CHAPTER IV

RESULTS AND DISCUSSION

Crude extract extracted from stem bark of *C. robustus* was purified and studied properties of the compounds eluted from column chromatography crude extract. Hexane crude extract obtained three compounds; Compound 1, 2 and 3. Furthermore, Compound 1was modified by methylation and reduction, respectively.

Structural elucidation of the isolated compounds from the stem barks of *C. robustus*

Structure elucidation of Compound 1

The IR spectrum of compound 1 (Fig.17) revealed the presence of carboxylic group according to the broad absorption band between 3300 to 2400 cm^{-1} and the strong absorption band at 1965 cm^{-1}due to the carboxylic acid carbonyl stretching. In the IR showed no unsaturation. The IR spectrum of Compound 1 was summarized in Table 3.

Table 3 The IR absorption bands assignment of Compound 1.

Wave number (cm^{-1})	Intensity	Vibration
3300-2400	Broad	O-H stretching vibration of acid
2924,2847	Strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1695	Strong	C=O stretching vibration of acid

The ^{1}H-NMR spectrum (Fig.18, Table 4) of Compound 1 showed no downfield signal. The presence of doublets at 1.23, 1.39 ppm; double of doublets at 0.82 and 1.0 ppm; double of double of doublets at 0.99, 1.68, 1.72, 1.84, 1.87 and

2.12 ppm; doublet of triplets at 0.58, 1.32, 1.45 and 1.75 ppm and three tertiary methyl groups [δ_H 0.88 (s, 3H-20), 1.14(s, 3H-17) and 1.21(s, 3H-18)].

The ^{13}C-NMR spectrum (Fig.19, Table 4) showed 20 lines. One signal of carboxylic acid appeared at 184.5 ppm.

DEPT 90 experiments (Fig.20), indicated the presence of four saturated methines at 57.0, 52.8, 24.3 and 20.5 ppm.

DEPT 135 spectrum (Fig.20) showed three methyl carbons at 28.8, 20.5 and 12.4 ppm and eight methylene carbons at 50.3, 39.4, 39.2, 37.8, 33.1, 19.7 and 18.7 ppm, which indicated that the carbon signals at 184.5, 43.7, 40.7, 38.9 and 22.4 ppm were quaternary.

Compound 1 showed a molecular ion with m/z 302 ($C_{20}H_{30}O_2$)(Fig.21) that indicated DBE of 6. These data precluded the possibility of unsaturation and therefore require Compound 1 to have a pentacyclic skeleton. The presence of double doublet signal at δ_H 0.82 J= 3.36 and 7.94 and double of triplet signal at δ_H 0.58 J= 2.4, 7.9 were typical of a cyclopropane system and suggested that Compound 1 belongs to the trachylobane series of diterpenes. Moreover the presence of a carboxylic group substituent indicated by data discussed above showed that Compound 1 was an acid trachyloban diterpenoid. The information from 2D-NMR techniques; HMQC correlation (Fig.22, Table 4), HMBC correlation (Fig.23, Table 4), COSY correlation (Fig.24, Table 5) and NOESY correlation (Fig.25) were used to assist the interpretation of the structure of Compound 1.

Table 4 The HMQC spectral data of Compound 1.

^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (Hz)
12.4(q)	0.88s
18.7(t)	1.35br,1.84ddd(*J*=6.4,10.1,13.7)
19.7(t)	1.67ddd(*J*=2.4, 7.3, 14.7),1.87ddd(J_x=J_y= 3.1,J_z= 11.4)
20.5(d)	1.14s
20.5(q)	0.58dt(*J*=2.4,7.9)
21.7(t)	1.72ddd(*J*=1.2,9.2,12.4),1.75dt(*J*=2.7,6.1)
22.4(s)	-
24.3(d)	0.82dd(*J*=3.3, 7.9)
28.8(q)	1.21s
33.1(t)	1.19m,2.05d(*J*= 11.6)
37.8(t)	0.99ddd(*J*=4.3, J_x=J_y=13.4),2.12ddd(J_x=J_y=3.4, J_z=7.3)
38.9(s)	-
39.2(t)	1.32dt(*J*= 4.9,13.1),1.45dt(*J*= 3.4,6.7)
39.4(t)	0.78dd(*J*=4.0,13.1),1.56br
40.7(s)	-
43.7(s)	-
50.3(t)	1.23d(*J*=11.3),1.39d(*J*=11.3)
52.8(d)	1.08br
57.0(d)	1.0dd(*J*=2.8,11.0)
184.5(s)	-

Table 5 The HMQC, HMBC and COSY spectral data of Compound 1.

Position	δ_C [a]	δ_H	HMBC (H to C)	COSY
1	39.4(t)	0.78dd 1.56br	C-9,C-2,C-10	H-1(1.56),H-2(1.35,1.84) H-1(0.76),H-2(1.35,1.84)
2	18.7(t)	1.35br 1.84ddd	C-18	H-1(0.78,1.56),H-2(1.84), H-3(0.99,2.12) H-1(0.78),H-3(2.12)
3	37.8(t)	0.99ddd 2.12ddd	C-2,C-19	H-2(1.35,1.84),H-3(2.12) H-2(1.35,1.84),H-3(0.99),H-5(1.0)
4	43.7(s)	-	-	-
5	57.0(d)	1.0dd	C-1,C-4,C-6,C-7,C-9, C-10,C-19,C-20	H-4(2.12),H-2(1.35,1.84)
6	21.7(t)	1.72ddd 1.75dt	C-7	H-5(1.0),H-7(1.32,1.45) H-5(1.0),H-7(1.32,1.45)
7	39.2(t)	1.32dt 1.45dt	C-8, C-15	H-6(1.75),H-7(1.45) H-6(1.72,1.75)
8	40.7(s)	-	-	-
9	52.8(d)	1.08br	C-8,C-10,C-11, C-14	H-11(1.67,1.87)
10	38.9(s)	-	-	-
11	19.7(t)	1.67ddd 1.87ddd	C-9,C-12,C-13, C-16	H-9(1.18),H-12(0.58) H-9(1.08)H-11(1.67),H-12(0.58), H-13(0.82)
12	20.5(d)	0.58dt	C-9, C-17	H-11(1.67,1.87),H-13(0.82)
13	24.3(d)	0.82dd	C-12,C-14, C-17	H-12(0.58),H-14(1.19),H-15(1.23)
14	33.1(t)	1.19m 2.05d	C-9,C-13, C-15	H-13(0.82),H-14(2.05) H-14(1.19)
15	50.3(t)	1.23d 1.39d	C-9,C-11,C-12,C-13, C-16	H-15(1.39) H-13(0.82)
16	22.4(s)	-	-	-
17	20.5(q)	1.14s	C-12,C-13, C-15, C-16	H-15(1.39),H-11(1.67)
18	28.8(q)	1.21s	C-2,C-6,C-19,C-20	-
19	184.5(s)	-	-	-
20	12.4(q)	0.88s	C-1,C-5,C-9,C-10,C-18	H-1(0.78)

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

Compound 1 showed spectral data identical to that of trachyloban-19-oic acid reported in the literature [17-21]. The signal of ^{13}C-NMR spectra of this compound was compared as shown in Table 6.

Table 6 Comparison of ^{13}C-NMR spectra of Compound 1 with trachyloban-19-oic acid.

Position	δ_C (ppm)	
	Compound 1	Trachyloban-19-oic acid
1	39.4(t)	39.5(t)
2	18.7(t)	18.7(t)
3	37.8(t)	37.8(t)
4	43.7(s)	43.7(s)
5	57.0(d)	57.0(d)
6	21.7(t)	21.8(t)
7	39.2(t)	39.2(t)
8	40.7(s)	40.8(s)
9	52.8(d)	52.2(d)
10	38.9(s)	38.9(s)
11	19.7(t)	19.7(t)
12	20.5(d)	20.6(d)
13	24.3(d)	24.3(d)
14	33.1(t)	33.1(t)
15	50.3(t)	50.4(t)
16	22.4(s)	22.4(s)
17	20.5(q)	20.6(q)
18	28.8(q)	28.9(q)
19	184.5(s)	184.7(s)
20	12.4(q)	12.5(q)

The chemical shift comparison of carbon of Compound 1 with trachyloban-19-oic acid, the spectra showed close similarity in Table 6. Thus, compound 1 and trachyloban-19-oic acid had similar structure. The NOESY correlation (Fig.4, 8) was confirmed that no correlation was observed between the proton at H-9 (1.08 ppm) and the protons of methyl (H-20), the proton at H-5 (1.0 ppm) and the protons of methyl (H-20), and the protons of methyl (H-18) and the protons of methyl (H-20), too.

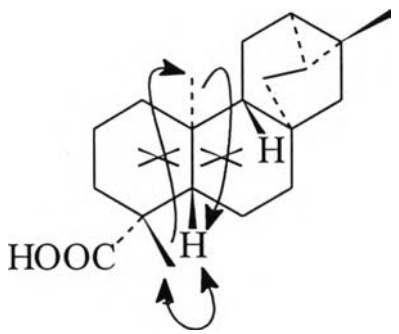


Figure 4 The NOESY correlation of trachyloban-19-oic acid

The above structures were shown by the NOESY correlation of Compound 1 and trachyloban-19-oic acid. It was the first record of a trachyloban diterpene from the *C. robustus*.

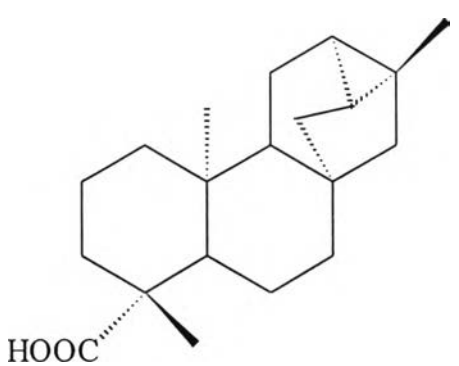


Figure 5 The structure of Compound 1

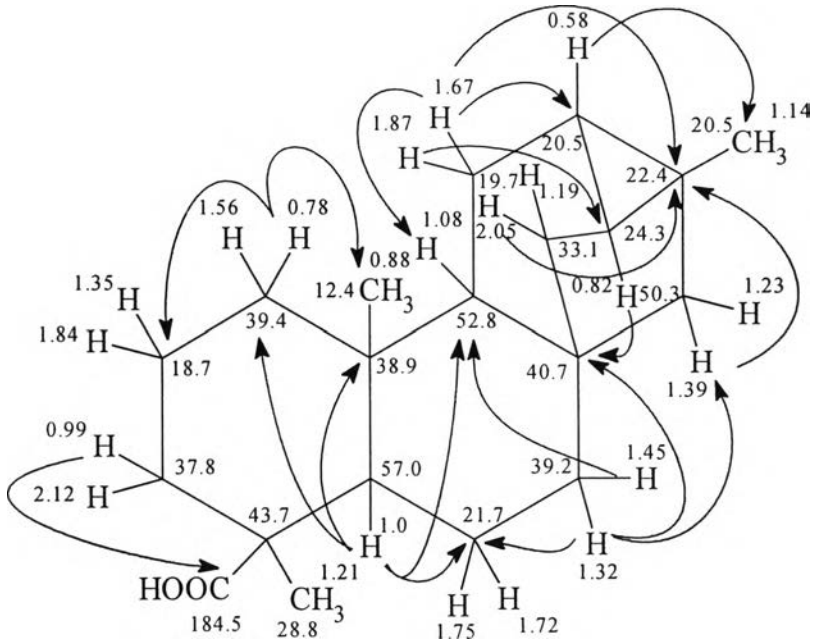


Figure 6 The HMBC correlation of Compound 1

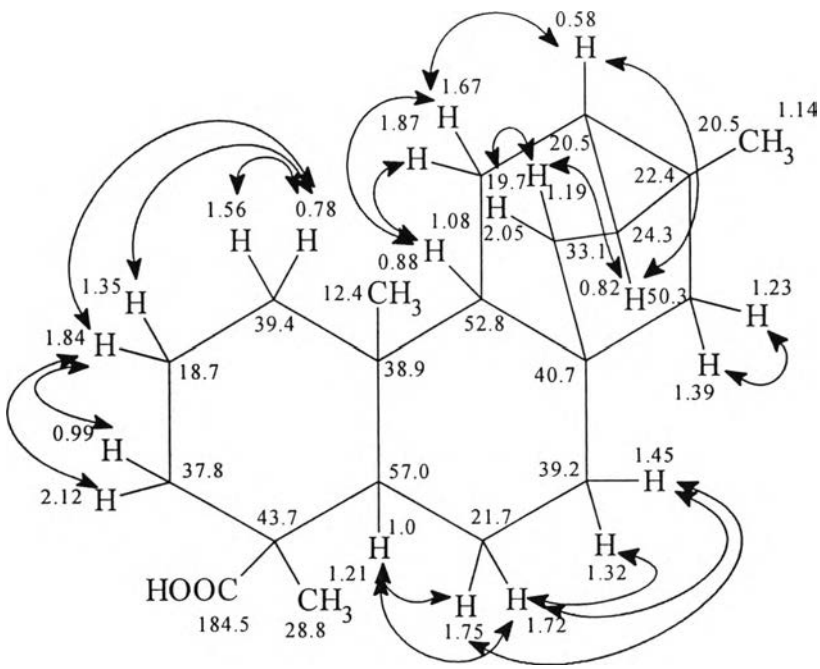


Figure 7 The COSY correlation of Compound 1

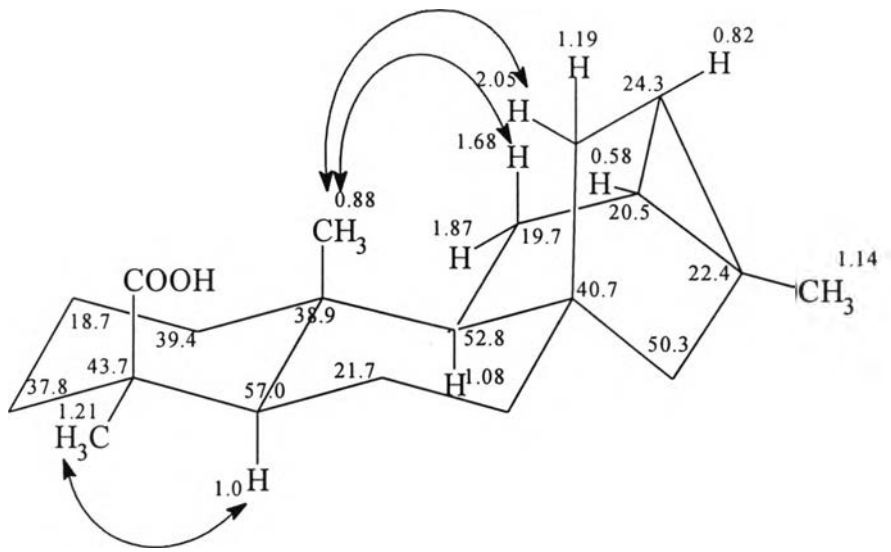


Figure 8 The NOESY correlation of Compound 1

Structure elucidation of Compound 2

The IR spectrum of Compound 2 (Fig.26) showed the presence of a carboxylic group to the broad absorption band between 3500 to 2200 cm^{-1} and the strong absorption band at 1697 cm^{-1}due to the carboxylic acid carbonyl stretching. The IR spectrum of Compound 2 was summarized in Table 7.

Table 7 The IR absorption bands assignment of Compound 2.

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

The ^{1}H-NMR spectrum (Fig.27) of Compound 2 possessed an isopropyl group at δ 0.75(3H), 0.80(3H) and 0.84(3H) ppm two olefinic methyl group at 1.66 and 1.82 ppm and five olefinic protons at 5.0-6.2 ppm.

The ^{13}C-NMR spectrum (Fig.28, Table 8) showed 20 lines which the carbonyl group of carboxylic acid corresponding to the signal at 173.3 ppm. The eight signals of olefinic carbon appeared at 147.6, 135.1, 131.3, 130.9, 130.4, 128.8, 127.9 and 125.7 ppm.

DEPT 90 experiments (Fig.29) indicated the presence of five sp^2 methine carbons at 147.6, 131.3, 130.4, 127.9 and 125.7 ppm and two saturated methines at 47.8 and 32.7 ppm.

DEPT 135 spectrum (Fig.29) showed five methylene carbons at 38.9, 32.1, 28.9, 26.2 and 25.8 ppm and four methyl carbons at 20.9, 19.9, 19.3 and 14.4 ppm, which indicated that the carbon signals at 173.3, 135.1, 130.9 and 128.8 ppm were quaternary.

Compound 2 showed a molecular ion with m/z = 302 ($C_{20}H_{30}O_2$)(Fig.30) which indicated DBE of 6. Compound 2 must consist of one ring in addition to the four double bonds and a carboxyl group. These data indicated that Compound 2 could be cembranoid possessing a 14-membered ring diterpene skeleton.

It could be concluded that Compound 2 exhibited the ^{13}C-NMR chemical shifts similar to poilaneic acid [6,14]. The ^{13}C-NMR chemical shift of Compound 2 and poilaneic acid were compared in Table 8. Therefore, Compound 2 was assigned as poilaneic acid which was previously isolated from *C. poilanei* [14].

Table 8 Comparison of ^{13}C-NMR spectra of Compound 2 and poilaneic acid.

	δ_C (ppm)	
	Compound 2	Poilaneic acid
1	14.4 (q)	14.5 (q)
2	19.3 (q)	19.4 (q)
3	19.9 (q)	19.9 (q)
4	20.9 (t)	20.9 (t)
5	25.8 (t)	25.9 (t)
6	26.2 (t)	26.3 (t)
7	28.9 (t)	29.5 (t)
8	32.1 (t)	32.7 (t)
9	32.7 (d)	32.7 (d)
10	38.8 (t)	38.6 (t)
11	47.8 (d)	47.8 (d)
12	125.7 (d)	125.7 (d)
13	127.9 (d)	128.0 (d)
14	128.8 (s)	128.8 (s)
15	130.4 (d)	130.5 (d)
16	130.9 (s)	131.0 (s)
17	131.3 (d)	131.3 (d)
18	135.1 (s)	135.2 (s)
19	147.6 (d)	147.8 (d)
20	173.3 (s)	173.7 (s)

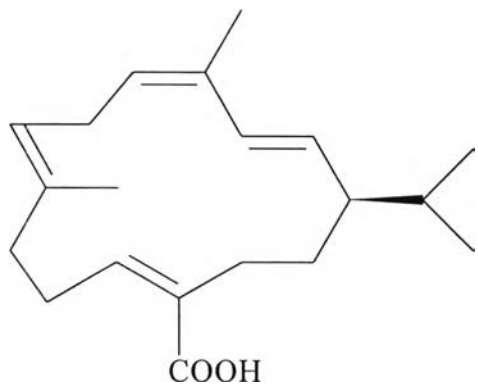


Figure 9 The structure of Compound 2

Structure and elucidation of Compound 3

The IR spectrum of Compound 3 (Fig.31) showed the presence of a hydroxy group according to the broad and strong absorption band at 3500 to 3200 cm^{-1}. The IR spectrum of Compound 3 was summarized in Table 9.

Table 9 The IR absorption bands assignment of Compound 3.

Wave number (cm^{-1})	Intensity	Vibration
3500-3200	broad, strong	O-H stretching vibration of alcohol
2942,2858	strong	C-H stretching vibration of $-CH_3$, $-CH_2$
1439	weak	O-H bending vibration of alcohol

The molecular formula of Compound 3 was assigned to be $C_{20}H_{32}O$ and EIMS $[M^+]$ (m/z = 288)(Fig.35) which indicated 5 DBE. The ^{13}C-NMR spectrum (Fig.33, Table 10) of Compound 3 was similar to that of Compound 1 except for the downfield position of C-19 (65.6 ppm) compared with that of Compound 1 (184.5ppm). ^{1}H-NMR spectrum (Fig.35) showed double doublet signal (δ_H =3.39, J=0.92, 10.99) and

doublet signal (δ_H =3.69, J =10.68) of 2H-19. Comparison of spectral data including ^{1}H-NMR and ^{13}C-NMR including DEPT analysis, HMQC correlation (Fig.36, Table 10), HMBC correlation (Fig.11, 37, Table 11), COSY correlation (Fig.12, 38, Table 11) and NOESY correlation (Fig.13, 39), of this compound with that of Compound 1 demonstrated that Compound 3 differed from 1 only in having a hydroxy group attached to C-19. Based on the spectral data discussed above and shown below, the structure of Compound 3 was assigned to be 19-trachylobanol [17,19].

Table 10 The HMQC spectral data of Compound 3.

^{13}C-NMR (ppm)	^{1}H-NMR (ppm), coupling constant (Hz)
15.1(q)	0.89s
17.8(t)	1.31m, 1.48m
19.9(t)	1.61ddd(J=2.4,7.0,14.6), 1.85ddd(J=3.1,11.29,1.6)
20.3(t)	1.13m, 1.54m
20.5(q)	1.1s
20.6(d)	0.54dt(J=2.4,7.9)
22.4(s)	-
24.2(d)	0.78dd(J=3.17.9)
26.8(q)	0.91s
33.4(t)	1.12m, 2.0d(J=11.9)
35.7(t)	0.88m, 1.73ddt(J=1.5,1.8,11.9)
38.2(s)	-
38.3(s)	-
39.3(t)	0.72ddd(J=3.7,13.4), 1.5m
39.4(t)	1.28dd(J=3.4,9.2), 1.4ddd(J=3.7,6.7,13.1)
40.7(s)	-
50.3(t)	1.21d(J=11.6), 1.37d(J=11.3)
53.4(d)	1.08m
56.8(d)	0.86dd(J=1.5,3.7)
65.6(t)	3.39dd(J=0.9, 10.9), 3.69d(J=10.7)

Table 11 The HMQC, HMBC and COSY spectral data of Compound 3.

Position	δ_C[a]	δ_H	HMBC(H to C)	COSY
1	39.3(t)	0.72dd 1.5m	C-2,C-3,C-9,C-10,C-20	H-1(1.5),H-2(1.31),H-20(0.89)
2	17.8(t)	1.31m 1.48m	C-3,C-5	H-1(0.72),H-3(1.73)
3	35.7(t)	0.88m 1.73ddt	C-1	H-1(1.5),H-2(1.31,1.48)
4	38.3(s)	-	-	-
5	56.8(d)	0.86dd	C-4,C-6,C-10,C-19,C-20	H-1(0.72)
6	20.3(t)	1.13m 1.54m	C-5, C-7	H-7(1.28,1.4)
7	39.4(t)	1.28dd 1.4dd	C-5,C-6,C-8	H-6(1.54),H-7(1.4)
8	40.7(s)	-	-	-
9	53.4(d)	1.08m	C-11,C-12,C-13,C-15	H-11(1.61,1.85),H-13(0.78), H-14(2.0)
10	38.2(s)	-	-	-
11	19.9(t)	1.61ddd 1.85ddd	C-8,C-10,C-12,C-13, C-16	H-9(1.08),H-11(1.85),H-14(1.12) H-9(1.08),H-11(1.61),H-12(0.54)
12	20.6(d)	0.54dt	C-9	H-11(1.85),H-13(0.78)
13	24.2(d)	0.78dd	C-8,C-12	H-12(0.54),H-14(1.12)
14	33.4(t)	1.12m 2.0d	C-8,C-13,C-15,C-16	H-14(1.12)
15	50.3(t)	1.21d 1.35d	C-7,C-8,C-9, C-13,C-14,C-16	H-15(1.35)
16	22.4(s)	-	-	-
17	20.5(q)	1.1s	C-12,C-14,C-16	H-13(0.78),C-15(1.21)
18	26.8(q)	0.91s	C-3,C-6,C-19	H-3(1.73)
19	65.6(t)	3.39dd 3.69d	C-4,C-9,C-18	H-19(3.69) H-19(3.39)
20	15.1(q)	0.89s	C-1,C-9,C-10	H-1(1.5)

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

Another way of confirmation was carried out by methylation of trachyloban-19-oic acid then reduced methyl trachyloban-19-oate (Compound 1a) to the primary alcohol (trachyloban-19-ol). Comparison ^{13}C-NMR chemical shifts of both compounds indicated Compound 3 and Compound 1a [Fig.43] as in Table 12.

Therefore, Compound 3 was assigned as trachyloban-19-ol which was previously reported that it was modified from trachyloban-19-oic acid isolated from sunflower flower [17,19].

Table 12 Comparison of ^{13}C-NMR spectra of Compound 3 with reduction of Compound 1a.

Position	δ_C (ppm)	
	Compound 3	Trachyloban-19-ol
1	39.3(t)	39.3(t)
2	17.8(t)	17.8(t)
3	35.7(t)	35.7(t)
4	38.3(s)	38.3(s)
5	56.8(d)	56.8(d)
6	20.3(t)	20.4(t)
7	39.4(t)	39.4(t)
8	40.7(s)	40.7(s)
9	53.4(d)	53.4(d)
10	38.2(s)	38.2(s)
11	19.9(t)	19.9(t)
12	20.6(d)	20.6(d)
13	24.2(d)	24.2(d)
14	33.4(t)	33.4(t)
15	50.3(t)	50.3(t)
16	22.4(s)	22.4(s)
17	20.5(q)	20.5(q)
18	26.8(q)	26.8(q)
19	65.6(t)	65.6(t)
20	15.1(q)	15.1(q)

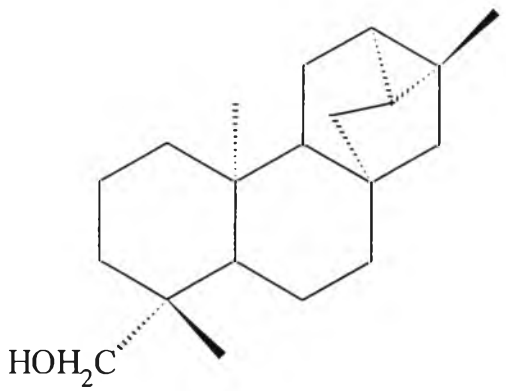


Figure 10 The structure of Compound 3

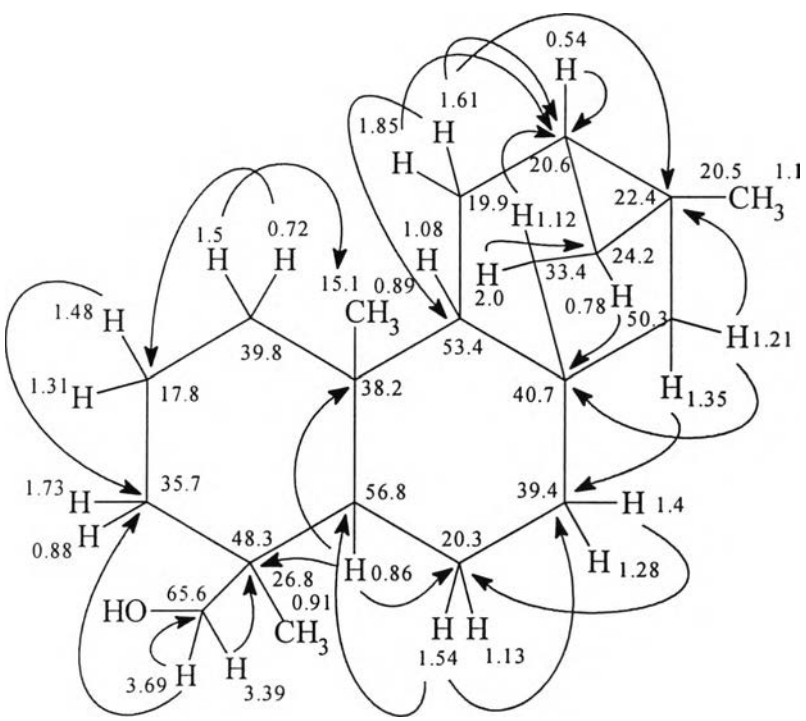


Figure 11 The HMBC correlation of Compound 3

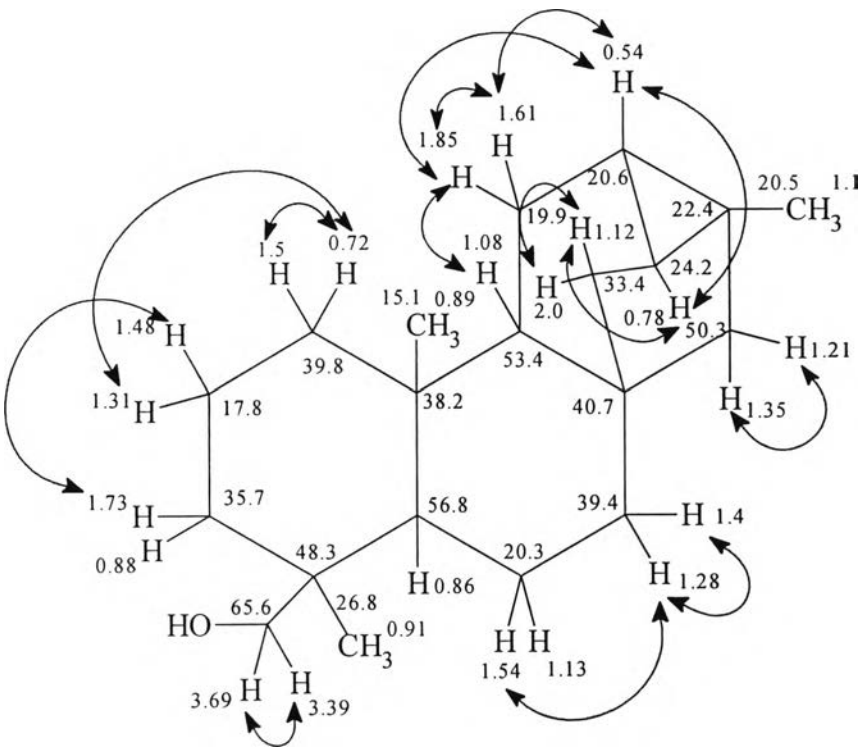


Figure 12 The COSY correlation of Compound 3

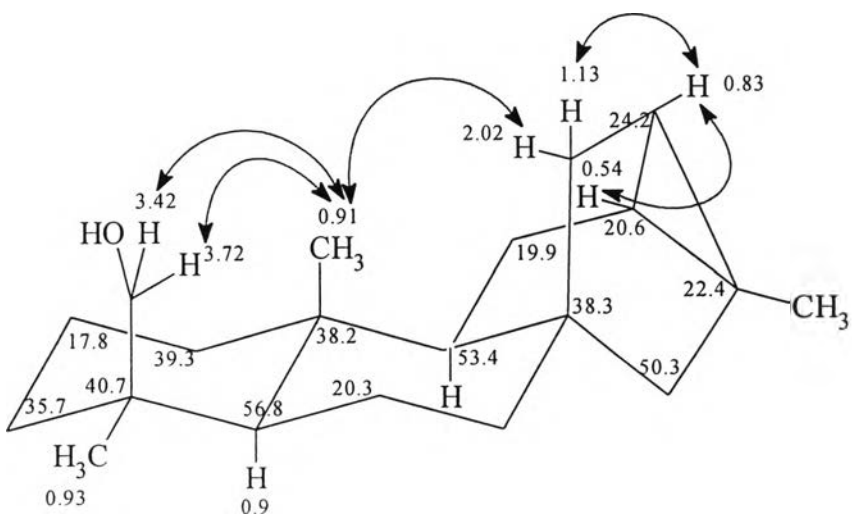


Figure 13 The NOESY correlation of Compound 3

Structure and elucidation of Compound 1a

Compound 1a was a methyl ester of Compound 1, which was generated by treatment of 1 with diazomethane in ether. The molecular formula of Compound 1a was indicated $C_{21}H_{32}O_2$ and showed molecular ion at m/z = 316 [Fig.42]. The ^{13}C-NMR spectrum was similar to that of Compound 1 except for the moving upfield position of C-19 carboxylate ester (δ_C = 178.0) compared with of Compound 1 (δ_C = 184.5) and revealed the presence of carbomethoxyl group [δ_H 3.6(3H, s, OMe); δ_C 51.0q, OMe][Fig.41].

It could be concluded that Compound 1a exhibited to the ^{13}C-NMR chemical shifts were similar to that of methyl trachyloban-19-oate. Comparison of data for Compound 1a with methyl trachyloban-19-oate which was already published showed good agreement [17, 19, 22]. The ^{13}C-NMR chemical shift of Compound 1a and trachyloban-19-oate could be compared as in Table 13.

Moreover methyl ester of Compound 1a was recrystallized from methanol and the structure of Compound 1a was confirmed by X-ray diffraction analysis in Fig.15. From X-ray diffraction data Table 14, 15, 16 and 17, indicated that methyl ester was an axial orientation carbomethoxyl group. The axial orientation carbomethyl group of 1a was assigned to C-19 on the basis of the following observations. The carbomethoxyl group (19 ester) at C-4 was the same side of molecule as methyl group (C-20). The relative configuration at C-19 methyl ester and H-20 was also indicated by orientation of cyclopropane system.

This phenomenon has been observed in other trachyloban diterpene series where the C-4 substitutent is axial.

Table 13 Comparison of ^{13}C-NMR spectra of Compound 1a with methyl trachyloban- 19-oate.

Position	δ_C (ppm)	
	Compound 1a	Methyl Trachyloban-19-oate
1	39.5(t)	39.5(t)
2	18.8(t)	18.8(t)
3	38.1(t)	38.1(t)
4	43.7(s)	43.7(s)
5	57.0(d)	57.0(d)
6	21.8(t)	21.8(t)
7	39.2(t)	39.3(t)
8	40.7(s)	40.8(s)
9	52.7(d)	52.7(d)
10	38.6(s)	38.6(s)
11	19.7(t)	19.7(t)
12	20.5(d)	20.5(d)
13	24.2(d)	24.2(d)
14	33.1(t)	33.1(t)
15	50.3(t)	50.4(t)
16	22.4(d)	22.4(d)
17	20.5(q)	20.5(q)
18	28.7(q)	28.7(q)
19	178.0(s)	177.8(s)
20	12.3(t)	12.3(t)
21	51.0(s)	51.0(s)

Table 14 Crystal data and structure refinement for 1a .

Empirical formula	$C_{21}H_{32}O_2$
Formula weight	316.47
Temperature	293(2) K
Wavelength	0.71073 A°
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 7.49000(10) A° alpha = 90 deg.
	b = 8.95230(10) A° beta = 90 deg.
	c = 26.7347(10) A° gamma = 90 deg.
Volume	1792.63(7) $A^{°3}$
Z, Calculated density	4, 1.173 Mg/m^3
Absorption coefficient	0.073 mm^{-1}
F(000)	696
Theta range for data collection	1.52 to 30.53 deg.
Index ranges	$-10 \leq h \leq 10, -12 \leq k \leq 7, -37 \leq l \leq 37$
Reflections collected / unique	13426 / 5111 [R(int) = 0.0244]
Completeness to 2theta = 30.53	96.1%
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	5111 / 0 / 336
Goodness-of-fit on F^2	1.065
Final R indices [I > 2sigma(I)]	R1 = 0.0438, wR2 = 0.1036
R indices (all data)	R1 = 0.0575, wR2 = 0.1120
Absolute structure parameter	0.9(13)
Largest diff. peak and hole	0.188 and -0.146 $e.A^{-3}$

Table 15 Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for 1a .

	X	Y	Z	U(eq)*
O(1)	7533(2)	-1952(1)	1898(1)	68(1)
O(2)	5610(2)	-402(1)	1533(1)	49(1)
C(1)	11414(2)	1339(2)	1719(1)	45(1)
C(2)	11018(2)	-10(2)	2052(1)	55(1)
C(3)	9419(3)	285(2)	2382(10	54(1)
C(4)	7715(2)	697(2)	2093(1)	39(1)
C(5)	8157(2)	2058(2)	1747(1)	33(1)
C(6)	6587(2)	2765(2)	1461(1)	41(1)
C(7)	7116(2)	4325(2)	1281(1)	45(1)
C(8)	8762(2)	4337(2)	945(1)	35(1)
C(9)	10290(2)	3413(2)	1183(1)	33(1)
C(10)	9796(2)	1840(2)	1399(1)	33(1)
C(11)	11946(2)	3410(2)	834(1)	47(1)
C(12)	11753(2)	4446(2)	391(1)	50(1)
C(13)	9989(3)	4537(2)	120(1)	54(1)
C(14)	8429(3)	3869(2)	396(1)	50(1)
C(15)	9486(2)	5949(2)	875(1)	39(1)
C(16)	10666(2)	5863(2)	418(1)	43(1)
C(17)	11326(3)	7279(2)	180(1)	57(1)
C(18)	6255(3)	1140(2)	2476(1)	56(1)
C(19)	7005(2)	-703(2)	1834(1)	41(1)
C(20)	9439(3)	648(2)	994(1)	45(1)
C(21)	4805(3)	-1654(2)	1286(1)	56(1)

*U(eq) is defined as one third of the trace of the orthogonalized

Table 16 Bond distances (A°) for 1a.

Bond Distances	Distances (A°)	Bond Distances	Distances (A°)
O(1)-C(19)	1.1977(19)	C(8)-C(14)	1.547(2)
O(2)-C(19)	1.3444(18)	C(8)-C(9)	1.5487(18)
O(2)-C(21)	1.435(2)	C(8)-C(15)	1.553(2)
C(1)-C(2)	1.529(2)	C(9)-C(11)	1.551(2)
C(1)-C(10)	1.551(2)	C(9)-C(10)	1.5556(19)
C(2)-C(3)	1.511(3)	C(10)-C(20)	1.544(2)
C(3)-C(4)	1.537(2)	C(11)-C(12)	1.512(2)
C(4)-C(19)	1.527(2)	C(12)-C(16)	1.509(2)
C(4)-C(18)	1.551(2)	C(12)-C(13)	1.509(3)
C(4)-C(5)	1.5653(19)	C(13)-C(14)	1.506(3)
C(5)-C(6)	1.537(2)	C(13)-C(16)	1.517(2)
C(5)-C(10)	1.5526(18)	C(15)-C(16)	1.510(2)
C(6)-C(7)	1.531(2)	C(16)-C(17)	1.510(2)
C(7)-C(8)	1.524(2)		

Table 17 Bond angles (deg) for 1a.

Angles	(A°)	Angles	(A°)
C(19)-O(2)-C(21)	116.46(14)	C(20)-C(10)-C(1)	108.84(12)
C(2)-C(1)-C(10)	113.47(13)	C(20)-C(10)-C(5)	111.75(12)
C(3)-C(2)-C(1)	110.84(15)	C(1)-C(10)-C(5)	108.90(11)
C(2)-C(3)-C(4)	113.93(13)	C(20)-C(10)-C(9)	113.82(11)
C(19)-C(4)-C(18)	108.70(13)	C(1)-C(10)-C(9)	106.19(11)
C(19)-C(4)-C(18)	105.31(13)	C(5)-C(10)-C(9)	107.11(10)
C(3)-C(4)-C(18)	108.25(14)	C(12)-C(11)-C(9)	113.20(13)
C(19)-C(4)-C(5)	116.42(11)	C(16)-C(12)-C(13)	60.32(12)
C(3)-C(4)-C(5)	108.00(12)	C(16)-C(12)-C(11)	121.97(13)
C(18)-C(4)-C(5)	109.89(13)	C(13)-C(12)-C(11)	119.53(15)
C(6)-C(5)-C(10)	111.06(11)	C(14)-C(13)-C(12)	115.00(14)
C(6)-C(5)-C(4)	116.87(11)	C(14)-C(13)-C(16)	108.22(14)
C(10)-C(5)-C(4)	115.00(11)	C(12)-C(13)-C(16)	59.83(11)
C(7)-C(6)-C(5)	109.51(12)	C(13)-C(14)-C(8)	103.48(13)
C(8)-C(7)-C(6)	113.67(12)	C(16)-C(15)-C(8)	104.80(12)
C(7)-C(8)-C(14)	115.22(14)	C(17)-C(16)-C(12)	120.78(16)
C(7)-C(8)-C(9)	110.64(11)	C(17)-C(16)-C(15)	119.49(15)
C(14)-C(8)-C(9)	111.36(13)	C(12)-C(16)-C(15)	113.40(12)
C(7)-C(8)-C(15)	111.12(12)	C(17)-C(16)-C(13)	123.25(15)
C(14)-C(8)-C(15)	101.11(12)	C(12)-C(16)-C(13)	59.85(12)
C(9)-C(8)-C(15)	106.72(11)	C(15)-C(16)-C(13)	105.59(13)
C(8)-C(9)-C(11)	110.19(11)	O(1)-C(19)-O(2)	121.92(15)
C(8)-C(9)-C(10)	117.17(11)	O(1)-C(19)-C(4)	125.86(15)
C(11)-C(9)-C(10)	114.11(11)	O(2)-C(19)-C(4)	112.13(12)

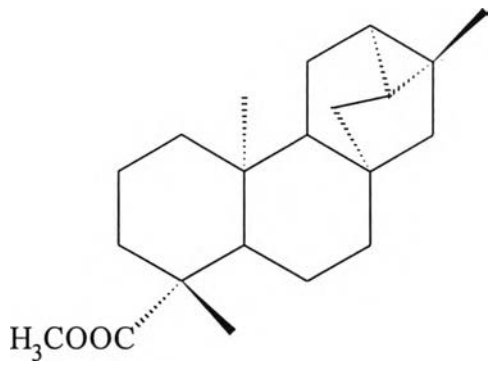


Figure 14 The structure of Compound 1a

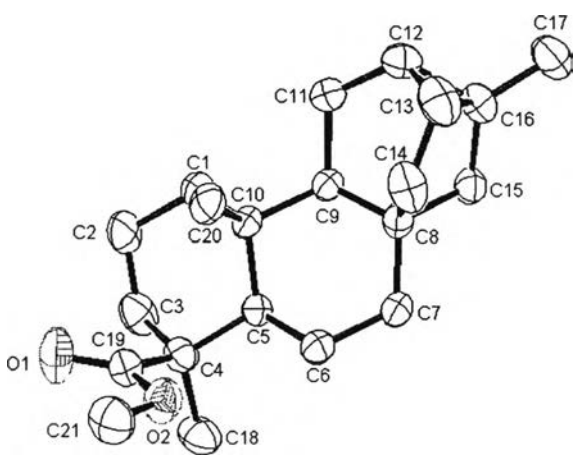


Figure 15 The crystal structure of Compound 1a

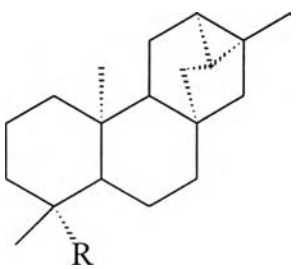
Literature reviews of trachyloban diterpene compounds

From the literature surveys, trachyloban diterpene, diterpene series containing a pentacyclic skeleton, has been widely studied and many diterpenoid compounds have been isolated and characterized as in the following examples.

In 1970, Pyrek, J. St. [17] isolated trachyloban-19-oic acid from the flower of *Helianthus annuus* L., and modified trachylobane derivative.

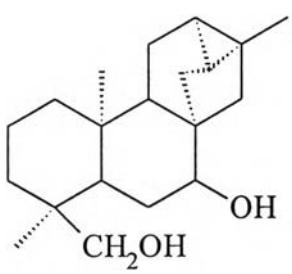
In 1976, Elliger, C. A. and Zinkel, D. F. [20-21] reported that thachyloban-19-oic acid which was isolated from sunflower inhibited larval growth.

In 1996, Costa, F. B. and Albuquerque, S. [19] isolated trachyloban-19-oic acid from the tuberous roots of *Viguiera aspillioides* Gardn. Trachyloban-19-oic acid and their modification were tested in vitro against *Trypanosoma cruzi.* The IC_{50} of trachyloban-19-oic acid showed IC_{50} value 500 μg/ml; 1.66 mM.

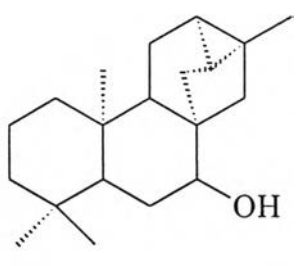


R	
COOH	trachyloban-19-oic acid
$COOCH_3$	methyl trachyloban-19-oic acid
CH_2OH	trachyloban-19-ol
CHO	19-oxotrachyloban
CH_3	trachyloban

In 1971, Gonzalez, A. G., Breton, J. L. and Fraga, B. M. [26] reported that the isolation of several diterpenes from *Sideritis canariensis* Ait., and the structure elucidation of two new compounds were 7β, 18-dihydroxy-trachilobane and 7β-hydroxy-trachilobane.

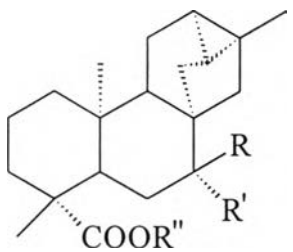


7β, 18-dihydroxy-trachilobane



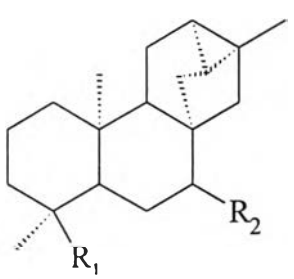
7β-hydroxy-trachilobane

In 1972, Bjeldanes, L. F. and Geissman, T. A. [25] isolated 7-α-hydroxy-4-epitrachylobanic acid from the dried and ground *Helianthus ciliaris* DC., but the others were modified from 7-α-hydroxy-4-epitrachylobanic acid.



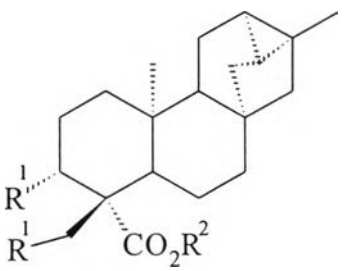
R"	R'	R	
H	OH	H	7-α-hydroxy-4-epitrachylobanic acid
H	OAc	H	7-α-acetoxy-4-epitrachylobanic acid
Me	OH	H	7-α-hydroxy-4-epitrachylobanic acid methyl ester

In 1982, Hasan, C. M. and Waterman, P. G. [17] isolated 7β-acetoxytrachyloban-18-oic acid from stem bark of *Xylopia quintasii* Engl., but the others were the synthetic derivative of 7β-acetoxytrachyloban-18-oic acid.



R_1	R_2	
OCOMe	COOH	7β-acetoxytrachyloban-18-oic acid
OCOMe	COOMe	7β-acetoxy-methyltrachyloban-18-oate
OH	COOH	7β-hydroxytrachyloban-18-oic acid
OH	CH_2OH	7β-hydroxytrachyloban-18-ol
OCOMe	CH_2OCOMe	7β,18-diacetoxytrachyloban

In 1989, Harrison, L. J. and Asakawa, Y. [22] isolated 3α,18-dihydroxytrachyloban-19-oic acid from the leverwort *Jungermannia exsertifolia* Steph. Subso. *Cordifolia.*, whereas the others were modified from 3α,18-dihydroxytrachyloban-19-oic acid.

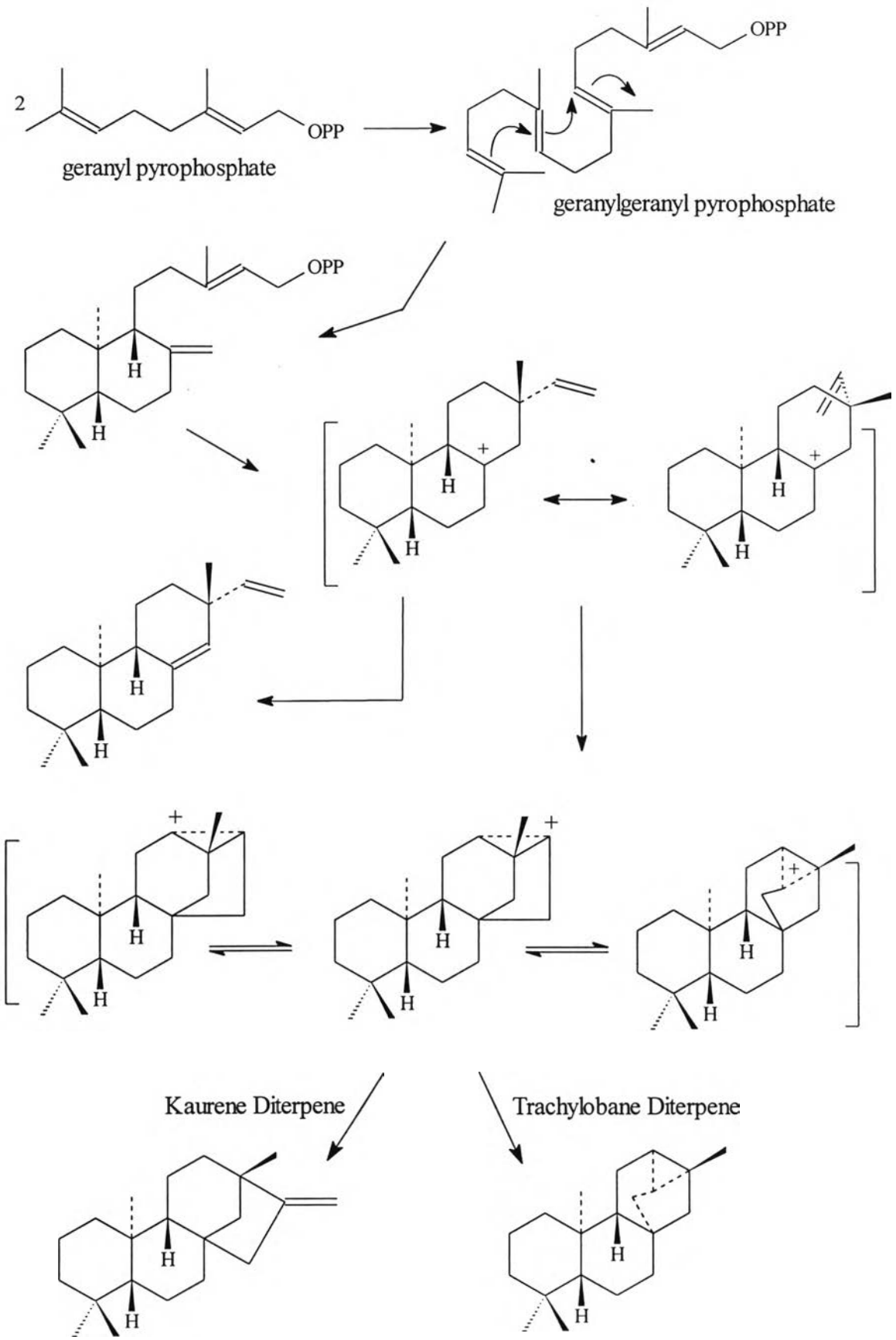


R^1	R^2	
OH	H	3α,18-dihydroxytrachyloban-19-oic acid
OAc	Me	3α,18-diacetoxytrachyloban-19-oate

Studies of other genus *Croton* have led to the isolation of diterpenoids belonging to the labdane, pimarane and kaurane diterpenoid which are biogenetically related to trachylobanes.

Biogenetic pathway of trachyloban diterpene was shown below.

Biogenetic of Trachylobane Diterpene [23-24]



The biogenetic scheme modified to permit skeletal rearrangements provides a concise rationale for skeletal patterns within this family of natural product. The diterpenes pimaradiene, kaurene and trachylobane fit into this biogenetic scheme.

Literature reviews in cytotoxic activity of cembranoid diterpene compounds of *C. oblongifolius* [5]

Previous studies by Singtothong, P., in Cytotoxic activity of cembranoid compounds from stem bark of *Croton oblongifolius* Roxb. against P 388 cell line and 6 tumor cell lines; S-102 (hepatoma), Hep-G2 (hepatoma), SW 620 (colon), Chago (lung), Kato-3 (gastric), BT 474 (breast) have been reported below.

Neocrotocembraneic acid, neocrotocembranal and poilaneic acid exhibited cytotoxic activity against P 388 cells *in vito*, with an IC_{50} value of 41.74, 6.48 and 42.49 μg/ml, respectively.

Their derivative of cembarnoid compounds; crotocambraneic acid, neocrotocembarneic acid and poilaneic acid exhibited cytotoxic activity against the 6 tumor cell lines mentioned above.

Moreover labdane diterpenoid and clerodane diterpenoid found in the stem bark of *Croton oblongifolius* Roxb. exhibited cytotoxic activity against tumor cell lines and anti bacterial, respectively.

Result of biological activity test

The *in vitro* activity of some compounds (10 μg/ml) from *Croton robustus* Kurz. against 6 cell lines, for example, Hs 27 (fibroblast), Kato-3 (gastric), BT 474 (breast), Chago (lung), SW 620 (colon) and Hep-G2 (hepatoma) cancer was reported in Table 18.

Table 18 Cytotoxic activity against tumor cell lines of some compounds from *C. robustus.*

Compound	IC 50 (μg/ml)*					
	Hs 27 (fibroblast)	Kato-3 (gastric)	BT474 (breast)	Chago (lung)	SW 620 (colon)	Hep-G2 (hepatoma)
1	>10	>10	>10	>10	>10	>10
2	>10	>10	>10	>10	>10	>10
3	>10	9.2	>10	>10	9.6	>10
1a	>10	8.3	>10	>10	9.1	>10

* IC_{50} was the minimum concentration of 50 % inhibitory activity.

Compound 3 and 1a showed weak cytotoxic activity against only Kato-3 (gastric) and SW 620 (colon) cancer. Compound 3 and 1a consisted of alcohol and methyl ester group, respectively. The cyctotoxicity of trachyloban-19 oic acid (Compound 1) and their derivative (Compound 3 and 1a) which were tested against *Trypanosoma cruzi.* have been reported previously [19]. The Compound 1 which inhibited larva growth has been reported previously [20-21]. These were report of the cytotoxicity of Compound 1, 3 and 1a against Hs 27, Kato-3, BT 474, Chago, SW 620 and Hep-G2 tumor cells for the first time. In addition, bioassay against P-388 of poilaneic acid (Compound 2) has been reported previously [5].