CHAPTER IV

CONCLUSION

During the course of this research, the antifungal activity of twenty-five essential oils was screened against three phytopathogenic fungi (*Fusarium oxysporum* 43-68, *Alternaria* sp. 43-89 and *Phytophthora* sp. 572). based on the antifungal activity results, all tested essential oils can be classified into three groups.

1. The essential oils exhibited excellent antifungal activity against all tested microorganisms are those from *Mentha cordifolia* Opiz., *Cinnamomum bejolghota* Sweet., *Litsea cubeba* and *Eugenia caryophyllus* Bullock & Harrison..

2. The essential oil which demonstrated selectively good antifungal activity against *Fusarium oxysporum* 43-68. is *Zingiber officinale* Roseoe.

3. The essential oils displayed discriminating antifungal activity against *Alternaria* sp. 43-89 are those from *Pelargonium graveolens, Ocimum gratissismum* Linn., *Ocimum sanctum* Linn., *Limnophila aromatica* Merr., *Kaempferia galanga* Linn. and *Zingiber cassumunar* Roxb.

Moreover, the active components were attempted to seek for in each active essential oil. It was found that the component of *Mentha cordifolia* Opiz., *Cinnamomum bejolghota* Sweet., *Litsea cubeba* and *Eugenia caryophyllus* Bullock & Harrison. exhibited clear zone on bioautographic plate. Two pure compounds, namely eugenol, a major component from *Eugenia caryophyllus* Bullock & Harrison. and cinnamaldehyde, a main compound from *Cinnamomum bejolghota* were tested. It was disclosed that both compounds possessed the antifungal activity, and could claim as active principle.

In addition, the structure-antifungal activity relationship was investigated. It was found that the phenolic compound gave potent activity than aldehyde, alcohol, carboxylic acid and hydrocarbon, respectively.

Moreover, cinnamon oil (*Cinnamomum bejolghota* Sweet) and clove oil (*Eugenia caryophyllus* Bullock & Harrison.) were further examined the possibility for the use in postharvest control. It was found that both essential oils demonstrated significant reduction in decay of 87 and 94%, respectively.

Proposal for future work

This research clearly addressed the antifungal activity of crude essential oils. Further studies should involve the elucidation of all active compounds from active crude essential oils, modify structure and study on structure activity relationship. Moreover, mode of action of essential oil on phytopathogenic fungi has not been illuminated. In addition, the essential oils are not as broad spectrum as synthetic pesticides, but their effectiveness can be improved by using them in conjunction with carefully designed packaging and developing new application methodology such as control release or encapsulation.

APPENDIX A

Fungal Culture Media

1. Potato Dextrose Agar

Potato extract	24 g/L
Agar Powder	0.15 % (w/v)
Distilled Water add to	1000 mL

2. Modified Potato Dextrose Agar

Potato extract	24 g/L
Agar Powder	0.15% (w/v)
Tween 20	0.5% (v/v)
Distilled Water add to	1000 mL

3. Potato Dextrose Broth

Potato extract	24 g/L
Distilled Water add to	1000 mL

4. Water Agar

Agar Powder	2% (w/v)
Distilled Water add to	1000 mL

Stained Solution

Acetic Acid	5% (v/v)
Lacto-phenol	1% (v/v)

Destained Solution

Acetic Acid	5% (v/v)

APPENDIX B

Table 1 The effect on growth inhibition of essential oils

plant	Colony diameter (mm)[a] ± SD				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Mentha cordifolia Opiz.	86.83 ± 0.289	86.33 ± 0.577	80.33 ± 2.517	73.00 ± 3.424	0 ± 0.00
Cinnamomum bejolghota Sweet.	87.00 ± 1.500	86.17 ± 0.764	84.33 ± 1.528	73.33 ± 0.577	0 ± 0.00
Litsea cubeba Pers.	81.33 ± 0.577	78.67 ± 4.163	77.33 ± 1.528	53.67 ± 0.577	0 ± 0.00
Eugenia caryophyllus Bullock & Harrison.	86.83 ± 0.289	85.33 ± 0.577	82.33 ± 0.764	53.17 ± 0.764	0 ± 0.00
Zingiber officinale Roseoe.	86.67 ± 0.577	84.50 ± 0.500	61.67 ± 0.577	33.33 ± 0.577	0 ± 0.00

[a] colony diameter of *Fusarium oxysporum* 43-68

$P < 0.05$

Table 2 The effect on growth inhibition of essential oils

plant	Colony diameter (mm)[a] ± SD				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Pelargonium graveolens	83.67 ± 1.528	72.67 ± 2.517	75.67 ± 4.042	71.00 ± 2.646	0 ± 0.00
Mentha cordifolia Opiz.	71.33 ± 0.577	66.00± 1.000	65.00 ± 0.577	69.00 ± 1.000	0 ± 0.00
Ocimum gratissimum Linn.	71.33 ± 0.577	60.33 ± 1.155	59.00 ± 2.000	55.67 ± 0.577	0 ± 0.00
Ocimum sanctum Linn.	73.00 ± 0.00	61.67 ± 2.886	64.67 ± 4.509	67.00 ± 0.00	0 ± 0.00
Cinnamomum bejolghota Sweet.	71.00 ± 0.00	68.33 ±0.577	67.67 ± 0.577	50.67 ± 0.577	0 ± 0.00
Litsea cubeba Pers.	71.00 ± 1.000	73.00 ± 0.00	73.33 ± 0.577	62.67 ± 5.508	0 ± 0.00
Eugenia caryophyllus Bullock & Harrison.	71.00 ± 1.000	66.67 ± 0.577	67.67 ± 1.154	54.00 ± 1.000	0 ± 0.00
Limnophila aromatica (Merr.)	71.00 ± 1.000	60.00 ± 2.000	59.33 ± 1.155	51.00 ± 3.000	0 ± 0.00
Kaempferia galanga Linn	71.00 ± 0.00	61.67 ± 2.517	63.00 ± 4.000	53.00 ± 1.732	0 ± 0.00
Zingiber cassumunar Roxb.	74.00 ± 0.00	72.00 ± 1.000	70.00 ± 2.000	49.33 ± 7.506	0 ± 0.00

[a] colony diameter of *Alternaria* sp. 43-89

$P < 0.05$

Table 3 The effect of eugenol, cinnamaldehyde and their derivative and some monoterpene on growth inhibition of *Fusarium oxysporum* 43-68

compound	Colony diameter (mm)[a] ± SD				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Eugenol	80 ± 0.5774	62 ± 2.0000	56 ± 0.5774	22 ± 0.5774	0 ± 0.00
Eugenol methy ether	80 ± 0.5774	74 ± 2.3094	73 ± 0.5774	60 ± 0.5774	0 ± 0.00
Cinnamaldehyde	80 ± 0.5774	71± 1.0000	64 ± 0.5774	20 ± 2.0000	0 ± 0.00
Cinnamyl alcohol	80 ± 0.5774	70 ± 2.0000	64 ± 1.0000	59 ± 1.1547	7 ± 4.0415
Cinnamic acid	69 ± 0.5774	7 ± 0.5774	69 ± 0.5774	65 ± 0.5774	0 ± 0.00
4-methoxy cinnamic acid	69 ± 0.5774	72 ± 1.0000	69 ± 0.5774	65 ± 2.3094	36 ± 2.0000
Limonene	70 ± 0.00	69 ± 1.0000	70 ± 2.5166	69 ± 1.1547	67 ± 1.0000
γ - terpinene	70 ± 0.00	69 ± 1.0000	69 ± 2.3094	67 ± 1.1547	67 ± 0.00
α - terpinene	70 ± 0.00	69 ± 1.1547	70 ± 0.5774	70 ± 0.00	65 ± 0.5774

[a] colony diameter of *Fusarium oxysporum* 43-68

$P < 0.05$

Table 4 Effect of essential oil on postharvest disease control

	Total area (cm^2)	Infected area (cm^2) [a]	Level of disease severity [b]
Un-inoculated control	155 128 149	5 7 8	2 2 2
	125 149 120	4 4 6	2 2 2
	136 138 129	15 4 5	2 2 2
	160 144 118	5 6 7	2 2 2
	117 135 148	3 9 6	2 2 2
Inoculated control	137 120 123	35 20 22	4 4 4
	102 116 110	26 32 18	4 4 4
	130 110 125	17 9 14	4 4 4
	122 112 102	9 5 27	4 4 4
	132 113 135	24 20 12	4 4 4
Cinnamomum bejolghota	80 92 99	1 3 5	1 1 1
Sweet.	82 76 75	2 0 2	1 1 1
	81 70 81	1 4 1	1 1 1
	80 86 85	2 1 2	1 1 1
	85 86 91	1 4 3	1 1 1
Eugenia caryophyllus	91 93 110	0.5 1 0	1 1 1
Bullock & Harrison.	86 91 98	0 1 1	1 1 1
	94 101 90	2 1 0	1 1 1
	100 92 90	0.5 1 1	1 1 1
	87 92 90	0 0 0	1 1 1

[a] infection area based on tissue necrosis

[b] level 1 = cottony hyphae less than 10 % of total fungal colony

level 2 = cottony hyphae 10 -25 % of total fungal colony

level 3 = cottony hyphae 25 - 50 % of total fungal colony

level 4 = cottony hyphae more than 50 % of total fungal colony

un-inoculated control : banana without inoculated fungal spore and without treated the crude essential oil

inoculated control : banana inoculated with fungal spore but without treated the crude essential oil

APPENDIX C

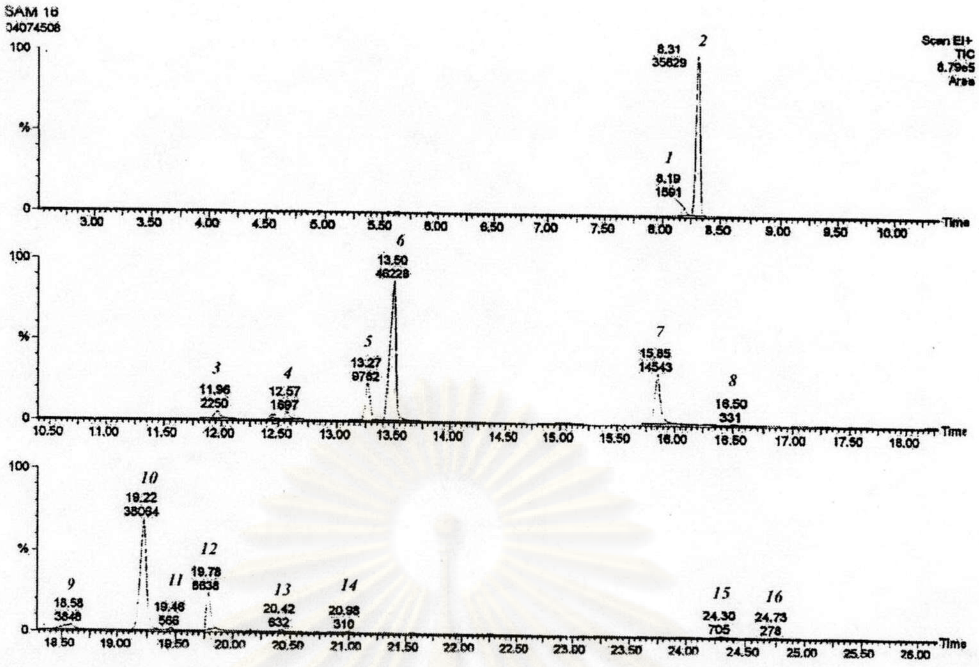


Fig. 1 GC-MS chromatogram of *Limnophila aromatica* Merr.

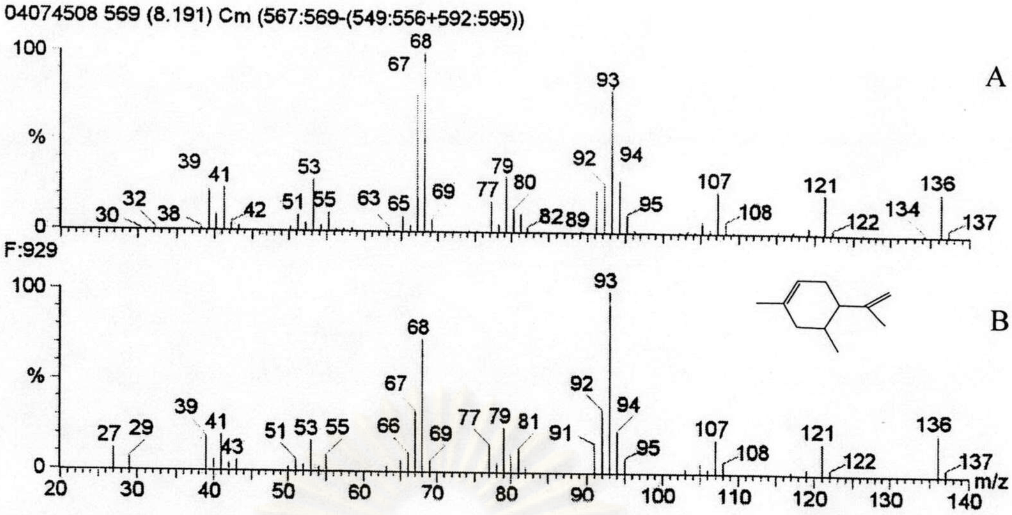
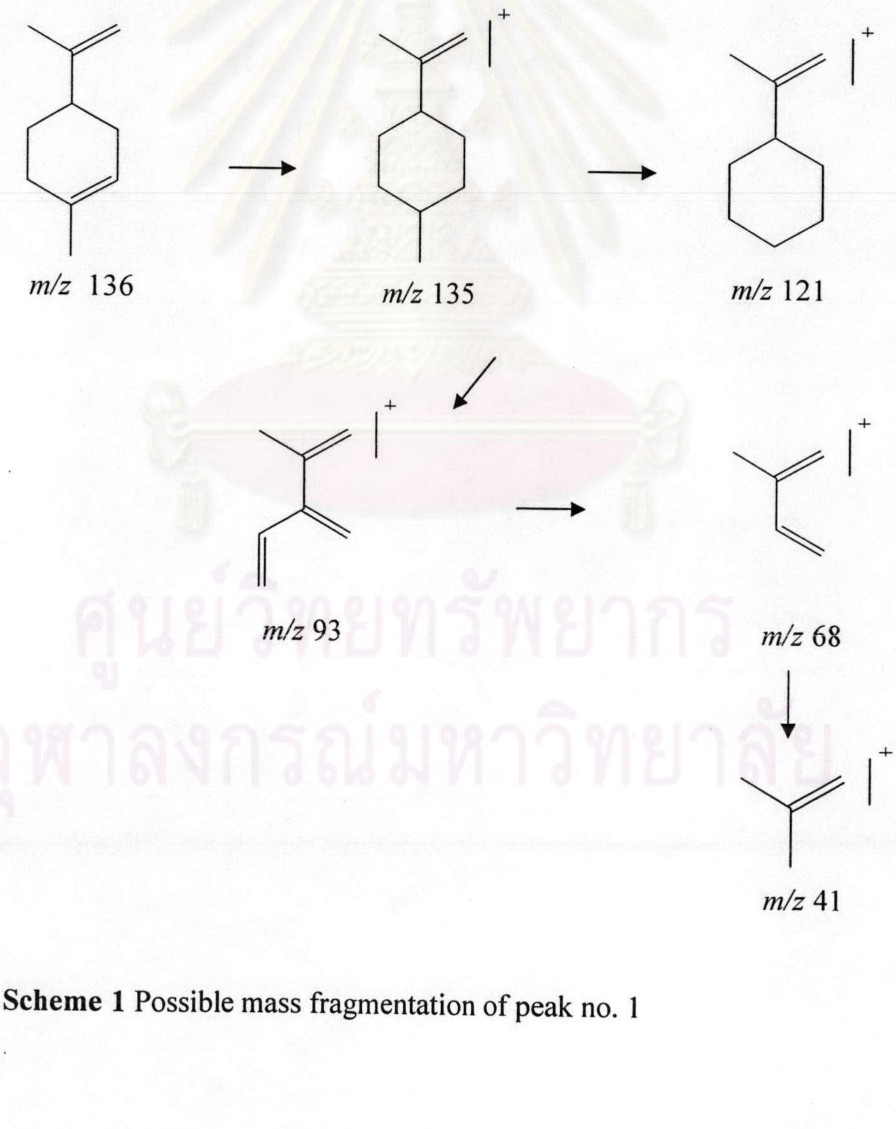


Fig. 2 Mass spectrum of peak no. 1 (A) and 1-methyl-5-(1-methylethenyl)-,(R) cyclohexene (B)



Scheme 1 Possible mass fragmentation of peak no. 1

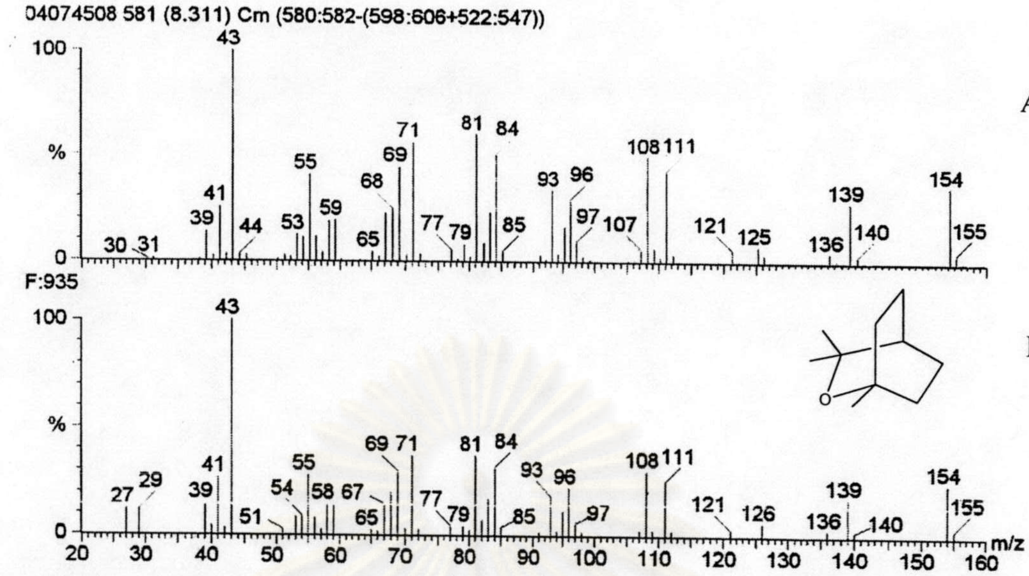
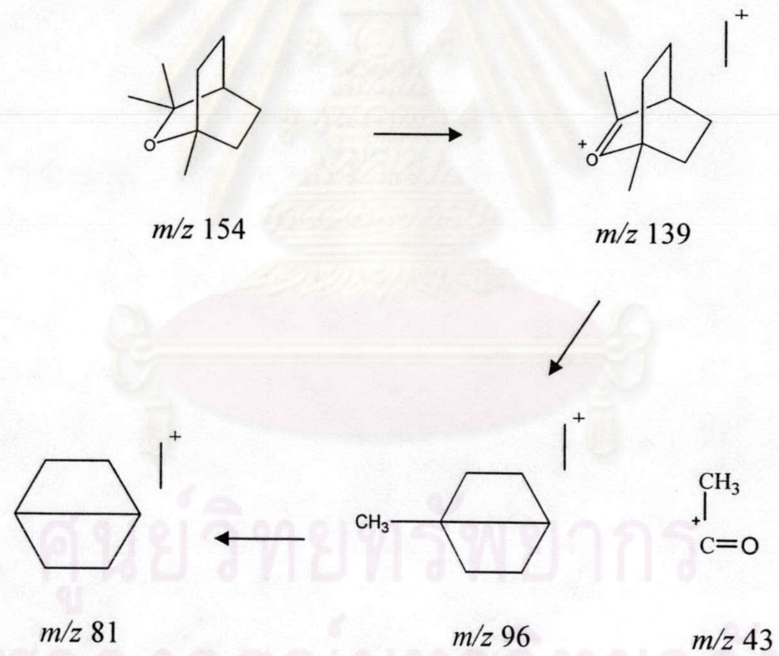


Fig. 3 Mass spectrum of peak no. 2 (A) and Eucalyptol (B)



Scheme 2 Possible mass fragmentation of peak no. 2

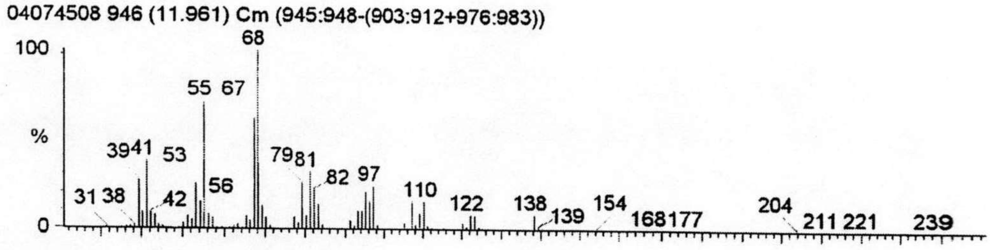


Fig. 4 Mass spectrum of peak no. 3

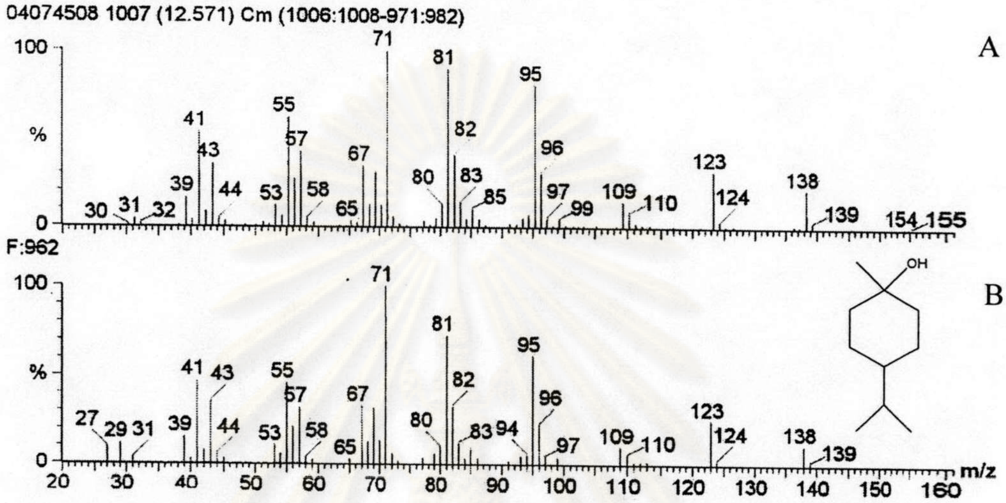
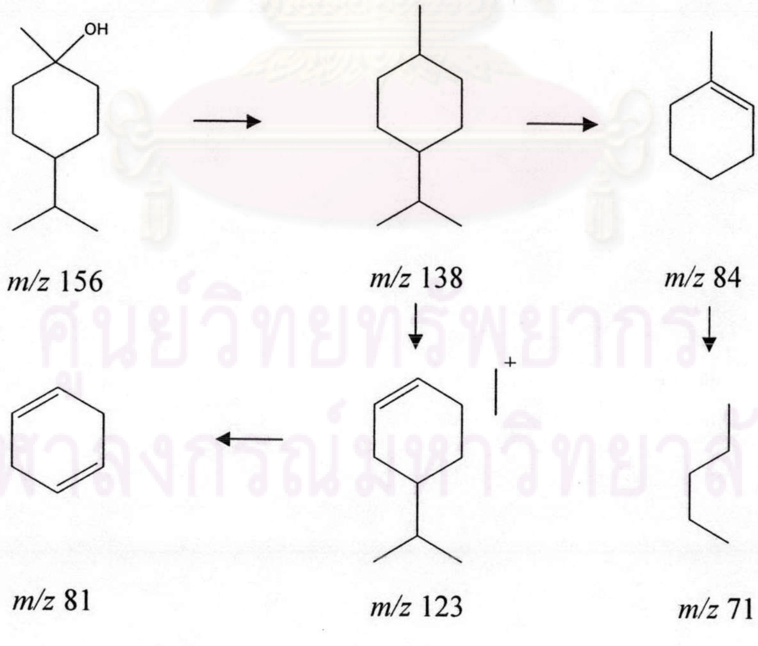


Fig. 5 Mass spectrum of peak no. 4 (A) and 1-methyl-4-(1-methylethyl)-cyclohexanol (B)



Scheme 3 Possible mass fragmentation of peak no. 4

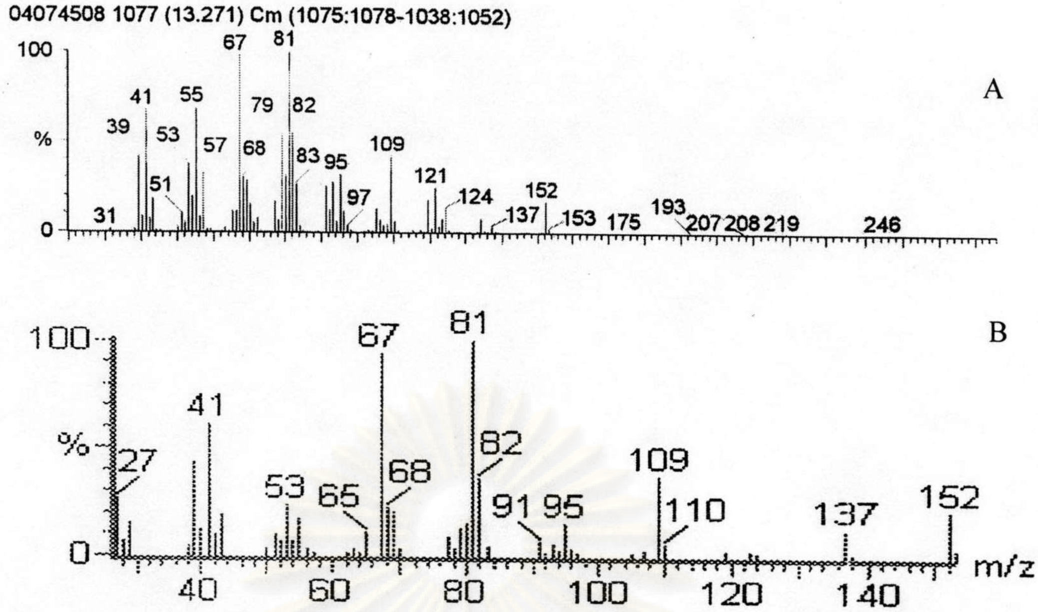


Fig. 6 Mass spectrum of peak no. 5 (A) and long-chain hydrocarbon (B)

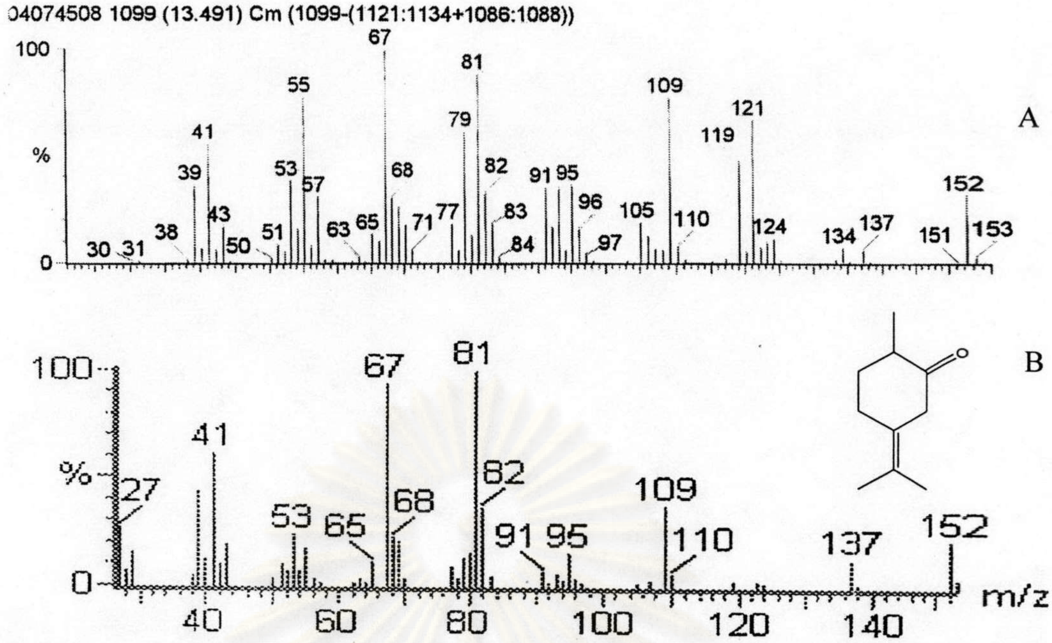
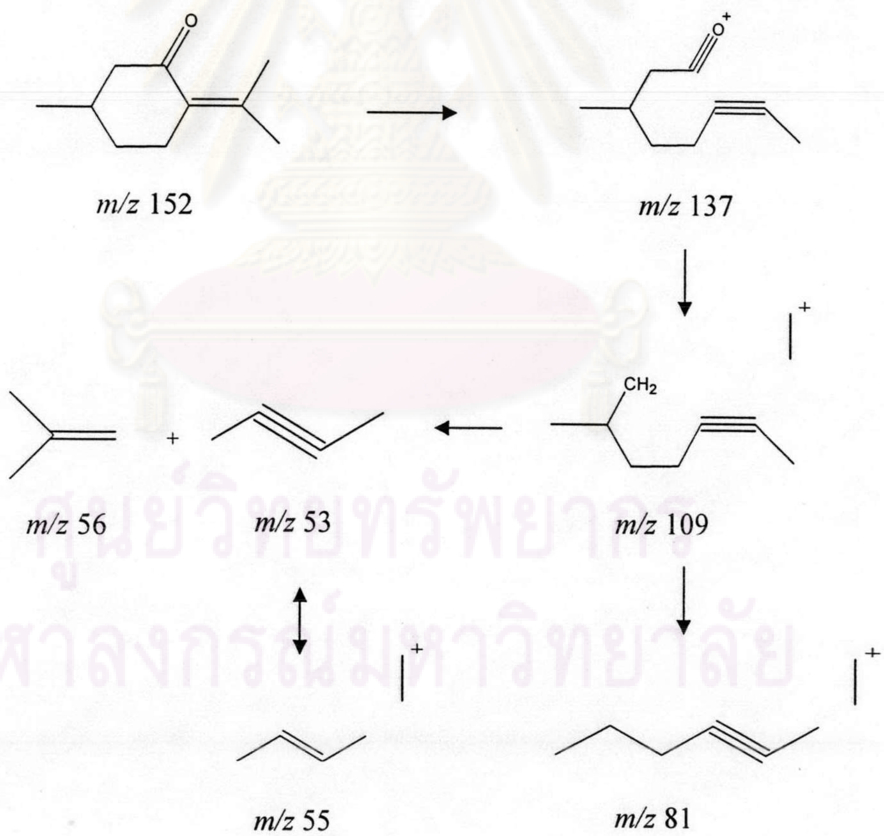


Fig. 7 Mass spectrum of peak no. 6 (A) and pulegone (B)



Scheme 4 Possible mass fragmentation of peak no. 6

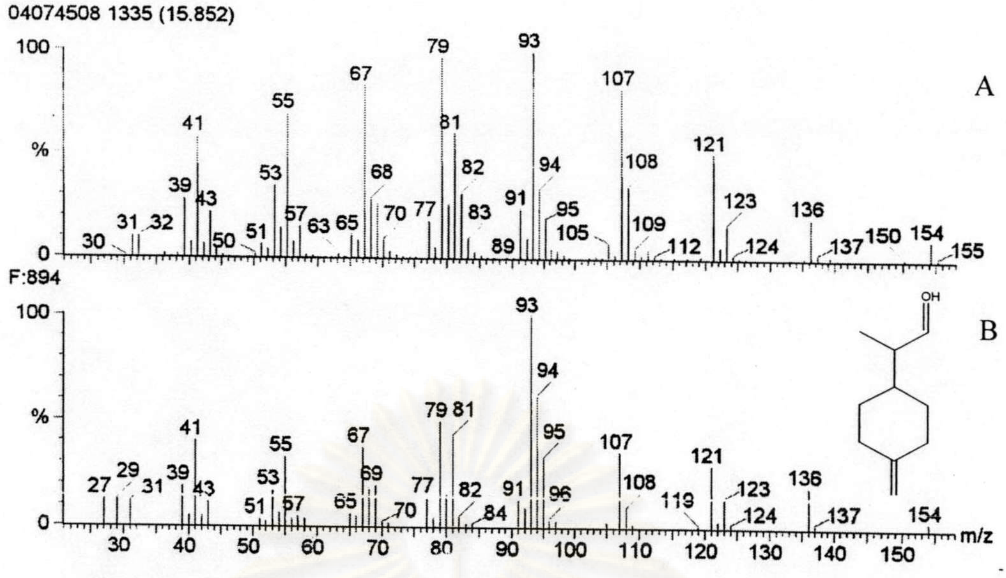
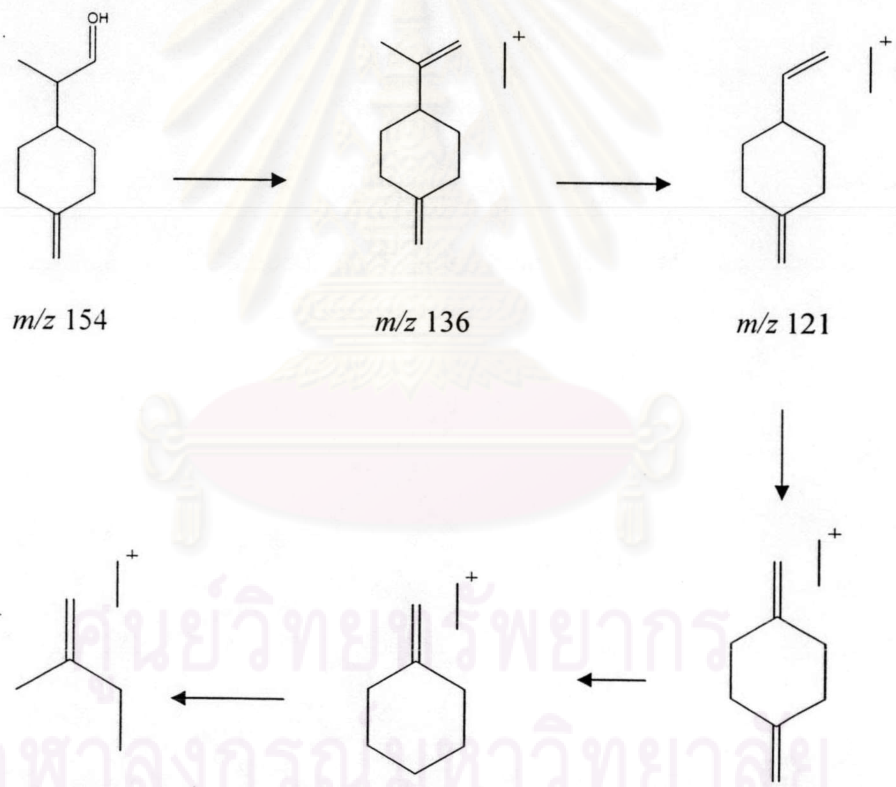


Fig. 8 Mass spectrum of peak no. 7 (A) and *p*-menth-1(7)-en-9-ol (B)



Scheme 5 Possible mass fragmentation of peak no. 7

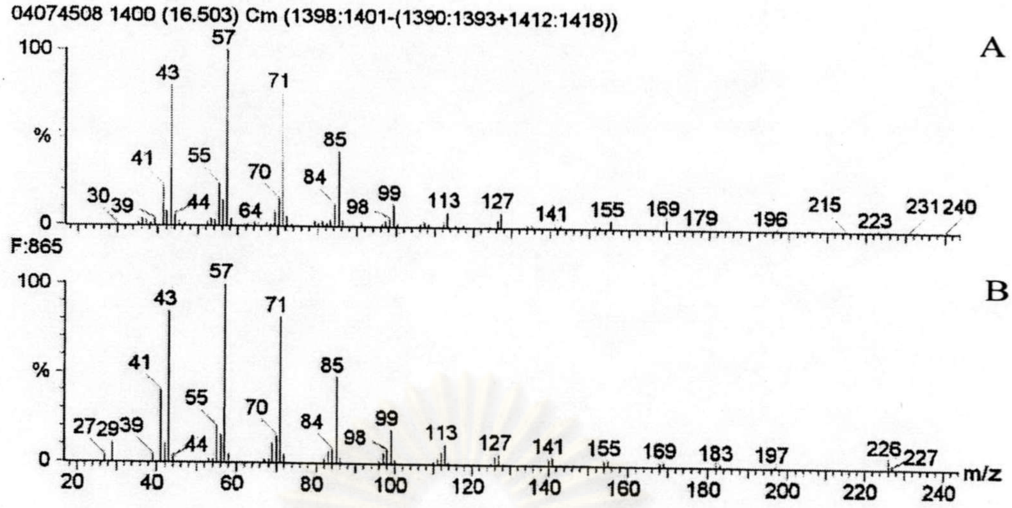


Fig. 9 Mass spectrum of peak no. 8 (A) and long chain hydrocarbon (B)

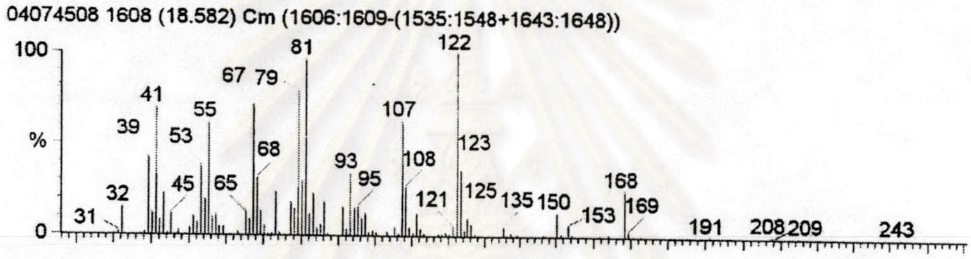


Fig. 10 Mass spectrum of peak no. 9

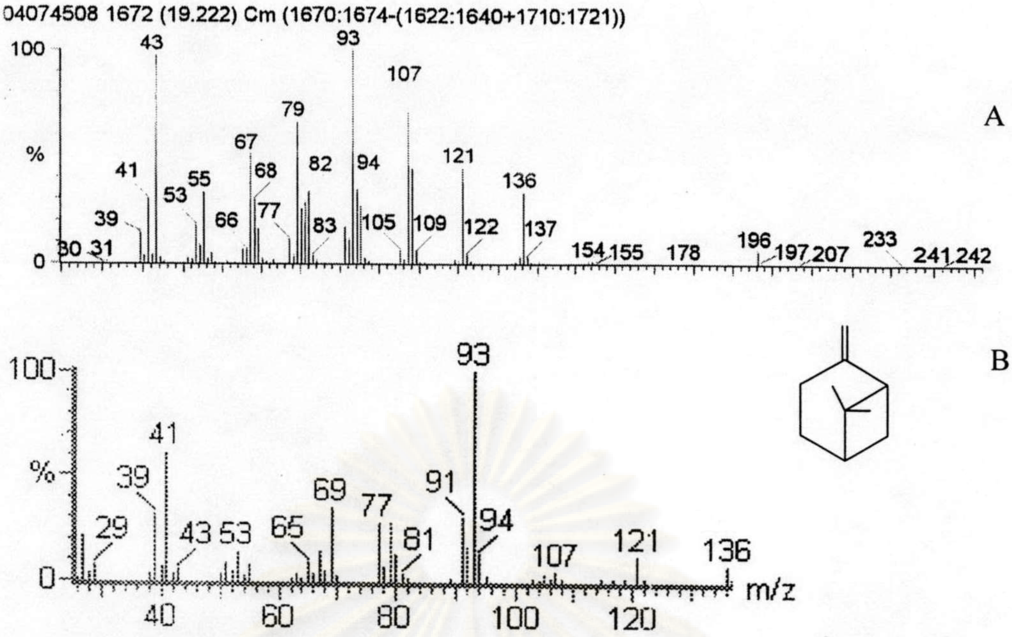
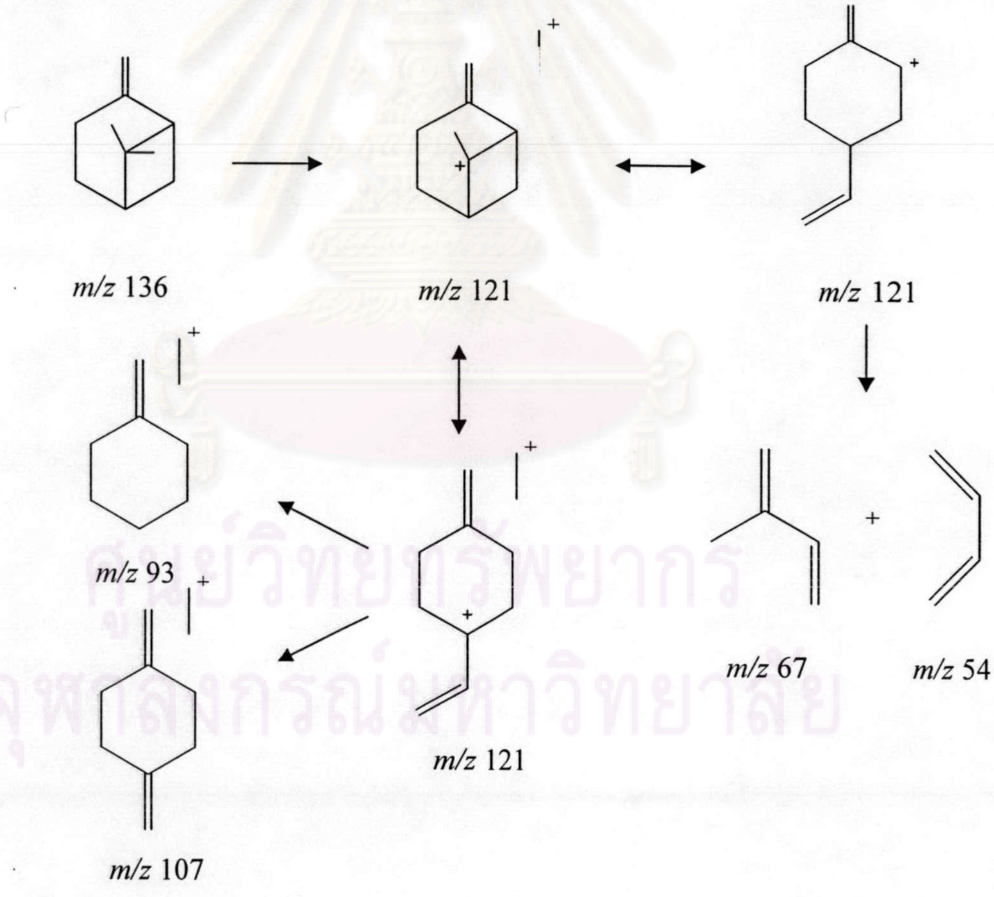


Fig. 11 Mass spectrum of peak no. 10 (A) and β-pinene (B)



Scheme 6 Possible mass fragmentation of peak no. 10

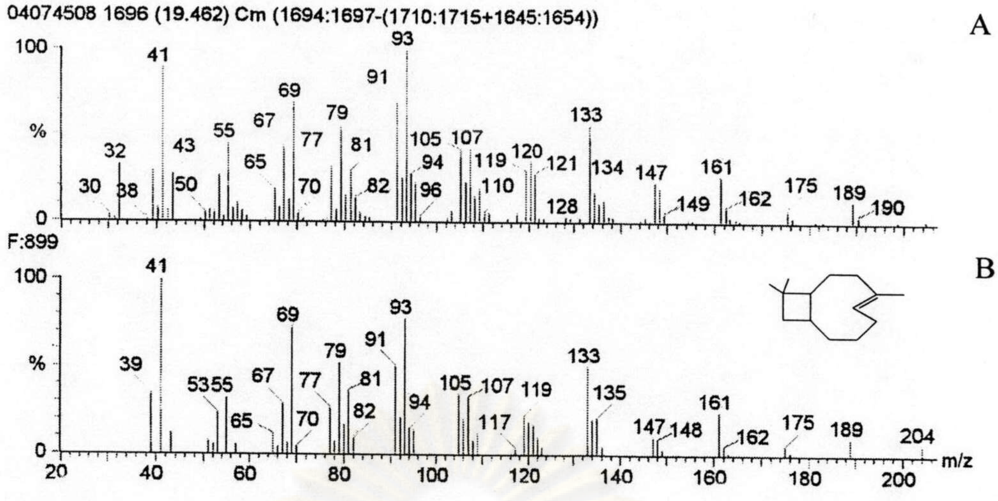


Fig. 12 Mass spectrum of peak no. 11 (A) and Isocaryophyllene (B)

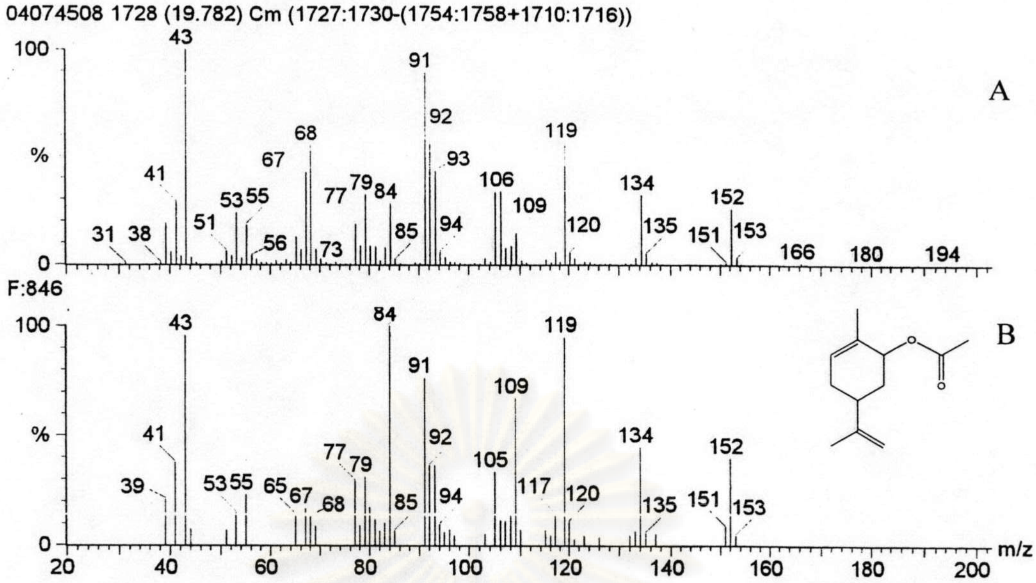
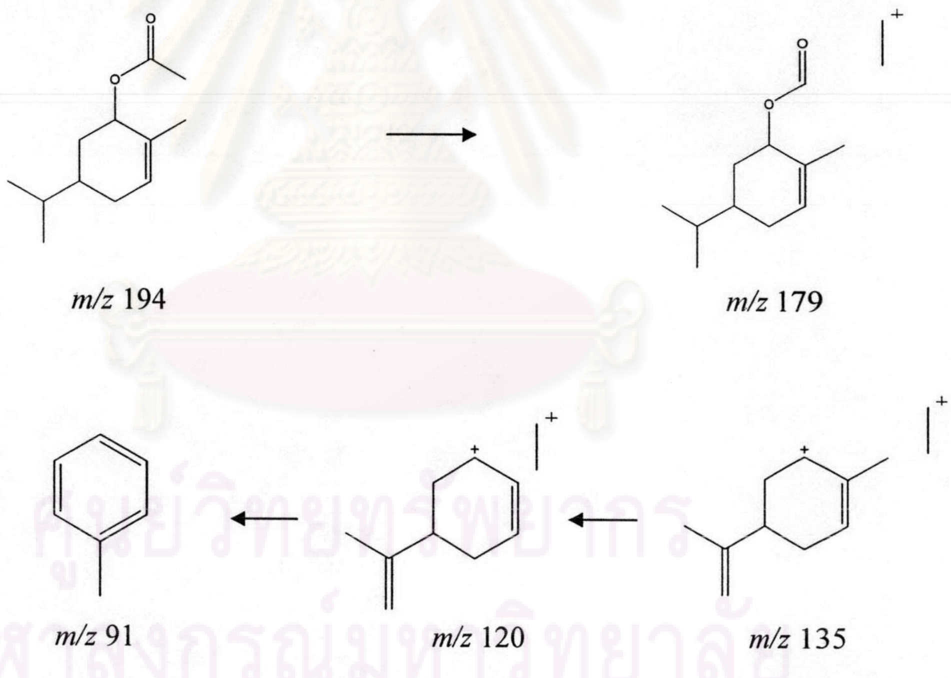


Fig. 13 Mass spectrum of peak no. 12 (A) and 2-menthyl-5-(1-methylethenyl) acetate, 2-cyclohexen-1-ol



Scheme 7 Possible mass fragmentation of peak no. 12

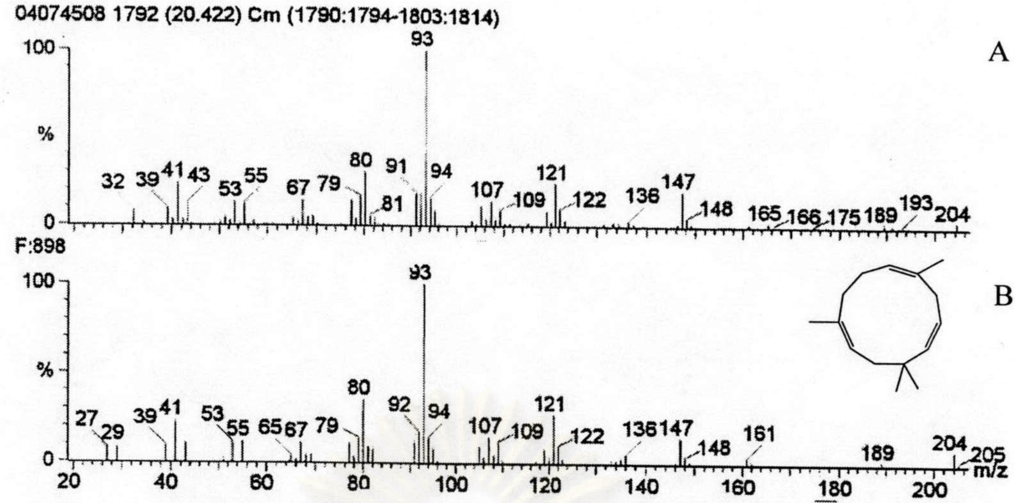


Fig. 14 Mass spectrum of peak no. 13 (A) and ∝-caryophyllene (B)

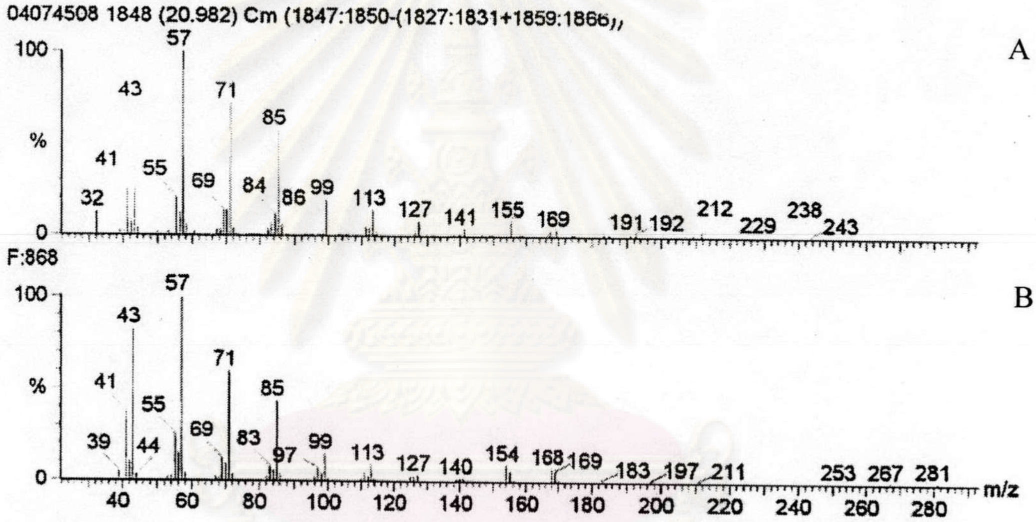


Fig. 15 Mass spectrum of peak no. 14 (A) and long-chain hydrocarcon (B)

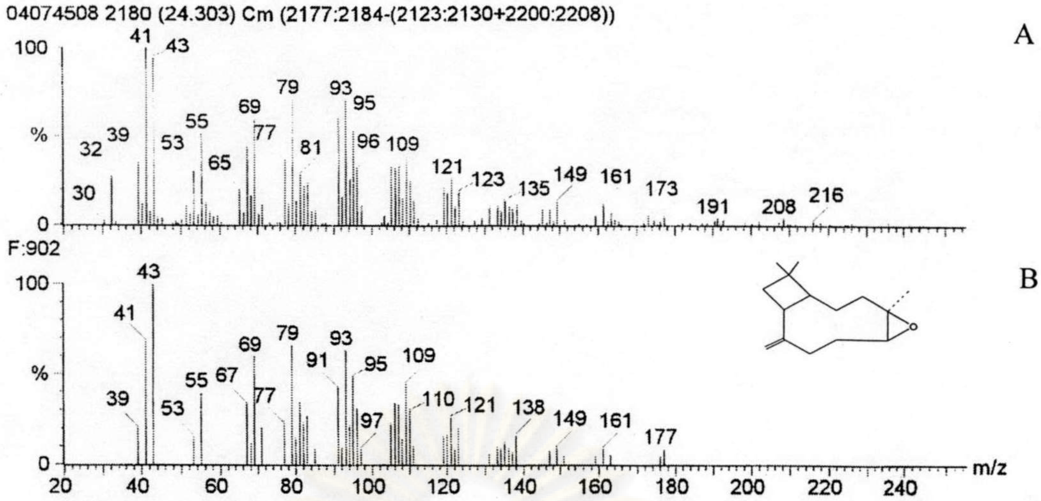


Fig. 16 Mass spectrum of peak no. 15 (A) and Caryophyllene oxide (B)

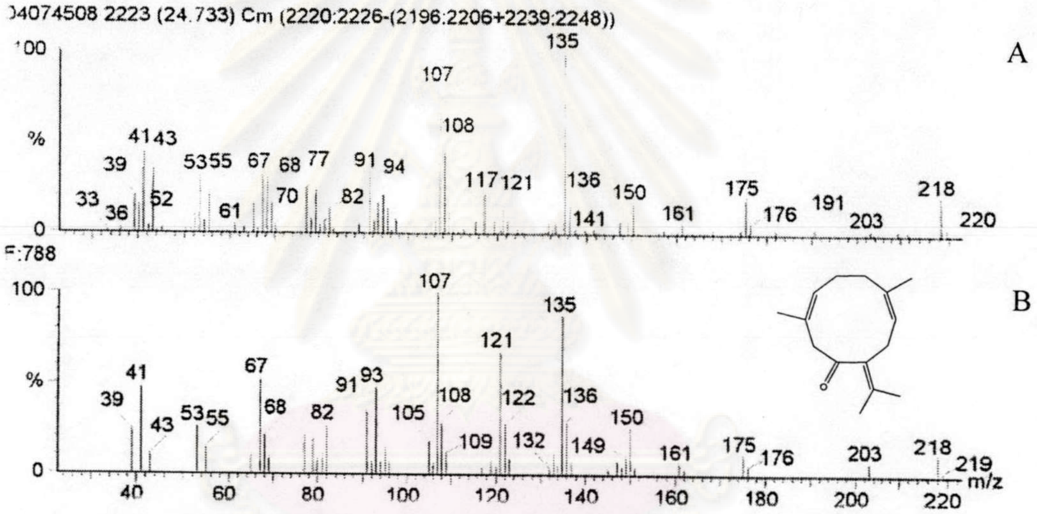


Fig. 17 Mass spectrum of peak no. 16 (A) and 3,7-dimethyl-10-(1-methylethyl)-3,7-cyclodecadien-1-one (B)