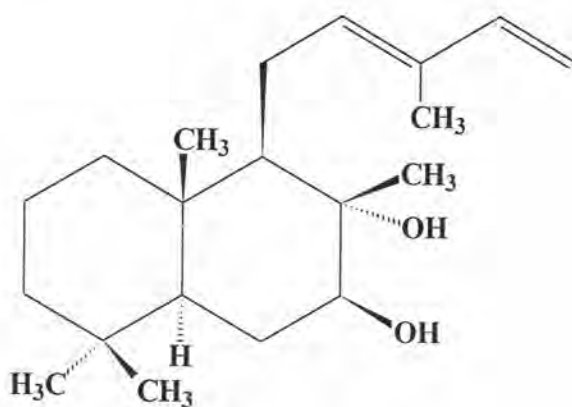


Fig. 23 The structure of compound 11

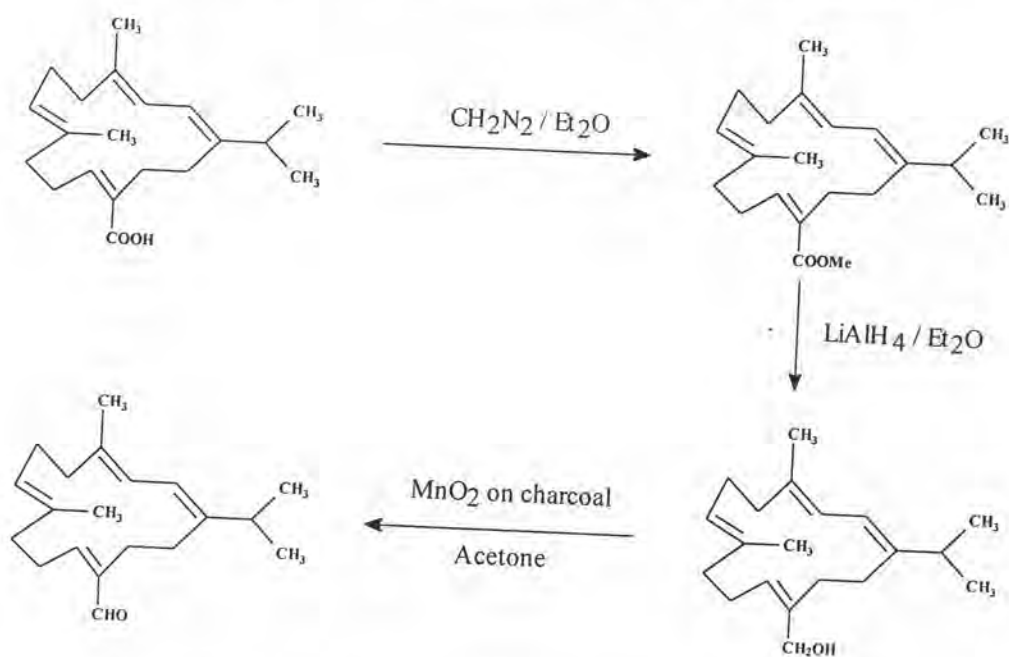
Table 23 $^1\text{H-NMR}$ chemical shifts of compound 11 and Nidorellol

Position	Compound <u>11</u>	Nidorellol
1	1.60	1.3-1.6
2	1.40	
3	1.35	
5	1.00 (dd, 2.1, 12.5)	
6 ex	1.26 (dd, 12.5, 12.8)	
6 eq	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)

4.2 Modification of compound from *Croton oblongifolius* Roxb.

4.2.1 Modification of compound 1

The pathway of modification of compound 1 is shown in Scheme 7.



Scheme 7 Modification pathway of compound 1

4.2.1.1 Methylation of compound 1

The compound 1 was methylated with diazomethane in diethyl ether to give methyl crotonembraneate, compound 1a, as a viscous transparent oil. The IR spectrum of compound 1a is shown in Fig. 105 and the absorption peaks were assigned as in Table 24. Its IR spectrum showed important absorption bands at 2960 , 2925 , and 2873 cm^{-1} (C-H stretching vibration), 1721 cm^{-1} (C=O stretching vibration of carbonyl group of ester), and 1644 cm^{-1} (C=C stretching vibration of olefin), and 1204 cm^{-1} (C-O stretching vibration of ester).

Table 24 The IR absorption band assignment of compound **1a**

Wave number (cm^{-1})	Intensity	Tentative assignment
2960, 2925, 2873	Medium	C-H stretching vibration of CH_3 - and $-\text{CH}_2$ -
1721	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin
1204	Medium	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 106) of compound **1a** showed that it possesses an isopropyl group (δ 0.98 ppm), two vinylic methyl groups (δ 1.50 and 1.69 ppm), one methyl ester group (δ 3.67 ppm) and four olefinic protons (δ 5.05 and 5.76-5.99 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 107) showed 20 signals. Eight signals of olefinic carbons appeared at δ 146.0, 143.2, 134.8, 133.9, 131.4, 125.4, 121.8, and 118.7 ppm. The signal at 168.6 ppm should be the carboxyl group of ester. There were sp^3 eleven carbon signals at δ 39.2 (t), 38.6 (t), 33.4 (d), 33.4 (t), 28.6 (t), 26.2 (t), 25.1 (t), 22.1 (2xq), 16.9 (q), and 15.7 (q) ppm and one methyl ester at δ 51.0 ppm.

Its molecular formula was established as $\text{C}_{21}\text{H}_{32}\text{O}_2$, which was confirmed by observing molecular ion at m/z 316 (Fig. 108).

4.2.1.2 Reduction of compound **1a**

The compound **1a** was reduced with lithium aluminium hydride in diethyl ether to give crotoembranol, compound **1b**, as a viscous transparent oil. The IR spectrum of compound **1b** is shown in Fig. 109 and the absorption peaks were assigned as in Table 25. Its IR spectrum showed important absorption bands at 3365 cm^{-1} (O-H stretching vibration of alcohol), 2960 , 2920 , and 2868 cm^{-1} (C-H stretching vibration), 1650 cm^{-1} (C=C stretching vibration of olefin), and 1009 cm^{-1} (C-O stretching vibration of primary alcohol).

Table 25 The IR absorption band assignment of compound 1b

Wave number (cm^{-1})	Intensity	Tentative assignment
3365	Broad	O-H stretching vibration of alcohol
2960, 2920, 2868	Strong	C-H stretching vibration of CH_3 - , - CH_2 -
1650	Weak	C=C stretching vibration of olefin
1009	Medium	C-O stretching vibration of primary alcohol

The ^1H -NMR spectrum (Fig. 110) of compound 1b showed that it possesses an isopropyl group (δ 1.03 ppm), two vinylic methyl groups (δ 1.48 and 1.71 ppm), one methylene alcohol protons (δ 4.00 ppm) and four olefinic protons (δ 4.97, 5.22, and 5.86-6.05 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 111) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 146.4, 138.3, 134.9, 133.8, 129.4, 125.1, 122.0, and 118.5 ppm. There were sp^3 eleven carbon signals at δ 39.3 (t), 38.8 (t), 33.5 (d), 33.0 (t), 28.6 (t), 25.4 (t), 24.3 (t), 22.2 (2xq), 16.8 (q), and 15.7 (q) ppm and one methylene carbon of alcohol at δ 61.5 ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 288 (Fig. 112).

4.2.1.3 Oxidation of compound 1b

The compound 1b was oxidized with manganese dioxide in acetone to give crotocebranal. compound 1c, as a viscous transparent oil. The IR spectrum of compound 1c is shown in Fig. 113 and the absorption peaks were assigned as in Table 26. Its IR spectrum showed important absorption bands at 2966, 2920, and 2873 cm^{-1} (C-H stretching vibration), 2756 (C-H stretching vibration of aldehyde), 1680 cm^{-1} (C=O stretching vibration of carbonyl group), and 1629 cm^{-1} (C=C stretching vibration of olefin).

Table 26 The IR absorption band assignment of compound **1c**

Wave number (cm^{-1})	Intensity	Tentative assignment
2966, 2920, 2873	Medium	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
2756	Weak	C-H stretching vibration of aldehyde
1680	Strong	C=O stretching vibration of carbonyl group
1629	Weak	C=C stretching vibration of alkene

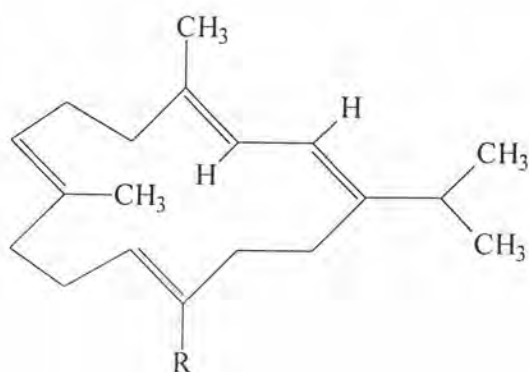
The $^1\text{H-NMR}$ spectrum (Fig. 114) of compound **1c** showed that it possesses an isopropyl group (δ 1.05 ppm), two vinylic methyl groups (δ 1.68 and 1.71 ppm), one proton of aldehyde group (δ 10.06 ppm) and four olefinic protons (δ 6.36, 5.97, 5.81 and 5.05 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 115) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 149.5, 145.8, 140.0, 134.5, 133.1, 126.0, 122.2, and 118.5 ppm. The signal at 191.2 ppm should be the carboxyl group of aldehyde. There were sp^3 eleven carbon signals at δ 39.4 (t), 38.4 (t), 32.7 (d), 28.1 (t), 27.7 (t), 25.3 (t), 23.4 (t), 22.1 (2xq), 16.6 (q), and 15.7 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}$, which was confirmed by observing molecular ion at m/z 286 (Fig. 116).

Table 27 ^{13}C -NMR chemical shift of compound **1** and derivatives (**1a**, **1b** and **1c**)

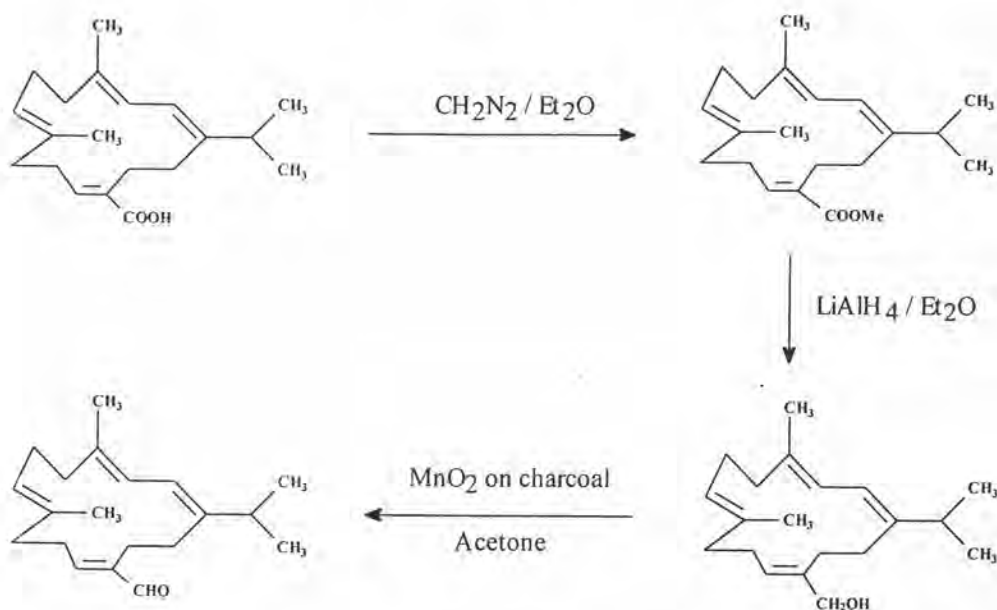
Position	Chemical shift of ^{13}C -NMR			
	Compound 1	Compound 1a	Compound 1b	Compound 1c
1	146.9 s	146.0 s	146.4 s	145.8 s
2	118.7 d	118.7 d	118.5 d	118.5 d
3	121.6 d	121.8 d	122.0 d	122.2 d
4	135.2 s	134.8 s	134.9 s	134.5 s
5	39.2 t	39.2 t	39.3 t	39.4 t
6	25.1 t	25.1 t	25.4 t	25.3 t
7	125.7 d	125.4 d	125.1 d	126.0 d
8	134.0 s	133.9 s	133.8 s	140.0 s
9	38.6 t	38.6 t	38.8 t	38.4 t
10	26.4 t	26.2 t	24.3 t	27.7 t
11	146.3 d	143.2 d	129.4 d	149.5 d
12	130.9 s	131.4 s	138.3 s	133.1 s
13	33.6 t	33.4 t	33.5 t	27.7 t
14	28.7 t	28.6 t	28.6 t	28.1 t
15	33.8 d	33.4 d	33.0 t	32.7 t
16, 17	22.1 q	22.1 q	22.2 q	22.1 q
18	17.0 q	16.9 q	16.8 q	16.6 q
19	15.8 q	15.7 q	15.7 q	15.7 q
20	174.1 s	168.9 s	61.5 t	191.2 d
OMe		51.0 q		



	R
1	COOH
1a	COOMe
1b	CH ₂ OH
1c	CHO

4.2.2 Modification of compound 2

The pathway of modification of compound 2 is shown in Scheme 8.



Scheme 8 Modification pathway of compound 2

4.2.2.1 Methylation of compound 2

The compound 2 was methylated with diazomethane in diethyl ether to give methyl neocrotocembranate, compound 2a, as a viscous transparent oil. The IR spectrum of compound 2a is shown in Fig. 117 and the absorption peaks were assigned as in Table 28. Its IR spectrum showed important absorption bands at 2966, 2925, and 2879 cm^{-1} (C-H stretching vibration), 1711 cm^{-1} (C=O stretching vibration of carbonyl group of ester), and 1639 cm^{-1} (C=C stretching vibration of olefin), and 1199 cm^{-1} (C-O stretching vibration of ester).

Table 28 The IR absorption band assignment of compound 2a

Wave number (cm^{-1})	Intensity	Tentative assignment
2966, 2925, 2879	Medium	C-H stretching vibration of CH_3- , $-\text{CH}_2-$
1711	Strong	C=O stretching vibration of carbonyl group
1639	Weak	C=C stretching vibration of olefin
1199	Medium	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 118) of compound 2a showed that it possesses an isopropyl group (δ 1.04 ppm), two vinylic methyl groups (δ 1.66 and 1.70 ppm), one methyl ester group (δ 3.68 ppm) and four olefinic protons (δ 5.12, 5.91, 5.96, and 6.72 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 119) exhibited 20 signals. Eight signals of olefinic carbons appeared at δ 146.5, 143.0, 135.4, 134.9, 132.5, 127.6, 120.1, and 118.6 ppm. The signal at 168.4 ppm should be the carboxyl group of ester. There were sp^3 eleven carbon signals at δ 38.6 (t), 37.8 (t), 34.8 (d), 30.4 (t), 29.0 (t), 27.2 (t), 24.7 (t), 22.1 (2xq), 17.9 (q), and 17.4 (q) ppm and one methyl ester at δ 51.5 ppm.

Its molecular formula was established as $\text{C}_{21}\text{H}_{32}\text{O}_2$, which was confirmed by observing molecular ion at m/z 316 (Fig. 120).

4.2.2.2 Reduction of compound 2a

The compound 2a was reduced with lithium aluminium hydride in diethyl ether to give, neocrotocembranol, compound 2b, as a viscous transparent oil. The IR spectrum of compound 2b is shown in Fig. 121 and the absorption peaks were assigned as in Table 29. Its IR spectrum showed important absorption bands at 3385 cm^{-1} (O-H stretching vibration of alcohol), 2960 , 2925 , and 2868 cm^{-1} (C-H stretching vibration), 1650 cm^{-1} (C=C stretching vibration of olefin), and 1015 cm^{-1} (C-O stretching vibration of primary alcohol).

Table 29 The IR absorption band assignment of compound 2b

Wave number (cm^{-1})	Intensity	Tentative assignment
3385	Broad	O-H stretching vibration of alcohol
2960, 2925, 2868	Strong	C-H stretching vibration of CH_3- , $-\text{CH}_2-$
1650	Weak	C=C stretching vibration of olefin
1015	Medium	C-O stretching vibration of primary alcohol

The $^1\text{H-NMR}$ spectrum (Fig. 122) of compound 2b showed that it possesses an isopropyl group (δ 1.05 ppm), two vinylic methyl groups (δ 1.68 and 1.72 ppm), one methylene alcohol protons (δ 3.98 ppm) and four olefinic protons (δ 5.08, 5.42, and 5.93–6.08 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 123) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 146.7, 139.3, 135.9, 135.1, 128.1, 127.2, 119.8, and 118.7 ppm. There were sp^3 eleven carbon signals at δ 39.2 (t), 37.5 (t), 34.8 (d), 28.9 (t), 28.8 (t), 28.3 (t), 24.8 (t), 22.2 (2xq), 18.2 (q), and 17.8 (q) ppm and one methylene carbon of alcohol at δ 67.3 ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 288 (Fig. 124).

4.2.2.3 Oxidation of compound 2b

The compound 2c was oxidized with manganese dioxide in acetone to give neocrotocembranal, compound 2c, as a viscous transparent oil. The IR spectrum of compound 2c is shown in Fig. 125 and the absorption peaks were assigned as in Table 30. Its IR spectrum showed important absorption bands at 2966, 2930, and 2873 cm^{-1} (C-H stretching vibration), 2720 (C-H stretching vibration of aldehyde), 1696 cm^{-1} (C=O stretching vibration of carbonyl group), and 1639 cm^{-1} (C=C stretching vibration of olefin).

Table 30 The IR absorption band assignment of compound 2c

Wave number (cm^{-1})	Intensity	Tentative assignment
2966, 2930, 2873	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
2720	Weak	C-H stretching vibration of aldehyde
1696	Strong	C=O stretching vibration of carbonyl group
1639	Weak	C=C stretching vibration of alkene

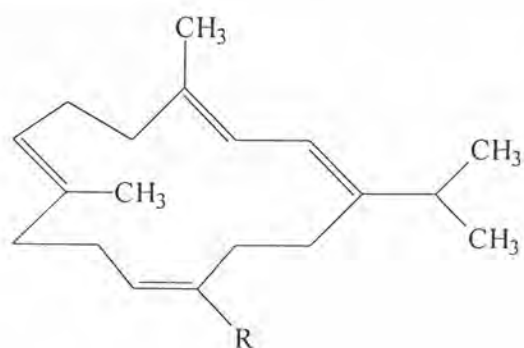
The ^1H -NMR spectrum (Fig. 126) of compound 2c showed that it possesses an isopropyl group (δ 1.05 ppm), two vinylic methyl groups (δ 1.68 ppm), one proton of aldehyde group (δ 9.29 ppm) and four olefinic protons (δ 6.40, 5.90, 5.84 and 5.12 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 127) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 155.2, 146.3, 143.9, 135.5, 134.1, 128.1, 119.8, and 118.4 ppm. The signal at 194.8 ppm should be the carboxyl group of aldehyde. There were sp^3 eleven carbon signals at δ 38.5 (t), 37.4 (t), 34.1 (d), 30.0 (t), 28.7 (t), 24.8 (t), 24.1 (t), 22.1 (2xq), 18.1 (q), and 17.3 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}$, which was confirmed by observing molecular ion at m/z 286 (Fig. 128).

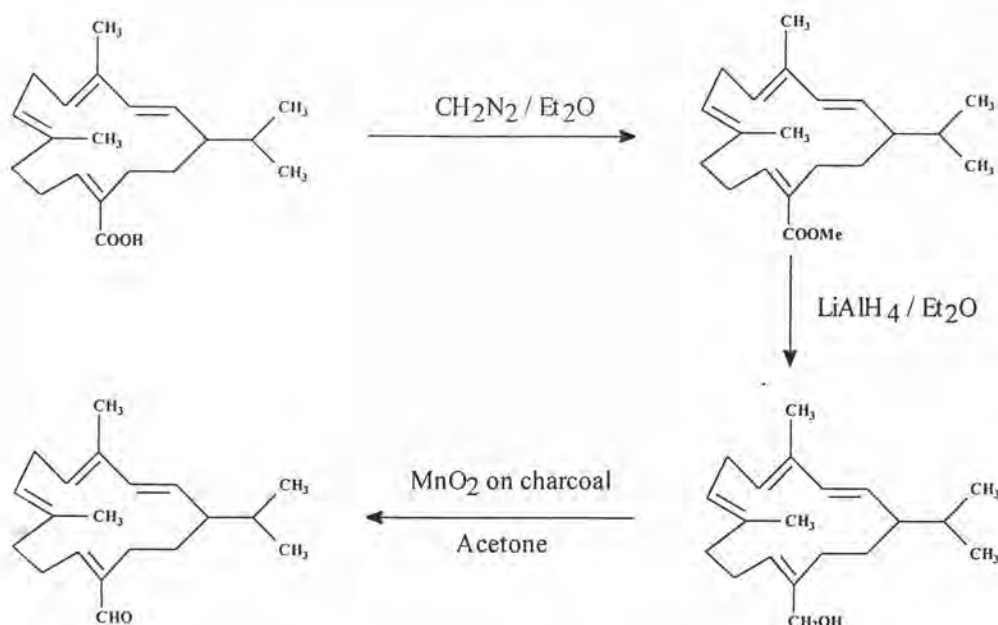
Table 31 ^{13}C -NMR chemical shift of compound 2 and derivatives (2a, 2b and 2c)

Position	Chemical shift of ^{13}C -NMR			
	Compound <u>2</u>	Compound <u>2a</u>	Compound <u>2b</u>	Compound <u>2c</u>
1	146.5 s	146.5 s	146.7 s	146.3 s
2	118.6 d	118.7 d	118.7 d	118.4 d
3	120.0 d	120.1 d	119.8 d	119.8 d
4	135.6 s	135.4 s	135.9 s	135.5 s
5	37.7 t	37.8 t	37.5 t	37.4 t
6	24.7 t	24.7 t	24.8 t	24.8 t
7	127.8 d	127.6 d	127.2 d	128.1 d
8	134.8 s	134.9 s	135.1 s	134.1 d
9	38.5 t	38.6 t	39.2 t	38.5 t
10	30.5 t	30.4 t	34.8 t	30.0 t
11	145.7 d	143.0 d	139.3 d	155.2 d
12	132.1 s	132.5 s	128.1 s	143.9 s
13	26.7 t	27.2 t	28.8 t	24.1 t
14	29.1 t	29.0 t	28.9 t	28.7 t
15	34.6 d	34.8 d	34.8 t	34.1 t
16, 17	22.1 q	22.1 q	22.2 q	22.1 q
18	18.0 q	17.9 q	18.2 q	18.1 q
19	17.4 q	17.4 q	17.8 q	17.3 q
20	173.5 s	168.4 s	67.3 t	194.8 d
OMe		51.5 q		



4.2.3 Modification of compound 5

The pathway of modification of compound 5 is shown in Scheme 9.



Scheme 9 Modification pathway of compound 5

4.2.3.1 Methylation of compound 5

The compound 5 was methylated with diazomethane in diethyl ether to give compound 5a as a viscous transparent oil. The IR spectrum of compound 5a is shown in Fig. 129 and the absorption peaks were assigned as in Table 32. Its IR spectrum showed important absorption bands at 2960, 2925, and 2873 cm^{-1} (C-H stretching vibration), 1721 cm^{-1} (C=O stretching vibration of carbonyl group of ester), and 1644 cm^{-1} (C=C stretching vibration of olefin), and 1204 cm^{-1} (C-O stretching vibration of ester).

Table 32 The IR absorption band assignment of compound 5a

Wave number (cm^{-1})	Intensity	Tentative assignment
2960, 2925, 2873	Medium	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
1721	Strong	C=O stretching vibration of carbonyl group
1644	Weak	C=C stretching vibration of olefin
1204	Medium	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 130) of compound 5a showed that it possesses an isopropyl group (δ 0.98 ppm), two vinylic methyl groups (δ 1.50 and 1.69 ppm), one methyl ester group (δ 3.67 ppm) and four olefinic protons (δ 5.05 and 5.76-5.99 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 131) showed 20 signals. Eight signals of olefinic carbons appeared at δ 146.0, 143.2, 134.8, 133.9, 131.4, 125.4, 121.8, and 118.7 ppm. The signal at 168.6 ppm should be the carboxyl group of ester. There were sp^3 eleven carbon signals at δ 39.2 (t), 38.6 (t), 33.4 (d), 33.4 (t), 28.6 (t), 26.2 (t), 25.1 (t), 22.1 (2xq), 16.9 (q), and 15.7 (q) ppm and one methyl ester at δ 51.0 ppm.

Its molecular formula was established as $\text{C}_{21}\text{H}_{32}\text{O}_2$, which was confirmed by observing molecular ion at m/z 316 (Fig. 132).

4.2.3.2 Reduction of compound 5a

The compound 5a was reduced with lithium aluminium hydride in diethyl ether to give a compound 5b as a white solid. The IR spectrum of compound 5b is shown in Fig. 133 and the absorption peaks were assigned as in Table 33. Its IR spectrum showed important absorption bands at 3365 cm^{-1} (O-H stretching vibration of alcohol), 2960 , 2920 , and 2868 cm^{-1} (C-H stretching vibration), 1650 cm^{-1} (C=C stretching vibration of olefin), and 1009 cm^{-1} (C-O stretching vibration of primary alcohol).

Table 33 The IR absorption band assignment of compound **5b**

Wave number (cm^{-1})	Intensity	Tentative assignment
3365	Broad	O-H stretching vibration of alcohol
2960, 2920, 2868	Strong	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1650	Weak	C=C stretching vibration of olefin
1009	Medium	C-O stretching vibration of primary alcohol

The $^1\text{H-NMR}$ spectrum (Fig. 134) of compound **5b** showed that it possesses an isopropyl group (δ 1.03 ppm), two vinylic methyl groups (δ 1.48 and 1.71 ppm), one methylene alcohol protons (δ 4.00 ppm) and four olefinic protons (δ 4.97, 5.22, and 5.86-6.05 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 135) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 146.4, 138.3, 134.9, 133.8, 129.4, 125.1, 122.0, and 118.5 ppm. There were sp^3 eleven carbon signals at δ 39.3 (t), 38.8 (t), 33.5 (d), 33.0 (t), 28.6 (t), 25.4 (t), 24.3 (t), 22.2 (2xq), 16.8 (q), and 15.7 (q) ppm and one methylene carbon of alcohol at δ 61.5 ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 288 (Fig. 136).

4.2.3.3 Oxidation of compound **5b**

The compound **5b** was oxidized with manganese dioxide in acetone to give compound **5c** as viscous transparent oil. The IR spectrum of compound **5c** is shown in Fig. 137 and the absorption peaks were assigned as in Table 34. Its IR spectrum showed important absorption bands at 2966, 2920, and 2873 cm^{-1} (C-H stretching vibration), 2756 (C-H stretching vibration of aldehyde), 1680 cm^{-1} (C=O stretching vibration of carbonyl group), and 1629 cm^{-1} (C=C stretching vibration of olefin).

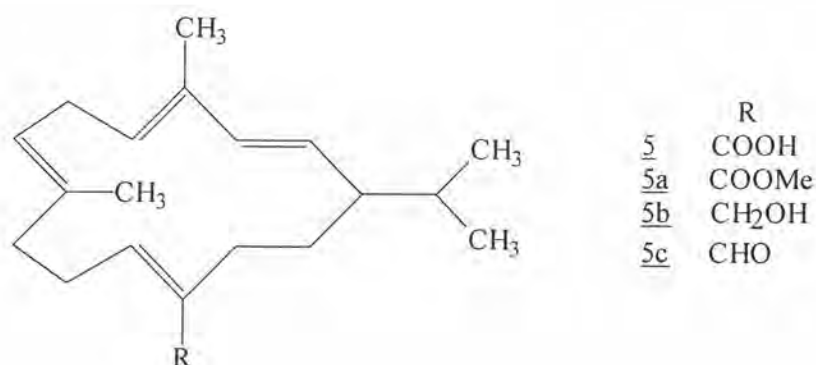
Table 34 The IR absorption band assignment of compound 5c

Wave number (cm^{-1})	Intensity	Tentative assignment
2966, 2920, 2873	Medium	C-H stretching vibration of CH_3 -, $-\text{CH}_2$ -
2756	Weak	C-H stretching vibration of aldehyde
1680	Strong	C=O stretching vibration of carbonyl group
1629	Weak	C=C stretching vibration of alkene

The $^1\text{H-NMR}$ spectrum (Fig. 138) of compound 5c showed that it possesses an isopropyl group (δ 1.05 ppm), two vinylic methyl groups (δ 1.68 and 1.71 ppm), one proton of aldehyde group (δ 10.06 ppm) and four olefinic protons (δ 6.36, 5.97, 5.81 and 5.05 ppm).

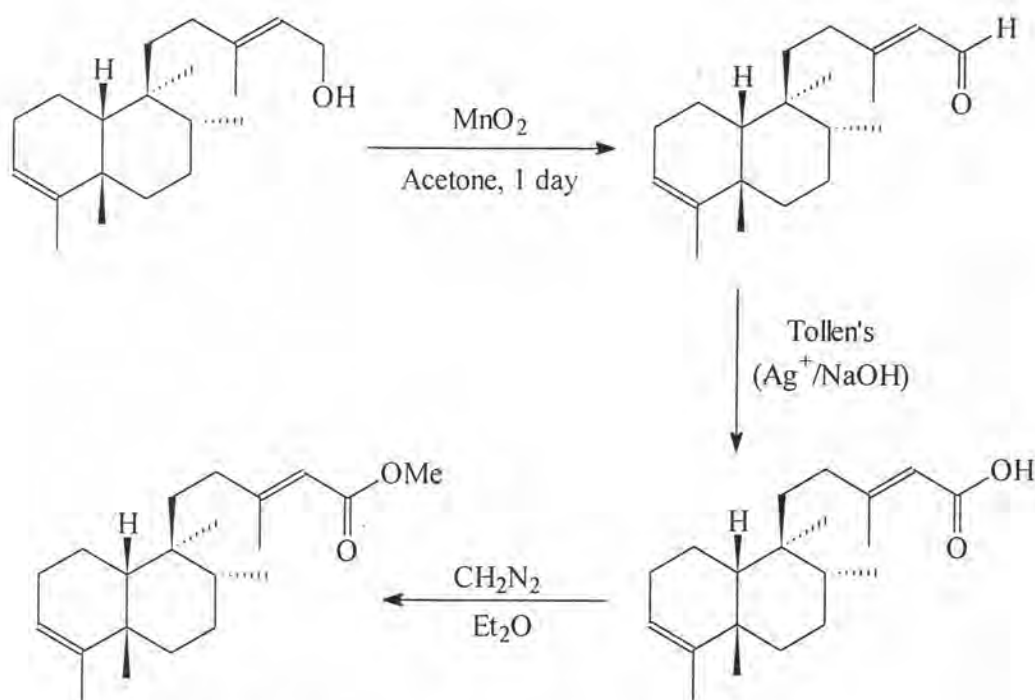
The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 139) exhibited 19 signals. Eight signals of olefinic carbons appeared at δ 149.5, 145.8, 140.0, 134.5, 133.1, 126.0, 122.2, and 118.5 ppm. The signal at 191.2 ppm should be the carboxyl group of aldehyde. There were sp^3 eleven carbon signals at δ 39.4 (t), 38.4 (t), 32.7 (d), 28.1 (t), 27.7 (t), 25.3 (t), 23.4 (t), 22.1 (2xq), 16.6 (q), and 15.7 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{30}\text{O}$, which was confirmed by observing molecular ion at m/z 286 (Fig. 140).



4.2.4 Modification of compound 6

The pathway of modification of compound 6 is shown in Scheme 10.



Scheme 10 Modification pathway of compound 6

4.2.4.1 Oxidation of compound 6

The compound 6 was oxidized with manganese dioxide in acetone to give compound 6a as viscous transparent oil. The IR spectrum of compound 6a is shown in Fig. 141 and the absorption peaks were assigned as in Table 35. Its IR spectrum showed important absorption bands at 2940, 2884, and 2863 cm^{-1} (C-H stretching vibration), 2766 (C-H stretching vibration of aldehyde), 1680 cm^{-1} (C=O stretching vibration of carbonyl group), and 1634 cm^{-1} (C=C stretching vibration of olefin).

Table 35 The IR absorption band assignment of compound **6a**

Wave number (cm^{-1})	Intensity	Tentative assignment
2935, 2879, 2858	Medium	C-H stretching vibration of CH_3 - , - CH_2 -
2766	Weak	C-H stretching vibration of aldehyde
1680	Strong	C=O stretching vibration of carbonyl group
1634	Weak	C=C stretching vibration of alkene

The ^1H -NMR spectrum (Fig. 142) of compound **6a** indicated that it possesses three methyl groups (δ 0.75, 0.81 and 1.02 ppm), two olefinic methyl groups (δ 1.66 and 2.16 ppm), two olefinic protons (δ 5.25 and 5.89 ppm), and a proton of aldehyde (δ 9.95 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 143) shown 20 signals. Four signals of olefinic carbons appeared at δ 165.5, 139.7, 127.1, and 123.1 ppm. The signal at 191.2 ppm should be carbonyl group of aldehyde. There were sp^3 fifteen carbon signals at δ 44.7 (d), 40.2 (s), 37.7 (t), 37.4 (d), 36.9 (s), 35.8 (t), 34.2 (t), 33.1 (q), 28.7 (t), 23.9 (t), 19.7 (q), 17.8 (t), 17.7 (q), 17.2 (q), and 15.9 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 288 (Fig. 144).

4.2.4.2 Oxidation of compound **6a**

The compound **6a** was oxidized with silver nitrate in sodium hydroxide (tollens) to give compound **6b** as viscous transparent oil. The IR spectrum of compound **6b** shown in Fig. 145 and the absorption peaks was assigned in Table 36. Its IR spectrum showed important absorption bands at 2400 – 3600 cm^{-1} (O-H stretching vibration of carboxylic acid), 2935, 2879, and 2858 cm^{-1} (C-H stretching vibration), 1690 cm^{-1} (C=O stretching vibration of carbonyl group), and 1639 cm^{-1} (C=C stretching vibration of olefin).

Table 36 The IR absorption band assignment of compound **6b**

Wave number (cm^{-1})	Intensity	Tentative assignment
2400 - 3600	Broad	O-H stretching vibration of carboxylic acid
2935, 2879, 2858	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1680	Strong	C=O stretching vibration of carbonyl group
1634	Weak	C=C stretching vibration of alkene

The $^1\text{H-NMR}$ spectrum (Fig. 146) of compound **6b** indicated that it possesses three methyl groups (δ 0.76, 0.80 and 1.02 ppm), two olefinic methyl groups (δ 1.66 and 2.17 ppm), two olefinic protons (δ 5.26 and 5.69 ppm).

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 147) shown 20 signals. Four signals of olefinic carbons appeared at δ 164.6, 139.7, 123.1 and 114.9 ppm. The signal at 172.5 ppm should be carbonyl group of carboxylic acid. There were sp^3 fifteen carbon signals at δ 44.6 (d), 40.2 (s), 37.7 (t), 37.4 (d), 36.9 (d), 35.8 (t), 34.8 (t), 33.1 (q), 28.7 (t), 24.0 (t), 19.8 (q), 19.5 (q), 17.7 (t), 17.2 (q), and 15.9 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 304 (Fig. 148).

4.2.4.3 Methylation of compound **6b**

The compound **6b** was methylated with diazomethane in diethyl ether to give compound **6c** as viscous transparent oil. The IR spectrum of compound **6c** is shown in Fig. 149 and the absorption peaks were assigned as in Table 37. Its IR spectrum showed important absorption bands at 2940, 2879, and 2858 cm^{-1} (C-H stretching vibration), 1729 cm^{-1} (C=O stretching vibration of carbonyl group of ester), 1650 cm^{-1} (C=C stretching vibration of olefin), and 1153 cm^{-1} (C-O stretching vibration of ester).

Table 37 The IR absorption band assignment of compound **6c**

Wave number (cm^{-1})	Intensity	Tentative assignment
2940, 2879, 2858	Strong	C-H stretching vibration of CH_3 - , - CH_2 -
1729	Strong	C=O stretching vibration of carbonyl group
1650	Medium	C=C stretching vibration of olefin
1153	Strong	C-O stretching vibration of ester

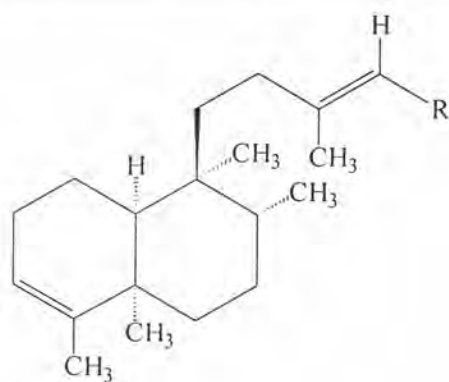
The ^1H -NMR spectrum (Fig. 150) of compound **6c** indicated that it possesses three methyl groups (δ 0.76, 0.80 and 1.02 ppm), two olefinic methyl groups (δ 1.66 and 2.17 ppm), two olefinic protons (δ 5.24 and 5.65 ppm).

The ^{13}C -NMR, DEPT-90, and DEPT-135 spectrum (Fig. 151) shown 20 signals. Four signals of olefinic carbons appeared at δ 161.9, 139.7, 123.1 and 114.7 ppm. The signal at 167.3 ppm should be carbonyl group of ester. There were sp^3 fifteen carbon signals at δ 44.6 (d), 40.2 (s), 37.7 (t), 37.4 (d), 36.9 (s), 36.1 (t), 34.5 (t), 33.1 (q), 28.7 (t), 24.0 (t), 19.7 (q), 19.2 (q), 17.7 (t), 17.2 (q), and 15.9 (q) ppm.

Its molecular formula was established as $\text{C}_{20}\text{H}_{32}\text{O}$, which was confirmed by observing molecular ion at m/z 318 (Fig. 152).

Table 38 ^{13}C -NMR chemical shifts of compound **6** and derivatives (**6a**, **6b** and **6c**)

Position	Chemical shift of ^{13}C -NMR			
	Compound 6	Compound 6a	Compound 6b	Compound 6c
1	17.7 t	17.7 t	17.7 t	17.7 t
2	24.0 t	23.9 t	24.0 t	24.0 t
3	123.1 d	123.1 d	123.1 d	123.1 d
4	139.8 s	139.7 s	139.7 s	139.7 s
5	36.8 s	36.9 s	36.9 s	36.8 s
6	37.7 t	37.7 t	37.7 t	37.7 t
7	28.7 t	28.7 t	28.7 t	28.7 t
8	37.3 d	37.4 d	37.4 d	37.4 d
9	40.0 s	40.2 s	40.2 s	40.2 s
10	44.6 d	44.7 d	44.7 d	44.6 d
11	36.4 t	35.8 t	36.1 t	36.1 t
12	32.7 t	34.2 t	34.8 t	34.5 t
13	140.9 s	165.5 s	164.6 s	161.9 s
14	122.8 d	127.1 d	114.9 d	114.7 d
15	59.4 t	191.2 d	172.5 s	167.3 s
16	16.5 q	17.8 q	19.5 q	19.2 q
17	15.9 q	15.9 q	15.9 q	15.9 q
18	17.2 q	17.2 q	17.2 q	17.2 q
19	33.0 q	33.1 q	33.1 q	33.1 q
20	19.7 q	19.7 q	19.8 q	19.7 q
OMe				50.7 q



	R
6	CH_2OH
6a	CHO
6b	COOH
6c	COOMe

4.2.5 Methylation of compound 7

The compound 7 was methylated with diazomethane in diethyl ether to give compound 7a as a viscous transparent oil. The IR spectrum of compound 7a is shown in Fig. 153 and the absorption peaks were assigned as in Table 39. Its IR spectrum showed important absorption bands at 2940, 2879 and 2843 cm^{-1} (C-H stretching vibration), 1737 cm^{-1} (C=O stretching vibration of carbonyl group of ester), and 1168 cm^{-1} (C-O stretching vibration of ester).

Table 39 The IR absorption band assignment of compound 7a

Wave number (cm^{-1})	Intensity	Tentative assignment
2940, 2879, 2843	Medium	C-H stretching vibration of CH_3 - , - CH_2 -
1737	Strong	C=O stretching vibration of carbonyl group
1168	Medium	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 154) of compound 7a indicated that it possesses three methyl groups (δ 0.86, 0.88 and 1.25 ppm), three olefinic protons of furanoid group (δ 7.31, 7.20 and 6.23 ppm) and one methyl ester group (δ 3.62 ppm)

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 155) showed 21 signals. Six signals of olefinic carbons appeared at δ 142.7, 138.4, 135.6, 131.7, 125.7 and 111.0 ppm. The signal at 178.2 ppm should be the carbonyl of ester. There were sp^3 thirteen carbon signals at δ 47.7 (d), 41.0 (t), 36.6 (t), 36.4 (t), 33.6 (d), 27.6 (t), 26.9 (t), 25.1 (t), 24.4 (q), 20.8 (q), 20.1 (q), 19.5 (q) and 16.1 (q) ppm and one methyl ester at δ 51.8 ppm.

Its molecular formula was established as $\text{C}_{21}\text{H}_{30}\text{O}_3$, which was confirmed by observing molecular ion at m/z 330 (Fig. 160).

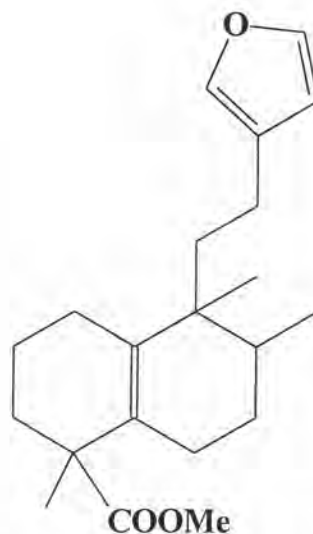


Fig. 24 The structure of compound 7a

4.2.6 Methylation of compound 9

The compound 9 was methylated with diazomethane in diethyl ether to give compound 9a as a viscous transparent oil. The IR spectrum of compound 9a is shown in Fig. 161 and the absorption peaks were assigned in Table 40. Its IR spectrum showed important absorption bands at 2940, 2879 and 2843 cm^{-1} (C-H stretching vibration), 1737 cm^{-1} (C=O stretching vibration of carbonyl group of ester), and 1168 cm^{-1} (C-O stretching vibration of ester).

Table 40 The IR absorption band assignment of compound 9a

Wave number (cm^{-1})	Intensity	Tentative assignment
2940, 2879, 2843	Medium	C-H stretching vibration of CH_3 - , $-\text{CH}_2$ -
1737	Strong	C=O stretching vibration of carbonyl group
1168	Medium	C-O stretching vibration of ester

The $^1\text{H-NMR}$ spectrum (Fig. 162) of compound 9a indicated that it possesses three methyl groups (δ 0.86, 0.88 and 1.25 ppm), three olefinic protons of furanoid group (δ 7.31, 7.20 and 6.23 ppm) and one methyl ester group (δ 3.62 ppm)

The $^{13}\text{C-NMR}$, DEPT-90, and DEPT-135 spectrum (Fig. 163) showed 21 signals. Six signals of olefinic carbons appeared at δ 142.7, 138.4, 135.6, 131.7, 125.7 and 111.0 ppm. The signal at 178.2 ppm should be the carbonyl of ester. There

were sp^3 thirteen carbon signals at δ 47.7 (d), 41.0 (t), 36.6 (t), 36.4 (t), 33.6 (d), 27.6 (t), 26.9 (t), 25.1 (t), 24.4 (q), 20.8 (q), 20.1 (q), 19.5 (q) and 16.1 (q) ppm and one methyl ester at δ 51.8 ppm.

Its molecular formula was established as $C_{21}H_{30}O_3$, which was confirmed by observing molecular ion at m/z 330 (Fig. 164).

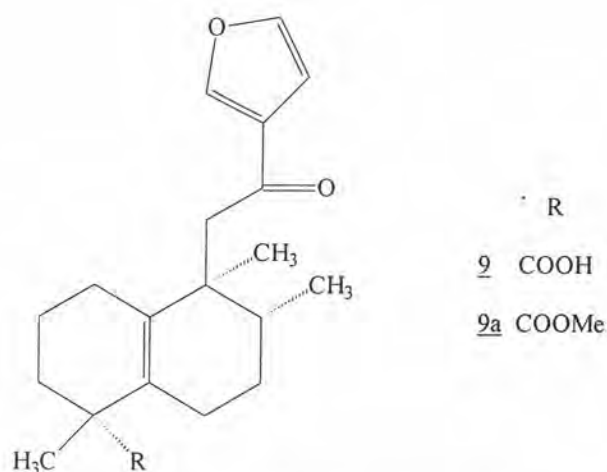


Fig. 25 The structure of compound 9a

4.3 Chemical diversity of *Croton oblongifolius* Roxb. in Thailand

With our in-house ^1H NMR spectra library of chemical previously isolated from *Croton oblongifolius* Roxb., we could use this as a screening tool to study chemical constituents of this plant. From the proton profile of the chemical constituent, which was extracted by hexane and then by a base solution, it was found that the chemical constituents of *Croton oblongifolius* Roxb. were different, even though, some of them may be similar.

It was discovered that the structure of the constituents found within these plants were different from those that were found in the plants from India. For example, *Croton oblongifolius* Roxb. from Amphur Vichienburi, Petchaboon province and Amphur Sai Yok, Kanchanaburi province was found to consist of crotocebraneic acid, neocrotocebraneic acid, and neocrotocebranal. These components fall under the cebranoid diterpene group. The specimen from Amphur Pranburi, Pachuabkirikhan province gave the Labdane group. [16] Overall, the chemical constituents found within *Croton oblongifolius* Roxb. in Thailand can be divided as follows :

1. Cebrane diterpenes. This group consists of compounds 1, 2, 3, and 5, which can be found in plants from Amphur Vichienburi, Petchaboon province, Amphur Pakchong, Nakornratchasima province, Amphur Sai Yok, Kanchanaburi province, Amphur Muang, Prachuabkirikhan province, Amphur Hang Dong, Chaingmai province, and Amphur Muang, Nakornpanom province. This group of chemical substances can be analyzed from ^1H -NMR profile. According to the profile, a signal is detected when there is a chemical shift between 5.00-6.50 ppm, which is a proton of the double bond that is positioned in the ring of the cebrane skeleton.

2. Clerodane diterpenes. The clerodane diterpene group includes compound 4, can be found in plants from Amphur Pakchong, Nakornratchasima province, compound 6, which can be found in plants from Amphur Sai Yok, Kanchanaburi province, and compound 10, which can be found in plants from Amphur Panusnikom, Chonburi province. These plants mainly grow along the mountain slope (the same as with the cembranoid diterpene group). This group of chemical constituents can also be analyzed from $^1\text{H-NMR}$ profile. According to the profile, the proton in the furan ring produces a signal at about 7.30, 7.10, 6.50, and 6.00 ppm. Furthermore, it has also been discovered that for plants from the same source, the constituents from the clerodane group can be found to exist with constituents from the cembranoid or labdane group. For example, in plants from Amphur Vicheienburi, Petchaboon province, Amphur Pakchong, Nakornratchasima province, Amphur Sai Yok, Kanchanaburi province, cembranoid diterpene co-exists with clerodane; in plants from Amphur Panusnikom, Chonburi province, clerodane co-exists with labdane. But, it has not been found yet for the cembranoid diterpene to co-exist along with the labdane diterpene group.

3. Labdane Diterpenes. This group consists of compound 11, which can be found in plant from Amphur Pranburi, Prachuabkirikhan province, Amphur Hun Car, Chainart province, Amphur Muang, Loei province, Amphur Muang, Sakornakorn province, Amphur Muang, Srisakate province and Amphur Nakornthai, Pitsanulok provinces. Most of these plants have been found to grow along flat land. The proton profile of these constituents are different from that of the cembranoid diterpene and clerodane diterpene groups. A proton signal in $^1\text{H-NMR}$ was found at about 5.00 – 7.00 ppm, which was quite a complicated pattern.

Currently, a new constituent, having a structure different from the three groups mentioned above, has been found in the stem bark of *Croton oblongifolius* Roxb. from Amphur Pakchong, Nakornratchasima province. Three pure chemical substances were extracted from this plant. These substances are compound 7, 8, and 9 and are categorized under the halimane diterpene compound. According to the proton NMR profile, these chemicals exhibit ¹H-NMR signals at about 7.30, 7.10, and 6.00 ppm.