CHAPTER IV

RESULTS AND DISCUSSION

1 Isolation of chemical constituents from *Ircinia* sp.

Sponge samples (900g) were macerated in MeOH and extracted twice with dichloromethane yielding 10g of crude extract for further purification. The crude extract was fractionated by a quick column technique using a sinterd glass filter (15 cm diameter) as a column. Silica gel 60 was used as an adsorbent. A column was eluted sequently with solvent systems as shown in Table 4.

Table 4. Isolation of compounds P1 and P4.

System	Volume (ml)	Fraction code	Weight (g)
hexane: $CHCl_3$			
10 : 0	500		
9 : 1	800	P1	1.0
4 : 1	500		
1 : 1	600		
2 : 3	1600	P2	2.0
3 : 7	1600		
1 : 4	800	P3	2.0
1 : 9	800	P4	1.2
0 : 10	1000	P5	0.6

1.1 Isolation of compound P1.

The proton NMR pattern of P1 showed that P1 contain only one component. This P1 appeared as a quenching spot on TLC under UV (254 nm) light, and changed to an orange spot after spraying with anisaldehyde reagent. Compound P1 was obtained as yellow oil (1.0g, .011%) after a silica gel quick column, eluted with 20 % of $CHCl_3$ in hexane. It had Rf value of 0.3 in a solvent system of hexane : toluene : EtOAc, (20:1:1, v/v) and

showed a quenching spot under UV 254 nm and orange color positive to anisaldehyde reagent.

1.2 Isolation of compounds P44 and P45.

A 2g portion of P2, obtained from a quick column method (Table 1), was further purified using a Sephadex LH - 20 gel filtration. A column with an internal diameter of 2.5 cm (46 cm long) was used. Chloroform was used as an eluent (Table 5).

Table 5. Purification of P2 with gel filtration on Sephadex LH - 20.

Number of Eluted	Volume (ml)	Fraction code	Weight (mg)
1 - 2	200	P14	93.
4	150	P15	260
5 - 8	300	P16	1170
9 - 14	300	P17	155

After routine TLC check up, fraction P16 (1170 mg) was purified with gel filtration on Sephadex LH -20. A column (2.60 internal diameters and 96.00 cm

long) was eluted with Chloroform. Detail of column chlomatography is shown in Table 6.

Table 6. Purification of P16 with gel filtration on Sephadex LH - 20.

Number of elution	Volume (ml)	Fraction code	Weight (mg)
1 - 3	150	P32	150
4 - 8	200	P33	161
9 - 12	150	P34	500

P34 (500 mg) was furthered purified by silica gel flash column (2 cm diameter and 13 cm long). The detailed of elution were showed in Table 7.

Table 7. Purification of P34 by a silica gel flash column.

Solvent system.	Volume (ml)	fraction code.	Fraction weight (mg).
Hexane : toluene			
4 : 1	800		
1 : 1	600	P35	164
0 : 1	100		
toluene : chloroform	200		
1: 4	200	P36	122
0 : 1	400		
Chloroform : methanol, 4:1	500	P37	148

P35 (164 mg) was further purified by MPLC with a silica gel C-18 reversed phase column (200 µl/injection). A mobile phase was methanol : water (8:2, v/v). An UV - detector was set at 230 nm. A fraction containing compounds (P38, 96.6 mg) was collected, then concentrated under vaccum. This fraction was subsequently purified by a preparative TLC technique with a solvent system of hexane : toluene : acetone (3:1:0.5, v/v). TLC plates were developed 6 times, and location of each band was visualized under UV - light at 254 nm and spraying with anisaldehyde reagent.

There were 2 major bands detected under UV light. Location containing each compound was collected and then eluted with chloroform : methanol (9:1, v/v). The major 2 compounds, namely P44 (6.30mg, 1.1×10^{-3} %) and P45 (10.70mg, 1.7×10^{-4} %), were obtained from this purification. P44 and P45 gave orange color with anisaldehyde reagent and showed quenching spots under UV light at 254 nm, and had Rf value of 0.5 and 0.3 with a system of hexane : toluene : EtoAc (3:1:0.5, v/v, 6 time developing), respectively.

1.3 Isolation of compound KP9.

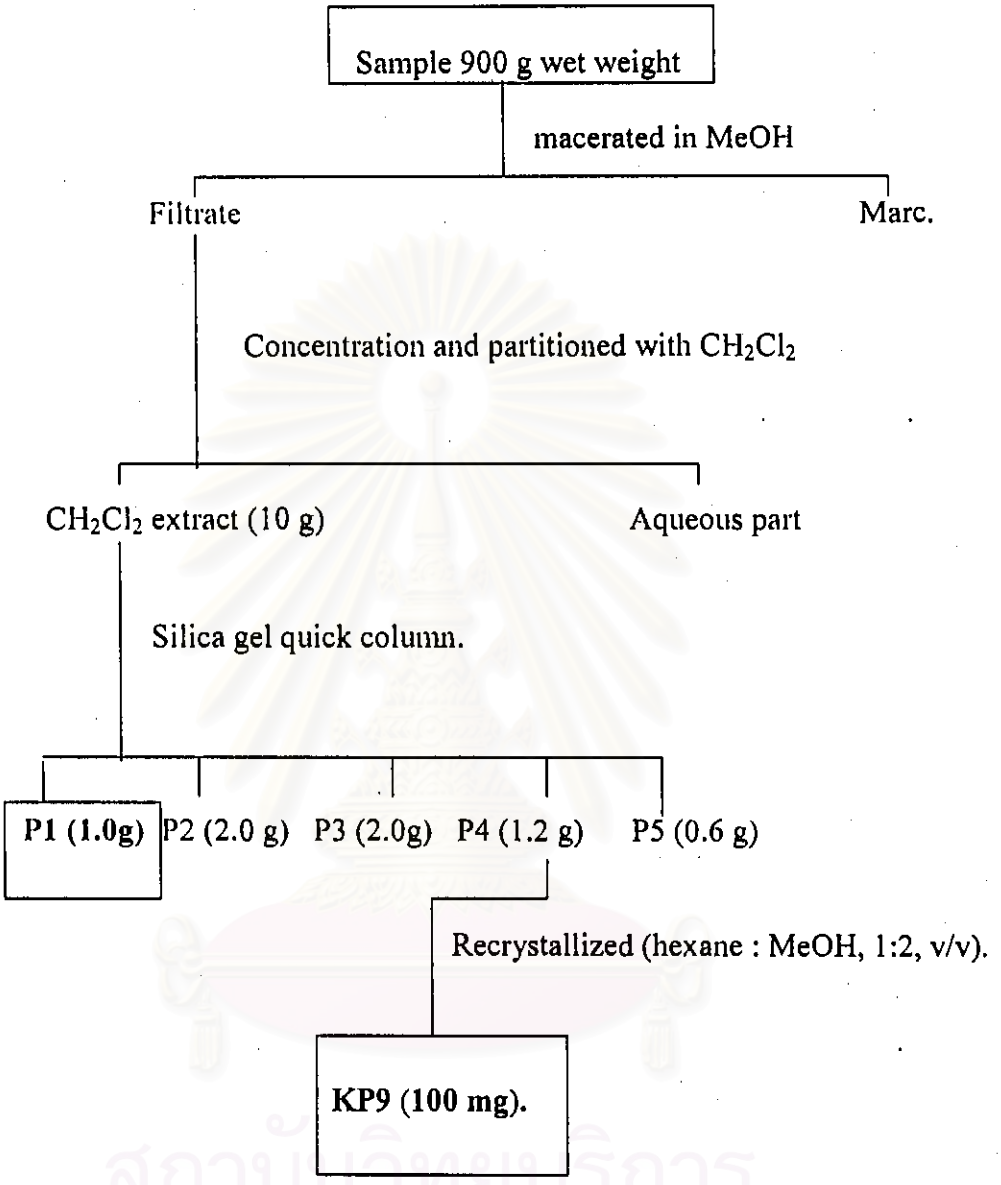
P4 which was obtained by a quick column technique eluted with solvent system shown in Table 1, was recrystallized in hexane : chloroform (1:9, v/v) to yield white needles (KP9, 100 mg). KP9 showed purple color positive to anisaldehyde reagent on TLC plate and had Rf value of 0.5 with a solvent system of hexane : $CHCl_3$ (1:2,v/v).

1.4 Acetylation reaction of compound KP9.

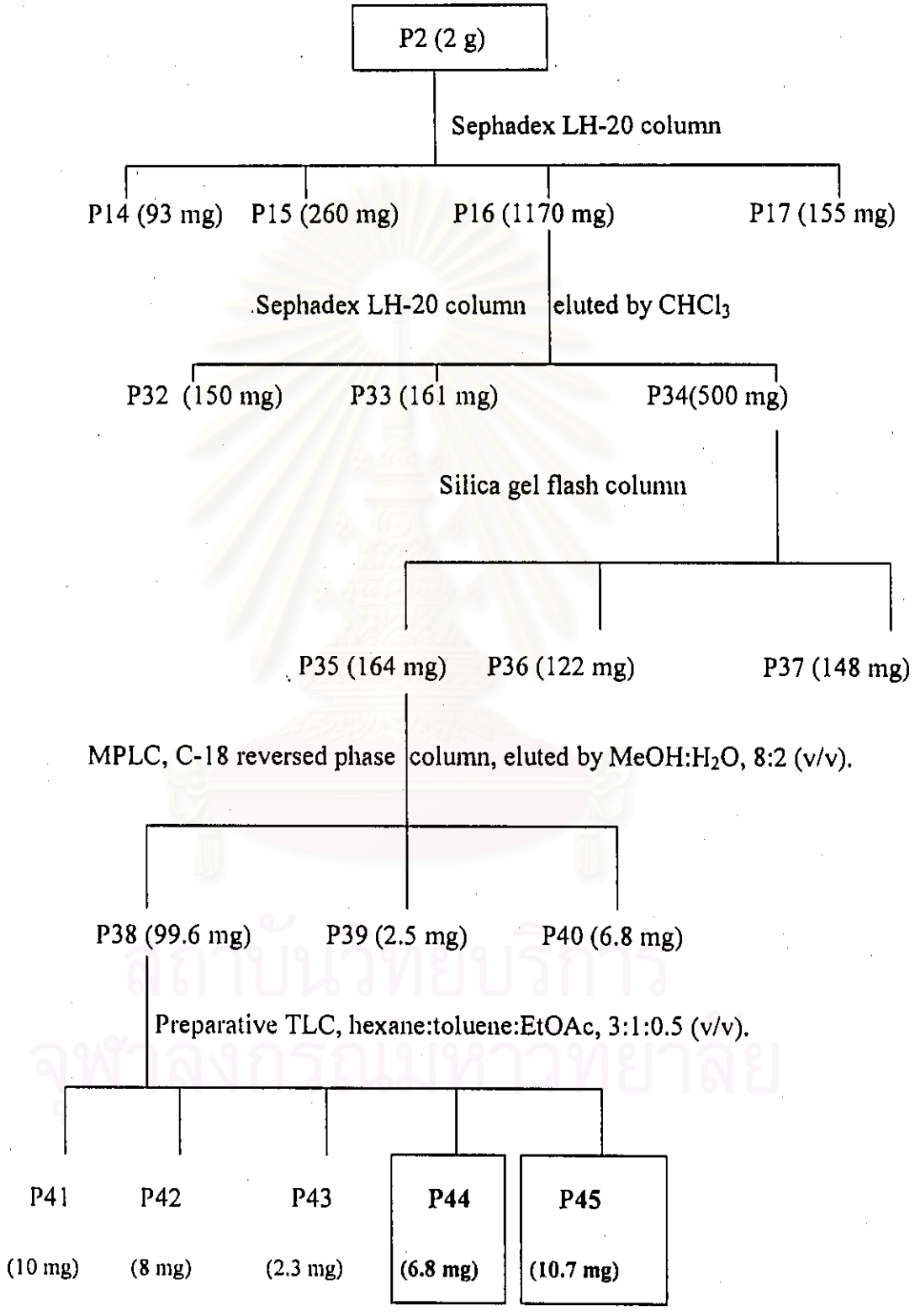
The NMR, IR and mass spectral data indicated the presence of a hydroxy group in compound KP9, so acetylation was performed to confirm the hydroxy group. The sample (6 g) was put in to the 5 ml round bottom flask with a magnetic bar inside. Pyridine and acetic acid anhydride 0.5 ml each, were added. The flask was sealed with rubber septum and the air inside was replaced by nitrogen

gas. The mixture was stirred at room temperature and reaction was monitored by TLC. The reaction was completed within 15 hrs. The reaction mixture was dried under reduced pressure process and purified by a small silica gel column chromatography using $CHCl_3$ an elutent, to yield 3.0 mg of an acetylation derivative.

The isolation of compounds P1, P44, P45 and KP9 was summerized in Scheme 2.



Scheme 2 Isolation and purification of the *Ircinia* sp.



Scheme 2. Continued.

2 Spectral data of the isolated compounds.

The isolated compounds were charactrerized by spectroscopic data including IR, UV, NMR and mass spectra.

Compound P1.

EIMS : m/z (relative intensity) (Figure 6).

312 (100, M^+), 297 (80), 203 (79), 175 (95), 163 (80), 145 (89), 133 (85), 121 (82), 93 (80) , 79 (95), 67 (92)

UV : λ_{max} nm (ε), in methanol (Figure 8)

: 220 (8780)

IR (NaCl disc) : √ cm^{-1}, (Figure 7)

2855, 2927, 1766.

^{1}H-NMR (300 MHz, in chloroform-d). : δ in ppm, J in Hz, (Figures 9 and 10)

1.64 (6H, brs), 1.17 (2H, p, J = 7.5), 2.06 (2H, t, J = 7.5), 2.06 (2H, t, J = 6.9), 2.13 (2H, t, 6.9), 2.30 (2H, q, J = 7.4), 2.41 (2H, t, J = 7.5), 2.49 (2H, t, J = 7.4), 5.16 (1H, brs, J = 6.9), 5.22 (1H, t, J = 7.4), 6.31 (1H, brs), 7.24 (1H, brs), 7.37 (1H, brs).

^{13}C-nmr(75 MHz, in chloroform-d) : δ in ppm (Figure 11).

142.38, 142.30, 138.61, 138.58, 135.45, 134.41, 124.48, 124.79, 124.41, 123.68, 110.94, 110.88, 39.73, 39.20, 28.52, 28.27, 26.58, 25.12, 24.31, 16.10, 15.97.

Compound P44.

EIMS : m/z (relative intensity), (Figure 23), 342 (5, M^+), 327 (5), 175 (20), 149 (40), 135 (55), 81 (100), 67 (82) , 55 (80).

IR (NaCl disc) : √ cm^{-1} (Figure 24), 3444, 1747.

UV : λ_{max} nm (ε), in methanol (Figure 25)

: 214 (7910)

^{1}H- NMR (300 MHz, in chloroform- d) : δ in ppm, J in Hz (Figures 26 and 27).

1.38 (2H, q), 1.45 (6H, m), 1.85 (2H, m), 1.86 (2H, m), 1.87 (2H, m), 1.96 (2H, q, J = 6.3), 2.11 (2H, q, J = 7.2), 2.31 (2H, t, J = 7.2), 2.90 (1H, brs), 4.63 (2H, brs), 4.95 (2H, brt), 5.03 (1H, brs), 5.40 (1H, brs), 6.14 (1H, brs), 7.07 (1H, brs), 7.20 (1H, brs).

^{13}C-NMR (75 MHz, in chloroform-d) : δ in ppm (Figure 28).

168.00, 142.37, 138.65, 135.46, 134.20, 133.93, 124.90, 124.81, 123.73, 115.25, 110.98, 71.12, 39.71, 39.10, 31.78, 30.14, 28.51, 26.61, 25.25, 25.15, 16.10, 15.97.

Compound P45.

EIMS : m/z (relative intensity), (Figure 33).

328 (19, M^+), 175 (20), 149 (79), 136 (60), 82 (79), 79 (90) , 67 (100), 53 (95).

UV : λ_{max} nm (ε), in methanol (Figure 35)

: 220 (8490)

IR. : √ cm^{-1} (Figure 34), 1780, 1747, 2930, 1780

^{1}H-NMR (300 MHz, in chloroform-d) : δ in ppm, J in Hz, (Figures 36 and 37).

1.57 (6H, brs), 1.68 (2H, p), 2.00 (4H, m), 2.06 (2H, m), 2.22 (2H, q, J = 7.5), 2.33 (2H, t), 2.43 (2H, t), 4.70 (2H, brs), 5.09 (1H, brs), 5.14 (1H, brs), 5.18 (1H, brs), 6.25 (1H, brs), 7.18 (1H, brs), 7.31 (1H, brs).

^{13}C-NMR (75 MHz, in chloroform-d). : δ in ppm (Figure 38).

175.25, 170.25, 142.37, 138.65, 135.37, 133.26, 125.57, 124.77, 123.79, 115.31, 110.97, 73.05, 39.63, 38.96, 28.49, 27.99, 26.59, 25.37, 25.13, 16.16, 15.87.

Compound KP9.

EIMS : m/z (relative intensity), (Figure 46), 474 (9, M^{+}), 446 (20), 428 (29), 414 (32), 401 (45), 371 (62), 229 (62), 271 (78), 215 (98), 203 (100).

UV : λ_{max} nm (ε), in methanol (Figure 48)

: 272 (9000)

IR (KBr disc) : √ cm^{-1}, (Figure 47).

3435, 1228, 1746, 1719.

^{1}H-NMR (300MHz, in chloroform-d). : δ in ppm, J in Hz, (Figures 49-51).

0.77 (1H, brs, J = 12.6), 0.81 (3H, brs), 0.87 (3H, brs), 0.95 (1H), 0.96 (3H, brs), 0.98 (3H, brs), 1.03 (1H, m), 1.10 (1H, m), 1.15 (2H, m), 1.20 (1H, brt), 1.42 (1H, m), 1.43 (2H, m), 1.55 (1H, m), 1.58 (1H, t), 1.82 (1H, m), 1.84 (1H, t), 1.99 (1h, brd, J = 12.6), 2.05 (4H, ddd, J = 11.0, 12.0 and 12.8), 2.15 (1H, d, J = 11.7), 2.25 (1H, brd, J = 12.8), 2.42 (3H, brs), 2.88 (1H, dd, J = 11.0 and 11.4), 3.65 (1H, dd, J =

3.5 and 12.0), 3.93 (1H, td, 5.2 and 10.0), 2.10 (1H, d, J = 12.0), 4.63 (1H, d, J = 12.0).

^{13}C-NMR (75 MHz, in Chloroform- d), δ in ppm (Figure 52)

210.41, 174.45, 170.52, 90.23, 74.67, 64.64, 61.22, 57.85, 56.78, 52.46, 51.64, 43.20, 41.81, 41.47, 41.42, 38.28, 35.37, 33.82, 33.47, 33.24, 29.69, 23.88, 21.90, 21.30, 18.41, 17.91, 17.79, 14.40.

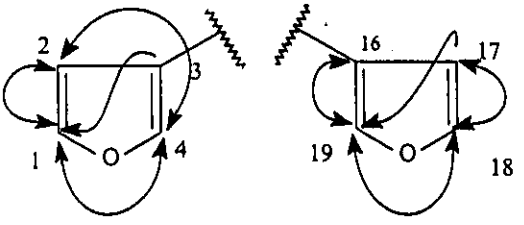
3 Structure elucidation of the isolated compounds

3.1 Compound P1

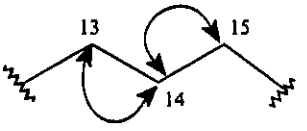
The molecular formula of compound P1 was established as $C_{21}H_{28}O_2$ based on the EIMS (Figure 6) showing a molecular ion peak at m/z 312, and the ^{13}C (Figure 11) and DEPT 135 (Figure 12) spectra showing 21 carbon signals which were 2 methyl carbons at δ 16.10 (C-20) and at δ 15.97 ppm (C-21), 7 methylene carbons at δ 25.12 (C-5), 28.52 (C-6), 39.73(C-9), 26.58 (C-10), 39.20 (C-13), 28.27 (C-14) and 24.31 ppm (C-15); 8 olefinic methine carbons at δ 142.38 (C-1), 110.94 (C-2), 138.58 (C-4), 123.68 (C-7), 124.41 (C-11), 138.61 (C-19), 110.88 (C-17) and 142.30 ppm (C-18) ; and 4 quaternary carbons at δ 124.48 (C-3), δ 134.41 (C-8), δ 135.45 (C-12) and δ 124.79 ppm (C-16).

From the NMR spectral data of ^{1}H-NMR, ^{13}C-NMR, DEPT 135, ^{1}H-^{1}H and C-H COSY and C-H long range correlation could be assigned as two furan rings of fragment A and as 3 fragments (B, C and D) of a linear skeleton.

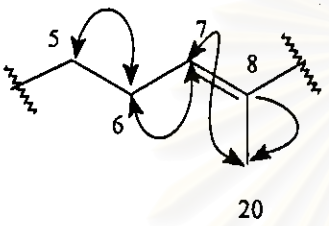
The ^{1}H-NMR spectrum of compound P1 in $CDCl_3$ (Figures 9 and 10) showed the presence of two furan rings. There are 6 protons attributable to protons of two typical β-substituted furan ring at δ 7.37 (brs, 2H, H-1 and H-18), 7.24 (brs, 2H, H-4 and H-19) and 6.31 (brs, 2H, H-2 and H-17). The HETCORR spectral data (Figures 13 and 14) which showed the C-H couplings, assisted the assignment of the carbon atoms in two furan rings : at δ 142.38 (C-1), 110.94 (C-2), 124.48 (C-3), 138.58 (C-4), 124.79 (C-16), 138.61 (C-19), 110.88 (C-17) and at 142.30 (C-18). The ^{1}H- ^{1}H COSY spectral data (Figures 15-17, table 9) exhibited correlations between the protons in two furan rings as follows: proton at δ 7.37 (2H, H-1 and 18) to protons at δ 7.24 (2H, H-4 and H-19) and at δ 6.31 (2H, H-2 and H-17); The C-H long range correlation spectra data (Figures 18-22, Table 8) showed a quaternary carbon at δ 124.48 (C-3) correlated to a proton at δ 7.37 (1H, H-1) and quaternary carbon at δ 124.79 (C-16) correlated to a proton at δ 7.24 (1H, H-19) in another one of furan ring. The IR spectrum revealed the presence of the furan rings at 1502, 1164, 1065, 1065 and 1025 cm^{-1}.

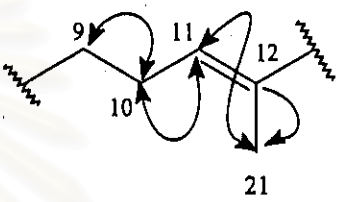


Fragment A



Fragment D.





Fragment B., ↶ (^{1}H-^{1}H COSY), ↷ (COLOC), Fragment C.

Table 8. ^{1}H-NMR, ^{13}C- NMR and C-H long range correlations data of compound P1.

Position	δ ^{1}H (ppm)	J-value (Hz)	δ ^{13}C (ppm)*	C-H long rang correlation in COLOC spectrum
1	7.37, brs,1H	-	142.38, d[a]	H-4
2	6.31, brs, 1H	-	110.94, d[b]	H-1
3	-	-	124.98, d[c]	H-1
4	7.24, brs, 1H	-	138.58,d[d]	H-2, H-1, H-5
5	2.49, t,2H	7.4	25.12, t	-
6	2.30, q, 2H	7.4	28.52, t	H-5 , H-10
7	5.22, t,1H	7.4	123.68, d	H-10
8	-	-	134.41, s[f]	H-9, H-10
9	2.06, t, 2H	6.9	39.73, t[e]	H-20, H-11
10	2.13, t , 2H	6.9	26.58, t	H-9, H-21
11	5.16, brs, 1H	6.9	124.41, d	H-9
12	-	-	135.45, s[f]	H-21, H-13
13	2.06, t, 2H	7.5	39.20, t[e]	H-11, H-21
14	1.71, p, 2H	7.5	28.27, t	H-15
15	2.41, t, 2H	7.5	24.31, t	H-13
16	-	-	124.79, s[c]	H-19
17	6.31, brs, 1H	-	110.88, d[b]	H-19
18	7.37, brs, 1H	-	142.30, d[a]	H-15, H-17
19	7.24, brs, 1H	-	138.61, d[d]	H-18
20	1.64, brs, 3H	-	16.10, q[g]	H-7, H-9
21	1.64, brs, 3H	-	15.97, q[g]	H-11, H-13

a, b, c, d, e, f, g interchangeable. * Multiplicity determine by DEPT 135 spectrum.

Table 9. ^{1}H-^{1}H COSY correlation of compound P1.

δ ^{1}H(ppm)	^{1}H-^{1}H correlation
7.37, H-1	H-4, H-2
6.31, H-2	H-1, H-4
7.24, H-4	H-1,H-2, H-5
2.49, H-5	H-6
2.30, H-6	H-5, H-7
5.22, H-7	H-6, H-20
2.06, H-9	H-10
2.13, H-10	H-11, H-9
5.16, H-11	H-10, H-21
2.06, H-13	H-14
1.71, H-14	H-13, H-15
2.41, H-15	H-14
6.31, H-17	H-18, H-19
7.37, H-18	H-19, H-17
7.24, H-19	H-15, H-18
1.64, H-20	H-7
1.64, H21	H-11

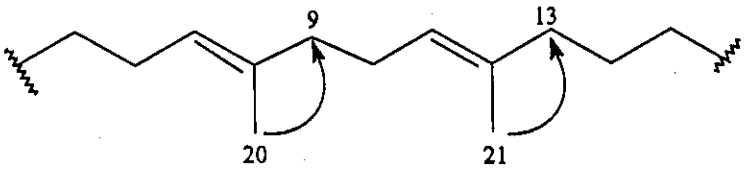
Fragment B is similarly to that of fragment C, because both consisted of CH_2CH_2HC=CMe groups. Fragment B was organized by ^{1}H-^{1}H COSY spectral data, showed the proton- proton correlations of methylene protons at δ 2.49 (H-5, J = 7.4 Hz) coupled to protons at δ 2.30 (H-6, J = 7.4 Hz) and these protons

coupled to an olefinic proton at δ 5.22 (H-7, J = 7.4 Hz), which in turn allylic coupled to methyl protons at δ 1.64 (20-CH_3). The COLOC pectrum showed the C-H long range correlation of a quaternary carbon at δ 134.41 ppm (C-8) to a broad singlet methyl resonance at δ 1.64 ppm, exhibited the location of the methyl proton.

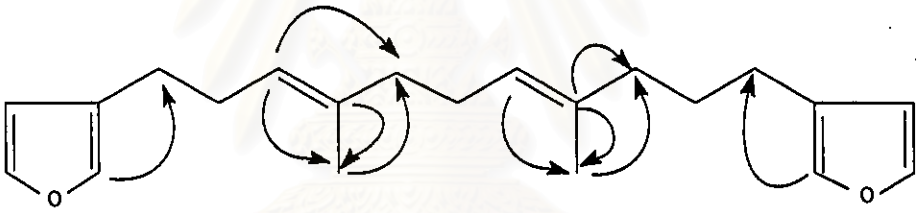
The correlation of methylene protons at δ 2.06 (H-9, J = 6.9 Hz) to protons at δ 2.13 (H-10, J = 6.9 Hz) which in turn coupled to proton at δ 5.16 (H-11, J = 6.9 Hz). The proton at δ 5.16 showed allylic coupling to methyl proton at δ 1.64 (H-21) and this proton was long range coupled by a quaternary carbon at δ 135.45 (C-12) in the COLOC spectra. As above data supported the assignment fragment C.

The ^{1}H-^{1}H COSY spectral data were used to establish fragment D as follow: methylene protons at δ 2.06 (H-13, J = 7.5 Hz) coupling to protons at δ 1.71 (H-14, J = 7.5 Hz) and further coupled to methylene protons at δ 2.41 (H-15, J = 7.5 Hz).

The C-H long range correlations data were used to link of all fragments, subsequently. Fragment B connected with fragment C by the correlation of the methyl carbon at δ16.10 (C-20) to the methylene protons at δ 2.06 (H-9) and fragment C was connected to fragment D by the correlation of the methyl carbon at δ 15.69 (C-21) to the methylene protons at δ 2.06 (H-13). These data gave a linear skeleton of P1 as shown below.

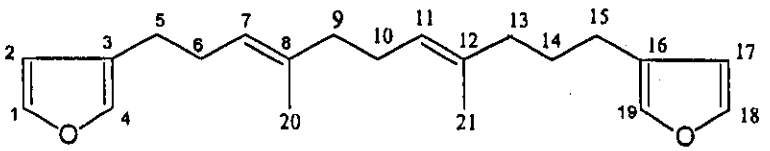


Finally, two furan rings connected to both ends of the linear skeleton by the carbons at δ 138.58 ppm (C-4) and at δ138.61 ppm (C-19) correlated to methylene protons at δ 2.49 (H-5) and at δ 2.41 (H-15). These information revealed that P1 has β-substituted furan ring at the both ends of a molecule.



Chemical structure of the compound P1 with C-H long range correlation.

By comparison with the published data P1 was identified as "anhydrofurospongin-1" previously isolated from a marine sponge *Spongia officinalis* (Cimino, Stefano and Minale, 1972).



Structure of P1 with the position of carbon atoms.

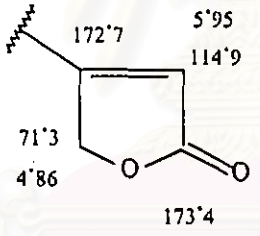
3.2 Compound P45.

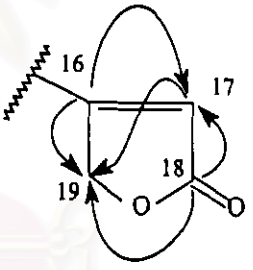
The electron impact (EI) mass spectrum (Figure 35) exhibited the molecular ion peak at m/z 328. The IR spectrum (Figure 34) revealed the presence of an α, β-unsaturated carbonyl stretching at ν_{max} 1781 cm^{-1} and 1748 cm^{-1} and also the presence of a furan ring at 1503, 1169, 1025, 874 and 782 cm^{-1}

The ^{13}C (Figure 38), DEPT 135 (Figure 39), and HETCORR (Figures 40 and 41) spectral data revealed that P45 contains 2 methyl carbons at δ 16.16 (C-20) and 15.87 (C-21), 6 olefinic methine carbons at δ 142.37 (C-1), 110.97 (C-2), 138.65 (C-4), 123.79 (C-7), 125.57 (C-11) and 115.31 (C-17); 8 methylene carbons at δ 25.13 (C-5), 28.49 (C-6), 39.63 (C-9), 26.59 (C-10), 38.96 (C-13), 25.37 (C-14), 27.99 (C-15) and 73.05 (C-19); 4 quaternary carbons at δ124.77 (C-3), 135.37 (C-8), 133.26 (C-12) and 170.25 (C-16); and 1 ester carbonyl carbon at δ 175.25 (C-18). From the above data established its tentative molecular formula as $C_{21}H_{28}O_3$.

Structure elucidation of compound P45 was organized by comparison their ^{13}C-NMR and ^{1}H-NMR data with those of compound P1. The ^{13}C and ^{1}H-NMR spectrum revealed that the structures of P45 and P1 are closely related; both had nearly the same pattern of ^{13}C and ^{1}H-NMR data. However, P45 had 4 carbons distinguishable from P1, including carbons at δ 170.25 (C-16), 115.31 (C-17), 175.25 (C-18) and 73.05 (C-19) which were attributable to a β-substituted α, β-unsaturated -γ-lactone ring, similar to a known lactone ring of luffracin-R reported previously by Capon and Bulter (1992). Structures of a lactone ring of luffracin-R

and a possible fragment of compound P45 (with C-H long range correlation pattern) are shown below. The ^{1}H-NMR spectra of compound P45 (Figures 36) showed protons in the lactone ring at δ 5.81 (brs, 1H, H-17) and δ 4.70 (brs, 2H, H_2-19). The ^{13}C-NMR spectrum showed the characteristic of the lactone carbonyl carbon at δ 175.25 (C-18). The COLOC technique revealed C-H long range correlations (Figures 42-44) of the carbonyl carbon (C-18) to protons at δ 5.81 (H-17) and at δ 4.70 (H-19); a carbon 115.31 (C-17) showed coupling to protons at δ 4.70 (C-19); a quaternary carbon of δ 170.25 (C-16) to protons at δ 5.81 (H-17) and at δ 4.70 (H-19). These data confirmed the presence of α,β-unsaturated- γ-lactone ring in a molecule.



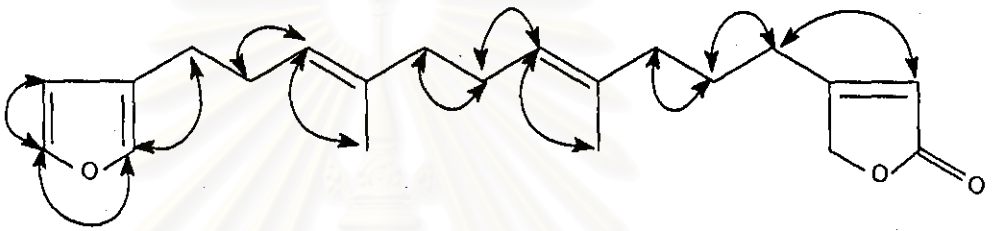


: (COLOC)

Lactone ring of luffracin-R Fragment A of compound P45

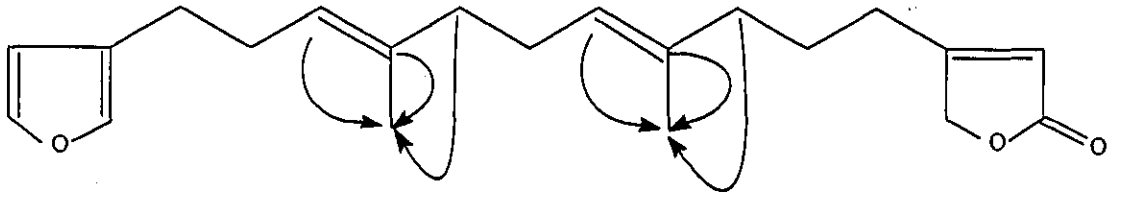
Furthermore, the ^{1}H and ^{13}C-NMR spectra of P45 showed typical characters of a β- substituted furan ring and a linear skeleton similar to those of P1, careful assignment of ^{1}H-^{1}H COSY (Figure 45) and C-H long range correlations in the COLOC spectra are summarized in Table 10.

The placements of β substituted furan ring and α,β-unsaturated -γ- lactone ring at both ends of the molecule were deduced by the observed allylic couplings in the ^{1}H-^{1}H COSY spectrum; olefinic proton at δ 5.81 (H-17) to methylene proton at δ 2.33 (H-15); olefinic proton at δ 7.18 (H-4) to methylene proton at δ 2.43 (H-5) are shown below.

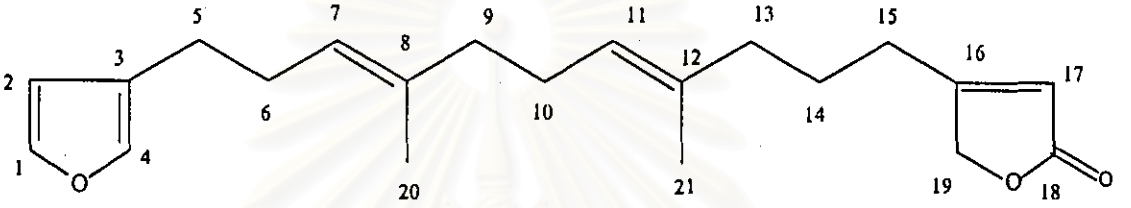


β-substituted furan ring and linear skeleton with ^{1}H-^{1}H correlation of P45.

Finally, a complete structure of P45 was organized as shown below. By comparison the chemical shifts of protons and carbons of P45 with that of the known compound of furospongolide (Kashman and Zviely, 1980) in Table 11, it is likely that P45 has the same chemical structure as that of furospongolide, which was previously isolated from a marine sponge, *Dysidea herbacea*.. P45 showed moderate anti HSV-1 activity at concentration of 20 μg/ml.



Structure of compound P45 with C-^{1}H long range correlation.



Chemical structure of compound P45 with the carbon atoms position.

Table 10. ^{1}H-NMR, ^{13}C-NMR with ^{1}H-^{1}H COSY and COLOC correlations of atoms in a molecule of compound P45.

Position	^{1}H-NMR ,δ ppm	*J*-value (Hz)	^{13}C-NMR,δ ppm	^{1}H-H correlation in COSY spectrum	C-H long range correlation in COLOC spectrum
1	7.31, brs, 1H	-	142.37,d	H-4, H-2	-
2	6.25, brs, 1H	-	110.97,d	H-1, H-4	-
3	-	-	124.77,s	-	-
4	7.18, brs, 1H	-	138.65,d	H-1, H-2, H-5	H-1
5	2.43, t, 2H	7.5	25.13, t	H-4, H-6	-
6	2.22, q, 2H	7.5	28.49,t	H-5, H-7	-
7	5.14, brt, 1H	7.5	123.79,d	H-6, 20-CH_3	20-CH_3
8	-	-	135.37[a],d	-	20-CH_3
9	2.00, m, 2H	-	39.63[b],t	H-10	20-CH_3
10	2.06, m, 2H	-	26.59,t	H-9, H-11	-
11	5.09, brt, 1H	-	125.57,d	H-9, 21-CH_3	21-CH_3
12	-	-	133.26[a],s	-	21-CH_3
13	2.00, m. 2H	-	38.96[b],t	H-14	21-CH_3
14	1.68, p, 2H	-	25.37, t	H13, H-15	-
15	2.33, t, 2H	-	27.99, t	H-14	-
16	-	-	170.25, t	-	19-CH_2, H-17
17	5.81, brs, 1H	7.6	115.31, d	H-15, H-19	19-CH_2
18	-	-	175.25, s	-	H-17, H-19
19	4.70, brs, 2H	-	73.05, t	-	-
20	1.57, brs, 3H	-	16.16[c],q	-	-
21	1.57, brs, 3H	-	15.87,q	-	-

* a, b and c assignment may be interchanged.

Table 11. The comparative of ^{1}H-NMR and ^{13}C-NMR between compound P45 and furospongolide (Kashman and Zviely, 1980).

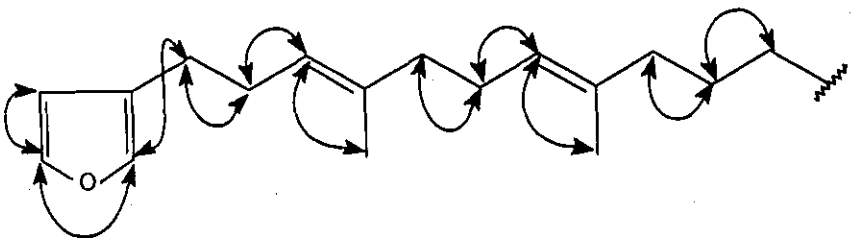
Position	Compound P45		Furospongolide	
	δH (ppm)	δC (ppm)	δH (ppm)	δC (ppm)
1	7.31, brs	142.37	7.33, brs	142.30
2	6.25, brs	110.97	6.27, brs	110.90
3	-	124.77	-	124.77
4	7.18, brs	138.65	7.20, brs	138.50
5	2.43, t	25.13	2.33, t	24.80
6	2.22,q	28.49	2.05, q	27.70
7	5.14, brt	123.79	5.16, t	123.70
8	-	135.37	-	135.30
9	2.00, m	39.63	2.05, m	38.70
10	2.06, m	26.59	2.05, q	26.20
11	5.09, brt	125.57	*	125.50
12	-	133.26	-	132.20
13	2.00, m	38.96	2.33, t	39.40
14	2.33, t	27.99	*	24.80
15	1.68, p	25.37	*	28.20
16	-	170.25	*	170.40
17	5.81, brs	115.31	5.83, brs	155.10
18	-	175.25	-	170.00
19	4.70, brs	73.05	4.72	72.90
20	1.57, brs	16.16	1.59, brs	15.70
21	1.57, brs	15.87	1.68	15.70

* Not reported in the literature.

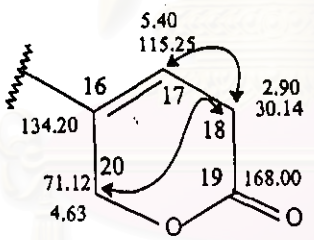
3.3 Compound P44.

The ^{13}C-NMR, DEPT 135 (Figure 29) and HETCOR (Figures 30 and 31) spectra of compound P44 revealed 22 carbons including, 2 methyl carbons at δ 16.10 (C-21) and 15.97 ppm (C-22), 9 methylene carbons at δ 25.15 (C-5), 28.51 (C-6), 39.71 (C-9), 26.61 (C-10), 123.73 (C-11), 39.10 (C-13), 25.25 (C-14), 31.78 (C-15) and 71.12 ppm (C-20); 6 olefinic methine carbons at 142.37, 110.98, 138.65, 124.90, 123.73, 115.25 and 4 quaternary carbons at δ 124.81 (C-3), 134.20 (C-8), 135.46 (C-12) and 133.90 ppm (C-16) and one a ester carbonyl carbon at δ 168.00 ppm. The IR spectrum (Figure 24) exhibited a lactone carbonyl adsorption at ν_{max} 1,748 cm^{-1} and a typical characteristic of a furan ring. The electron impact (EI) mass spectrum (Figure 23) indicated molecular ion peak at 342, suggesting a molecular formula of $C_{22}H_{30}O_3$.

The ^{1}H-NMR (Figures 28 and 29) and ^{13}C-NMR data of compound P44 are closely related to those of compound P45 (summarized in Table 14), suggesting that P44 has linear skeleton and a β- substituted furan ring similar to P45. The linear skeleton and β- substituted furan ring were assigned by ^{1}H-^{1}H COSY spectral data (Figures 32, Table11). The structure of β-substituted furan ring with linear skeleton is shown below.

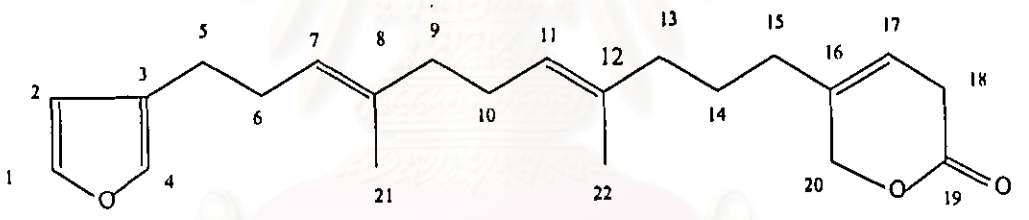


The presence of a lactone ring was deduced by data of ^{1}H-^{1}H COSY showing the methylene protons at δ 2.90 (brs, H-18) correlated to the olefinic proton at δ 5.40 (brs, H-17) and homoallylic coupling correlated to the methylene protons at δ 4.63 (brs, H-20) careful analysis of the ^{13}C-NMR and HETCOR spectra confirmed the β, γ unsaturated-δ-lactone ring; 133.90 (C-16), 115.25 (C-17), 30.14 (C-18) and 168.00 (C-19) ppm.



Proposed lactone ring of P44.

Finally, the lactone ring was connected to linear skeleton by the allylic coupling of proton signal at δ 1.85 (m, 2H, H-15) to proton signal at δ 5.40 (brs, 1H, H-17) in the ^{1}H-^{1}H COSY spectrum. After careful comparison of ^{1}H-NMR and ^{13}C-NMR data of P44 with the literature (Table 13), compound P44 was identified as furodendin which was previously isolated from a marine sponge, *Phyllospongia dendyi* (Kaslauskas, *et al*, 1980).



Chemical structure of P44 with an assignment of carbon position.

Table 12. Chemical shift of ^{1}H, ^{13}C and ^{1}H-^{1}H COSY of compound P44.

Position	^{1}H-NMR, δ ppm	^{1}H-^{1}H correlation	J-value (H_Z)	^{13}C-NMR, δ ppm[a]
1	7.20, brs,1H	H-2, H-4	-	142.37, d
2	6.14, brs,1H	H-1, H-4	-	110.98, d
3	-	-	-	124.81, s
4	7.07, brs,1H	H-1, H-2, H-5	-	138.65, d
5	2.31, t, 2H	H-4, H-6	7.2	25.15, t
6	2.11, q, 2H	H-5, H-7	7.2	28.51, t
7	5.03, brt, 1H	H-6, 21-CH_3	7.2	124.90, d
8	-	-	-	134.20, s
9	1.87, m, 2H	H-10	-	39.71,t
10	1.96, q, 2H	H-9, H-11	6.3	26.61, q
11	4.95, brt, 2H	H-10, 22-CH_3	6.3	123.73, d
12	-	-	-	135.46, s
13	1.86, m, 2H	H-14	-	39.10, t
14	1.38, q, 2H	H-13, H-15	-	25.25, t
15	1.85, m, 2H	H-14, H-17	-	31.78, t
16	-	-	-	133.90, s
17	5.40, brs, 1H	H-15, H-18, H-20	-	115.25, t
18	2.90, brs, 1H	H-17, H-20	-	30.14, t
19	-	-	-	168.00, s
20	4.63, brs, 2H	H-17	-	71.12, t
21	1.45, brs, 3H	H-11	-	16.10, q
22	1.45, brs, 3H	-	-	15.97, q

a: multiplicity detemined by the DEPT 135 spectrum

Table 13 The comparison of ^{13}C and ^{1}H-NMR data between P44 and Furodendin.

Position	Furodendin		Compound P44	
	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)	^{1}H-NMR (ppm)	^{13}C-NMR (ppm)
1	7.20	142.60	7.20	142.37
2	6.30	110.00	6.14	110.98
3	-	125.30	-	124.81
4	7.36	138.80	7.07	138.65
5	*	25.10	2.31	25.15
6	2.60	28.40	2.11	28.51
7	5.20	125.40	5.03	124.90
8	-	134.20	-	134.20
9	*	*	1.87	39.71
10	1.54	26.50	1.96	26.61
11	5.12	*	4.95	123.73
12	-	135.00	-	135.46
13	*	*	1.86	39.10
14	1.9	25.50	1.38	25.25
15	*	30.00	1.85	31.78
16	-	134.00	-	133.90
17	5.56	115.40	5.40	115.25
18	3.08	*	2.90	30.14
19	-	169.40	-	168.00
20	4.62	71.00	4.63	71.12
21	1.60	16.00	1.45	16.10
22	1.60	16.00	1.45	15.97

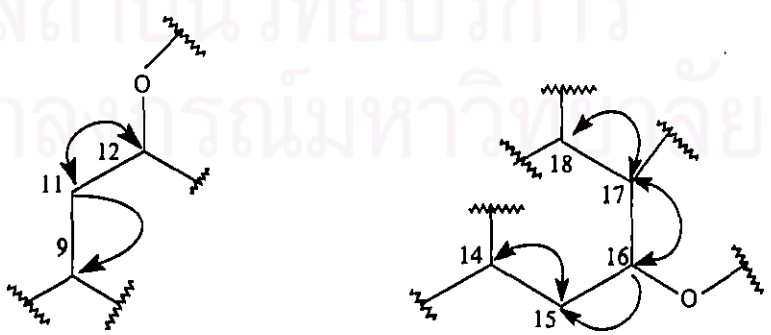
* Not reported in the literature.

3.4 Compound KP9.

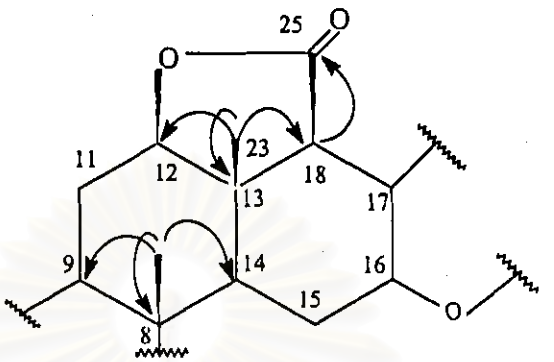
The ^{13}C-NMR spectrum of KP9 (Figure 52) exhibited 28 carbon atoms in a molecule consisting of 2 ester carbonyl carbons at δ174.45 and δ170.52 ppm and 1 keton carbonyl carbon at δ 210.41 ppm. The DEPT 135 spectral data (Figure 53) showed 6 methyl carbons at δ 33.82 (C-19), 21.90 (C-20), 17.91 (C-21), 14.40 (C-23), 33.24 (C-26) and 21.30 ppm (C-28); 8 methylene carbons Iat δ 35.37 (C-1), 18.41 (C-2), 41.42 (C-3), 41.47 (C-6), 17.79 (C-7), 23.88 (C-11), 29.69 (C-15) and 64.64 ppm (C-22); 7 methine carbons at δ 57.85 (C-5), 61.22 (C-9), 90.23 (C-12), 52.46 (C-14), 74.67 (C-16), 51.54 (C-17) and at δ 56.78 ppm (C-18); 4 quaternary carbons at δ 33.47 (C-4), 38.28 (C-8), 41.81 (C-10) and at δ 43.20 ppm (C-13). The IR spectrum (Figure 47) exhibited the presence of OH group at 3,435, ester carbonyl group at 1,746 and ketone carbonyl at 1,719 cm^{-1}. Combination of the above data with the EIMS showing a molecular ion peak at m/z 474 (Figure 46) suggested the tentative molecular formula of $C_{28}H_{42}O_6$ which indicated eight degrees of unsaturation.

The presence of 2 ester carbonyls and 1 ketone carbonyl suggested that the remaining unsaturations are pentacarbocyclic in nature. This data was used to guide for the elucidation of the molecular structure of KP9 using in combination with HSQC (Figures 54-57), HMBC (Figures 58-63) and ^{1}H-^{1}H COSY (Figures 64 and 65) techniques. The correlation in the ^{1}H-^{1}H COSY spectral data (summarized in Table 16) revealed the presence of two separate spin systems : fragment A and B. Fragments A was deduced by the following correlations: proton at

δ 2.25 (H_e -11) to δ 1.10 (H-9), proton at δ 2.05 (H_a - 11) to proton at δ 3.65 (H-12) and this proton coupling to a proton at δ 2.05 (H_a -11). Fragment B was also formed by the following correlations ; proton at δ 2.15 (H-18) to proton at δ 2.88 (H-17); proton at δ 3.93 (H-16) to protons at δ 2.88, 1.58 (H_a -15) and δ 1.86 ppm (H_e-15). The latter methylene protons were further coupled by proton at δ 1.20 (H-14). The HMBC spectral data (Figures, 58-63 and data summarized in Table 17) helped the connection of these two fragments as shown in fragment C: the methyl protons at 0.98 (H-23) correlated to carbons at δ 90.23 (C-12), and 56.78 ppm (C-18) and a quaternary carbon at δ 43.20 ppm (C-13). The methyl protons at δ 0.96 (H-21) correlated to two methine carbons at δ 61.22 (C-9) and 52.46 (C-14) and a quaternary carbon at δ 38.28 (C-8). In addition, the downfield signal of protons at δ 3.65 (H-12) and δ 3.93 ppm (H-16) indicated the oxymethine proton in nature. The proton at δ 2.15 (H-18) is connected to an ester carbonyl carbon to form a lactone ring which was observed by the correlation of this proton to the ester carbonyl carbon at δ 174.45 (C-25) in the HMBC spectrum data.

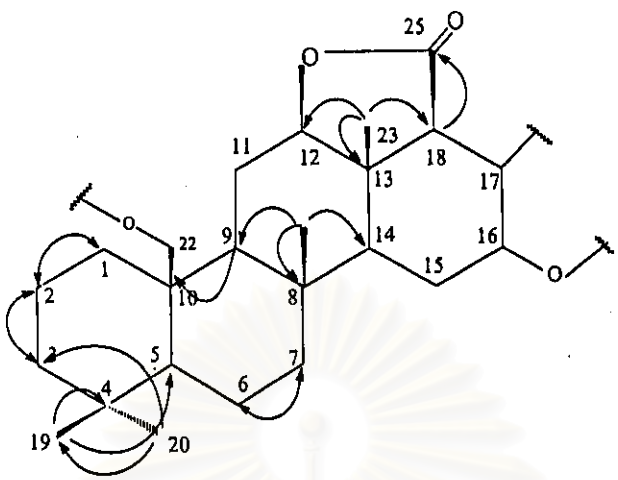


(A) : ^1H-^1H COSY (B)



(C), ↷ : HMBC

The remaining 2 saturated fused - rings fused was performed based on ^{1}H-^{1}H COSY, HSQC and HMBC spectral data. The HMBC spectra (Figure58-63) showed C-H long range correlations of oxymethylene protons at δ 4.63 and δ 4.10 ppm (H_a -22 and H_b -22) to a carbon at δ 35.77 (C-1), and then ^{1}H-^{1}H correlations of a proton at δ 0.77 (H_a -1) to δ 1.55 (H_e-2) which in turn coupled to a proton at δ 1.17 (H_e-3). The geminal methyl protons at δ 0.81 (brs, H-19) and δ 0.87 (brs, H-20) correlated to carbons at 33.47 (C-4), 57.85 (C-5) and 41.42 ppm (C-3). A proton at δ 1.03 ppm (H_a-6) showed C-H long range correlation to a methine carbon at δ 57.85 ppm (C-5). Furthermore, a proton at δ 1.03 (H_a -6) also showed ^{1}H-^{1}H coupling to δ 1.44 (H_a -7). The connectivity of fragment C and 2 ring systems was performed by correlations of proton at δ 1.10 ppm (H-9) to a carbon at δ 23.88 (C-11) and a quaternary carbon at δ 41.81 ppm (C-10). As above discussed, the complete assignment of the ring system is shown below.

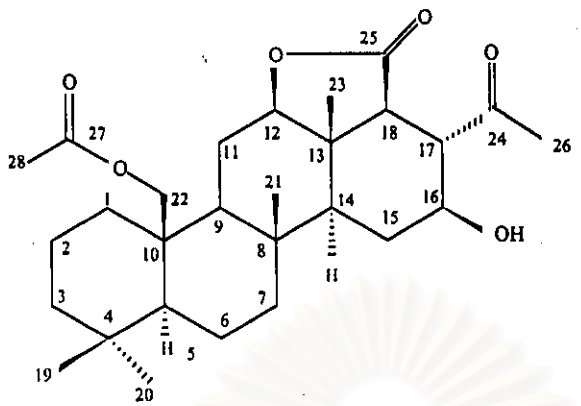


↷: HMBC, ↷: ^{1}H-^{1}H COSY

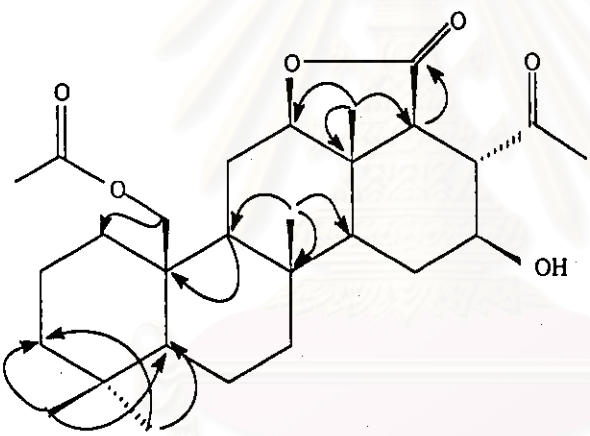
The presence of an acetoxyl group and a methyl ketone group were formed by HMBC technique. The methyl protons at δ 2.05(brs, H-28) correlated to an ester carbonyl carbon at 170.52 (C-27) and the methyl protons at δ 2.42 (brs, H-26) to a carbonyl ketone at δ 210.41 (C-24). According to the downfield shift of H-17 at δ 2.88, the methyl ketone group was placed at C-17 at δ 51.64 ppm.

The placement of the acetyl group and the free OH group was determined by acetylation reaction. The ^{1}H-NMR spectrum of the acetylation product of KP9 (Figure 67) showed a 3.97 ppm downfield shift of H-16 (δ 4.90) comparing to that of KP9 (δ 3.93). This information permitted the OH substituted at C-16 and hence, the acetoxyl at C-22.

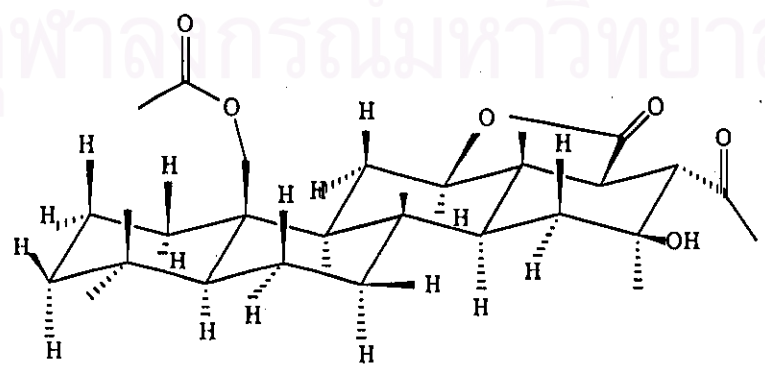
The final structure of KP9 was confirmed by comparison of its chemical shifts of ^{13}C-NMR and ^{1}H-NMR data with those of the known compound which has been reported by Kazlauskas *et al* (1982) (Table 17). Thus, compound KP9 was induced 22β-acetoxy-16β-hydroxy-24-methyl-24-oxoscalaran-25, 12β-olactone which was previously isolated from *Lendenfeldia* sp. However, the report is the first complete proton and carbon assignments for this compound.



Structure of compound KP9 and carbon position.



Structure of compound KP9 with C-H long range correlation.



Configuration of KP9.

Table 14 Chemical shift of ^{1}H and ^{13}C-NMR of compound KP9.

Position	δH (ppm)	multiplicity (J = Hz)	δC (ppm)*
1e	1.99	1H, brd, J = 12.6	35.37, t
1a	0.77	1H, brt, J = 12.6	
2e	1.55	1H, m	18.41, t
2a	1.42	1H, m	
3e	1.43	1H, m	41.42, t
3a	1.15	1H, m	
4	-	-	33.47, s
5	0.95	1H,	57.85, d
6e	1.82	1H, m	41.47, t
6a	1.03	1H, m	
7e	1.55	1H, m	17.79, t
7a	1.44	1H, m	
8	-	-	38.28, s
9	1.10	1H, m	61.22, d
10	-	-	41.81, s
11e	2.25	1H, brd , J = 12.8	23.88, t
11a	2.05	1H, ddd, J = 11.0, 12.0, 12.8,	
12	3.65	1H, dd, J = 3.5, 12.0	90.23, d
13	-	-	43.20, s
14	1.20	1H, brt	52.46, s
15e	1.84	1H, t	29.69, t
15a	1.58		
16	3.93	1H, td, J = 5.2, 10.0	74.67, d
17	2.88	1H, dd, J = 10.0, 11.4	51.64, d
18	2.15	1H, d, J = 11.7	56.78, d
19	0.81	3H, brs	21.90, q
20	0.87	3H, brs,	33.82, q
21	0.96	3H, brs	17.91, q
22a	4.63	1H, d, J = 12.0	64.64, t
22b	4.10	1H, d, J = 12.0	
23	0.98	3H, brs	14.40, q
24	-	-	210.41, s
25	-	-	174.45, s
26	2.42	3H, brs	33.24, q
27	-	-	170.52, s
28	2.05	3H. brs	21.30, q

* Multiplicity determined by the DEPT 135 spectrum.

Table 15 ^{1}H-^{1}H correlation of compound KP9.

Position	δH (ppm) of KP9	^{1}H-^{1}H correlation
1e	1.99	H_a-1
1a	0.77	H_e-1, H_e-2
2e	1.55	H_a-2, H_e-3
2a	1.42	H_e-2, H_e-3
3e	1.43	H_e-2
3a	1.15	H_e-3
4	-	-
5	0.95	-
6e	1.82	H_a-6, H_e-7
6a	1.03	H_e-6, H_a-7
7e	1.55	H_e-6
7a	1.44	H_e-6, H_e-7
8	-	-
9	1.10	H-5
10	-	-
11e	2.25	H-9, H_a-11
11a	2.05	H_e-11, H-12
12	3.65	H_a-11, H-18
13	-	-
14	1.20	H_e-15
15e	1.84	H-14
15a	1.58	H-14
16	3.93	H_a-15, H_e-15, H-17
17	2.88	H16, H18
18	2.15	H-17, H-26
19	0.81	-
20	0.87	-
21	0.96	-
22a	4.63	H_b-22
22b	4.10	H_a-22
23	0.98	-
24	-	-
25	-	-
26	2.42	-
27	-	-
28	2.05	-

Table 16. HMBC correlation of KP9.

Position	δH (ppm)	δC (ppm)	Correlation in HMBC spectrum(δ ppm)
1e	1.99	35.77	-
1a	0.77		
2e	1.55	18.41	-
2a	1.42		
3e	1.43	41.42	C-1,C-5
3a	1.15		C-9, C-19, C-20
4	-	33.47	-
5	0.95	57.85	-
6e	1.82	41.47	C-5
6a	1.03		
7e	1.55	17.79	C-6
7a	1.44		
8	-	38.28	-
9	1.10	61.22	C-8, C-10, C-11, C-21, C-22
10	-	41.81	-
11e	2.25	23.88	-
11a	2.05		
12	3.63	90.23	-
13	-	43.20	-
14	1.20	52.46	C-21
15e	1.84	29.69	C-14, C-16, C-17
15a	1.58		C-14, C-16, C-17
16	3.93	74.67	
17	2.88	51.64	C-15, C-16, C-18
18	2.15	56.78	C-13, C-17, C-23, C-25
19	0.87	33.82	C-3, C-4, C-5, C-20
20	0.96	21.90	C-3, C-4, C-5 C-19
21	0.81	17.91	C-8, C-9, C-10
22a	4.63	64.64	C-1, C-9, C-10
22b	4.10		
23	0.98	14.40	C-12, C-13, C-18
24	-	210.41	-
25	-	174.45	-
26	2.42	33.24	C-24
27	-	170.52	-
28	2.05	21.30	C-27

Table 17. Comparison of chemical shift of ^{13}C and ^{1}H-NMR data between compound KP9 and known compound .

Position	Compound KP9		22β-acetoxy-16β-hydroxy-24-methyl-24-oxo scalaran-25, 12β-olactone	
	δH (ppm)	δC (ppm)	δH (ppm)	δC (ppm)
1e	1.99	35.37	*	35.20
1a	0.7			
2e	1.55	18.41	*	18.30
2a	1.42			
3e	1.43	41.42	*	41.30
3a	1.15			
4	-	33.47	*	33.10
5	0.95	57.85	*	57.70
6e	1.82	41.47	*	17.70
6a	1.03			
7e	1.55	17.79	*	41.70
7a	1.44			
8	-	38.28	*	38.10
9	1.10	61.22	*	61.10
10	-	41.81	*	41.80
11e	2.25	23.88	*	23.70
11a	2.05			
12	3.65	90.23	3.69	90.30
13	-	43.20	*	43.0
14	1.20	52.46	*	52.30
15e	1.84	29.69	*	26.60
15a	1.58			
16	3.93	74.67	3.88	74.40
17	2.88	51.64	2.88	51.50
18	2.15	56.58	2.18	56.70
19	0.81	33.82	0.83	21.80
20	0.87	21.90	0.88	33.70
21	0.96	17.91	0.98	17.70
22a	4.63	64.64	4.64	64.70
22b	4.10		4.09	
23	0.98	14.40	0.98	14.20
24	-	210.41	*	210.80
25	-	174.45	*	174.70
26	2.42	33.24	2.40	33.40
27	-	170.52	*	170.70
28	2.05	21.30	2.06	21.10

* * Not previously assigned,

4 Bioactivities of the isolated compounds

Compound P1, P45 and KP9 were subjected to test for brine shrimp lethality assay and anti - HSV activity except P44 due to its limited quality.

4.1 Compound P1 is yellow oilly which has toxicity to brine shrimps (LD_{50} at 2.38 μg/ml) and moderate active to HSV-1 at concentration of 20 μg/ml.

4.2 Compound P45 showed moderated active to HSV-1 at concentration of 20μg/ml

4.3 KP9 was not active in both bioactivities test.

Therefore, compound P1 was responsible for the activity of the dichloromethane extract against brine shrimp. This is the first report for anti-HSV activity of linear furanoterpenes.