23rd ISCE Annual Meeting
Symposium on Insect Semiochemicals I
Analysis, Structures, Synthesis, ……

Jena, Germany (July 23, 2007)

GC-MS and LC-MS analyses for unraveling the diversity of lepidopteran communication systems

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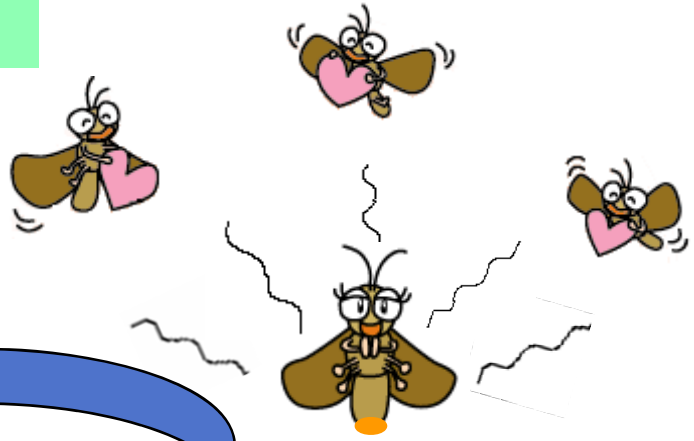
Lepidopteran sex pheromones



Produced by ♀ to attract ♂

Main factor for reproductive isolation

→ Species specific

Pheromones of 580 species
have been reported.

Lepidoptera: ca. 150,000 species

→ Variety of chemical structures

Blending of multiple components

Small insects → Low content

Identification by GC-EAD

GC-MS

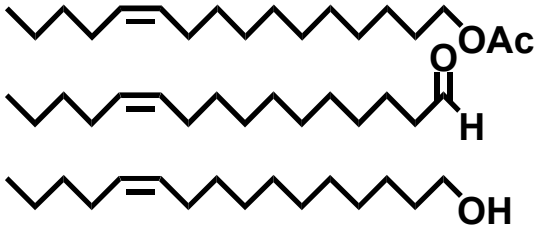
HPLC and LC-MS ?

Type I sex pheromones

Pest insects in Japan

i) Monoenyl compounds

Diamondback moth



Z11-16:OAc

Z11-16:Ald

Z11-16:OH

ii) Dienyl compounds

Pine caterpillar

Z5,E7-12:OH

Cherry treeborer

Z3,Z13-18:OAc

E3,Z13-18:OAc

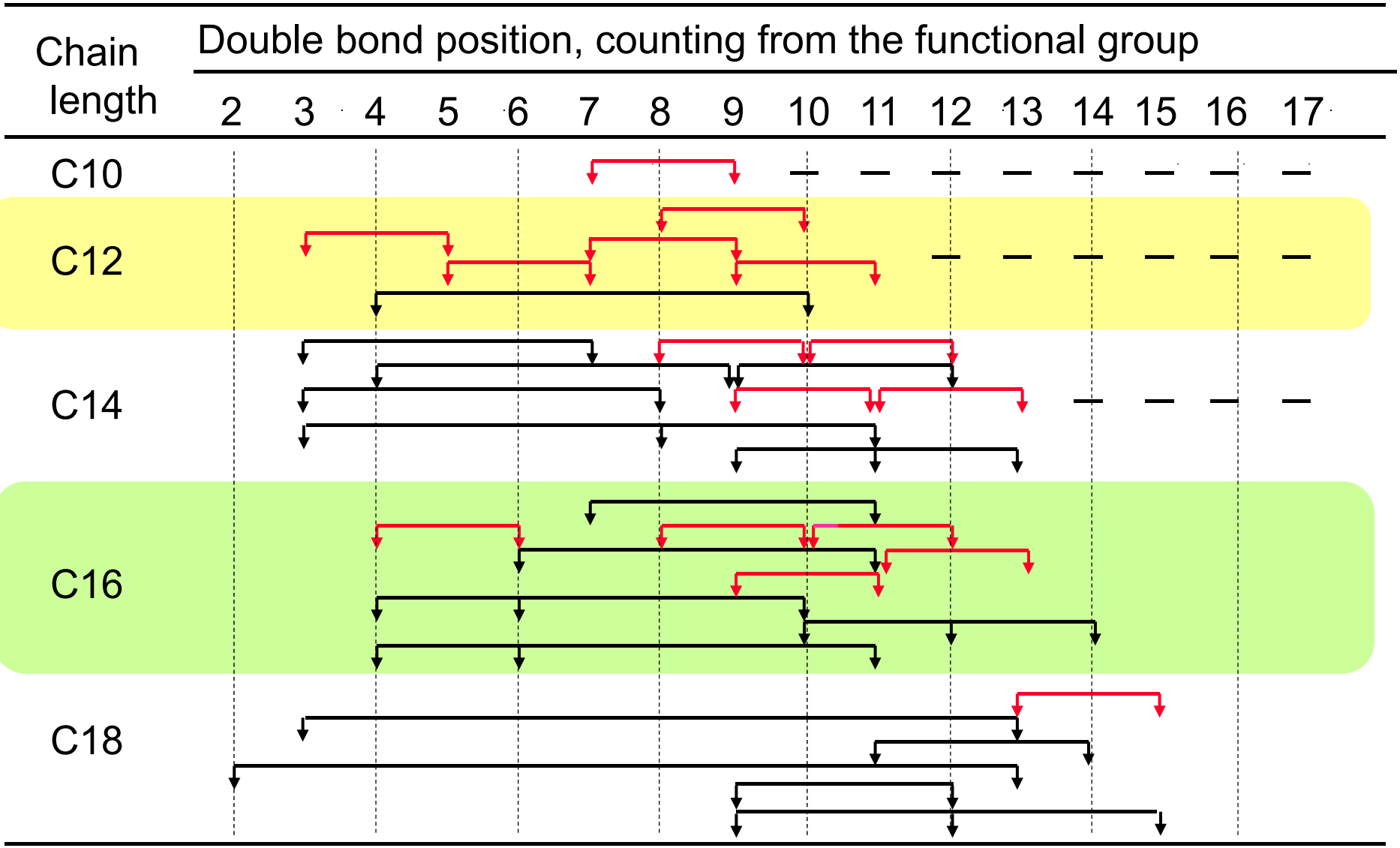
iii) Trienyl compounds

Mulberry pyralid

E10,E12,Z14-16:OAc

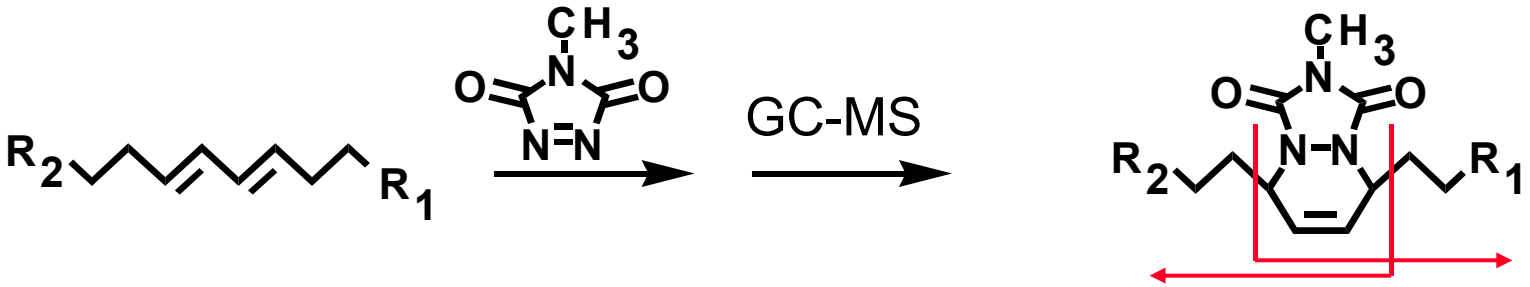
Double-bond positions of dienes and trienes

(C_{13} and C_{15} chain compounds are not included.)

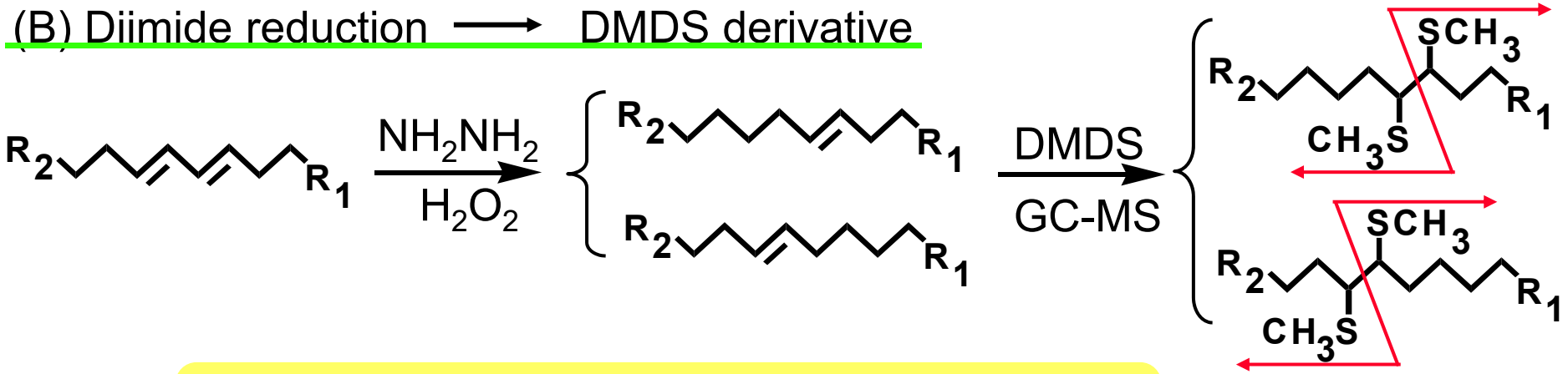


Determination of double-bond positions

(A) MTAD (4-methyl-1.2.4-triazoline-3.5-dione) derivative



(B) Diimide reduction ⟶ DMDS derivative



Derivatization is difficult
for natural pheromones in a trace amount.

If conjugated dienes specifically show characteristic fragment ions on the direct GC-MS analysis, derivatization is not necessary.

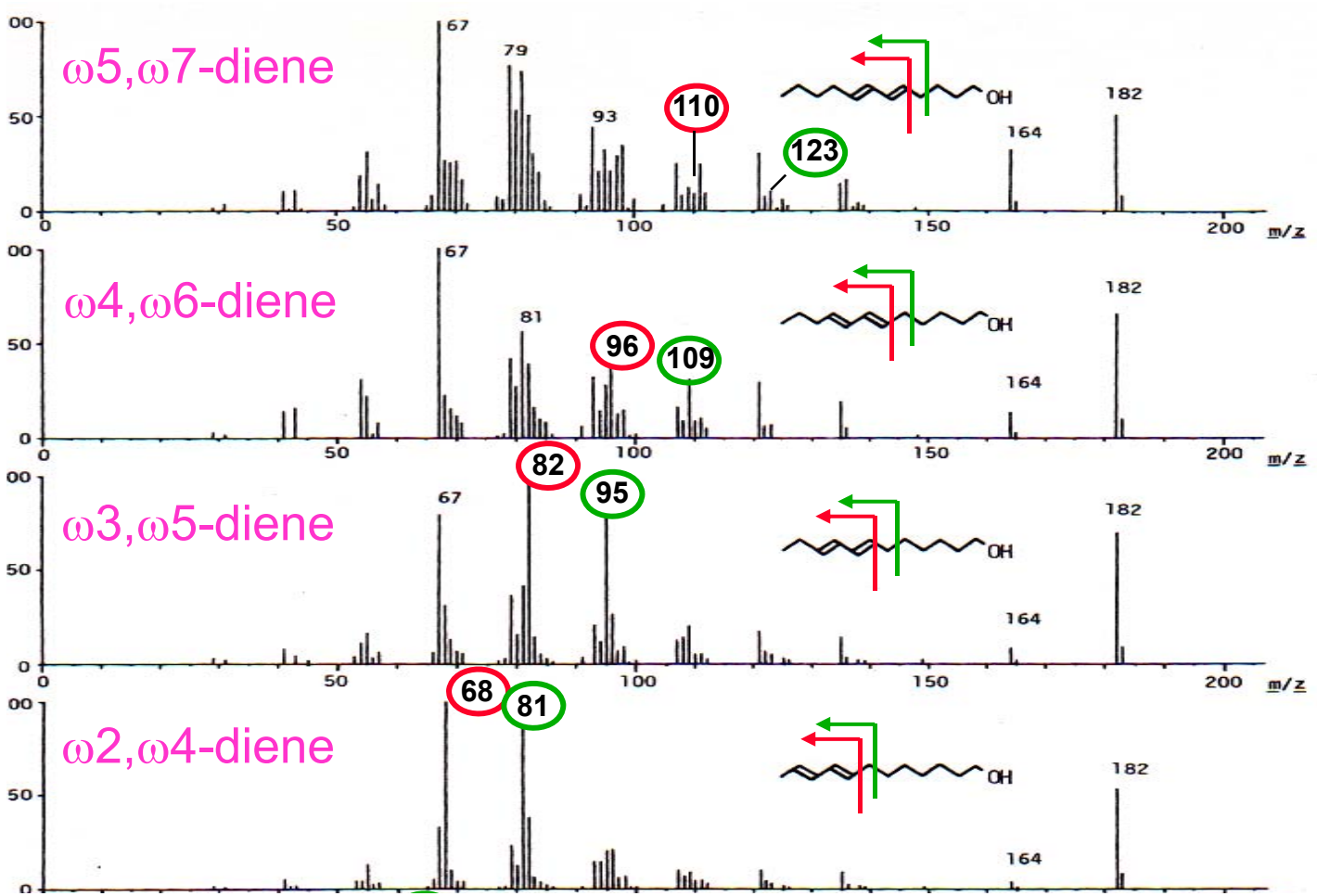
Mass spectra of conjugated dienes (C_{12} alcohols)

5,7-diene

6,8-diene

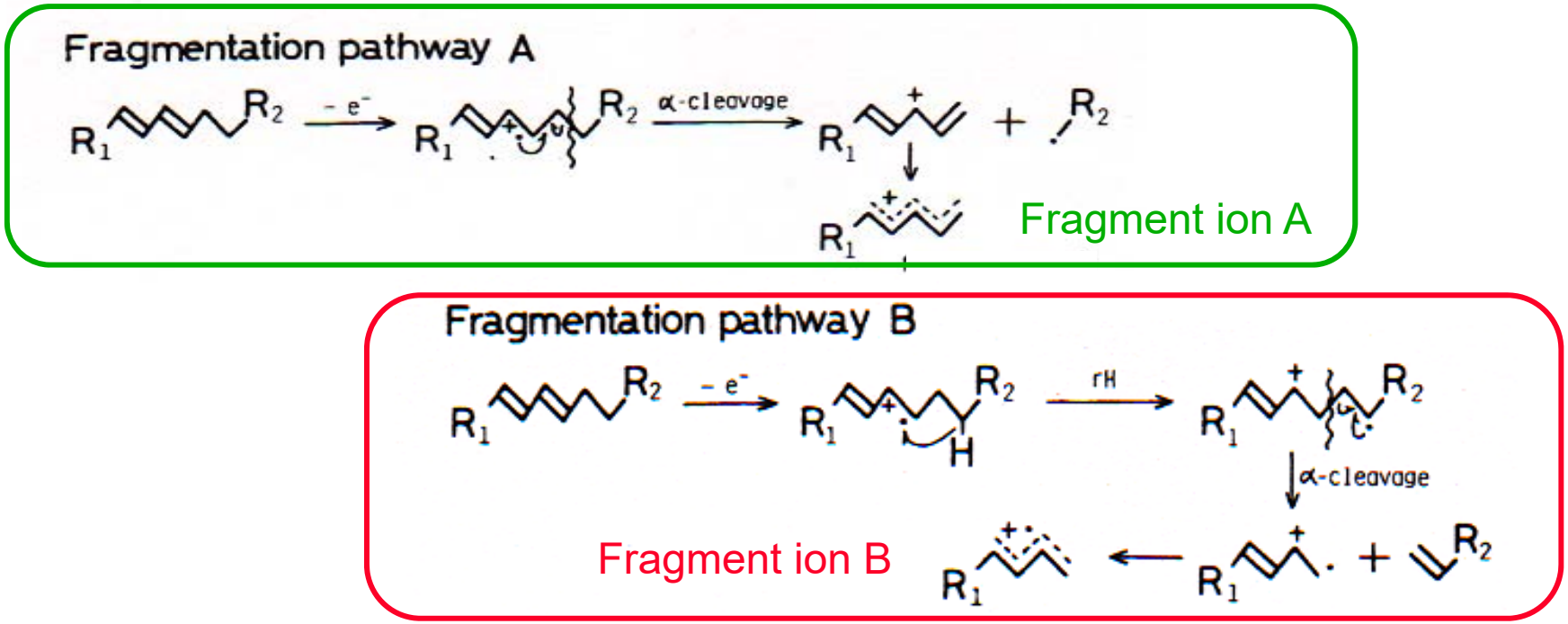
7,9-diene

8,10-diene



In the case of the a terminal conjugated diene, identification of a natural pheromone can be accomplished without derivatization.

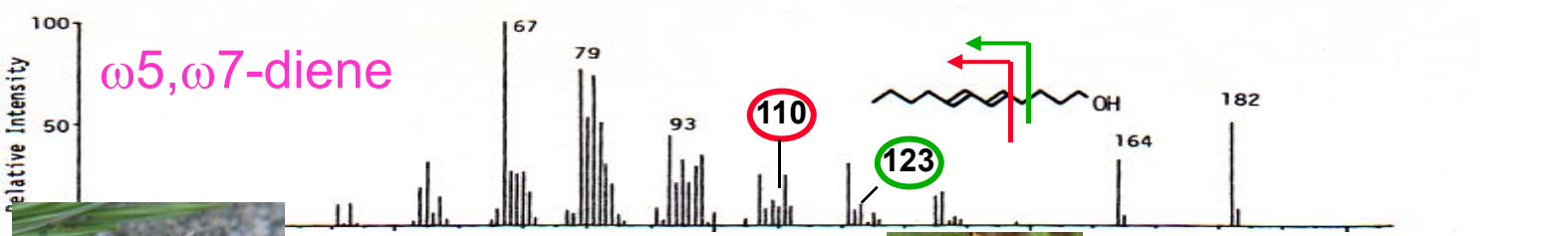
Diagnostic ions of conjugated dienes



	ω5,ω7-diene	ω4,ω2-diene	ω3,ω5-diene	ω2,ω4-diene	ω1,ω3-diene
A^+ (*m/z*)	**123**	**109**	**95**	**81**	**67**
B^+ (*m/z*)	**110**	**96**	**82**	**68**	**54**

In the case of the a terminal conjugated diene, identification of a natural pheromone can be accomplished without derivatization.

Identification of dienyl pheromones



Z5,E7-12:OH

Pine caterpillar [Lasiocampidae]

E5,Z7-12:OAc

Chrysanthemum golden plusia [Noctuidae: Plusiinae]

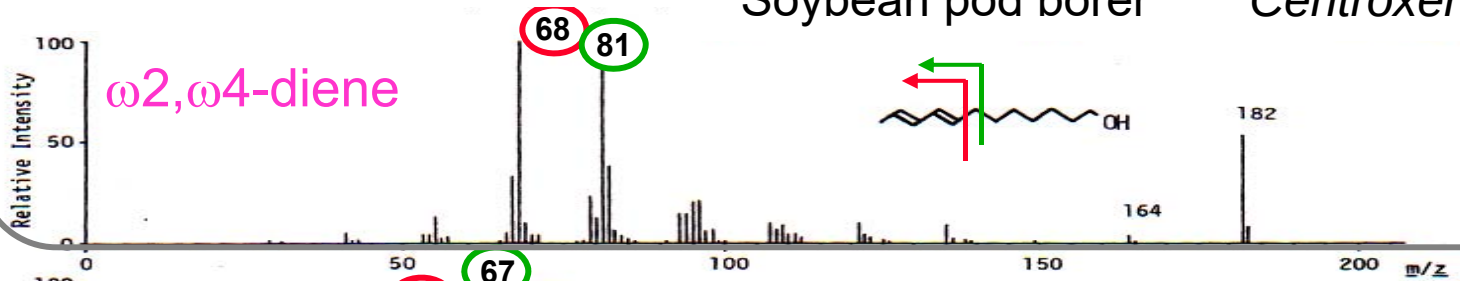
OAc

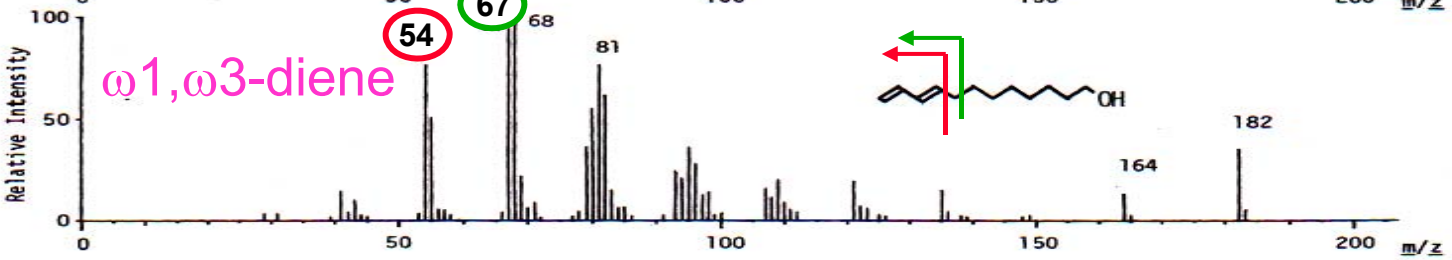
E8,E10-12:OAc

[Tortricidae: Olethreutinae]

Soybean pod borer

Centroxena sp.





Identification of ω1,ω3-dienes

[Limacodidae] Nettle moths

Parasa lepida

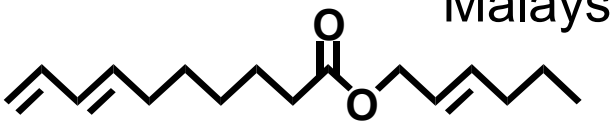
Z7,9-10:OH

Oil palm defoliators

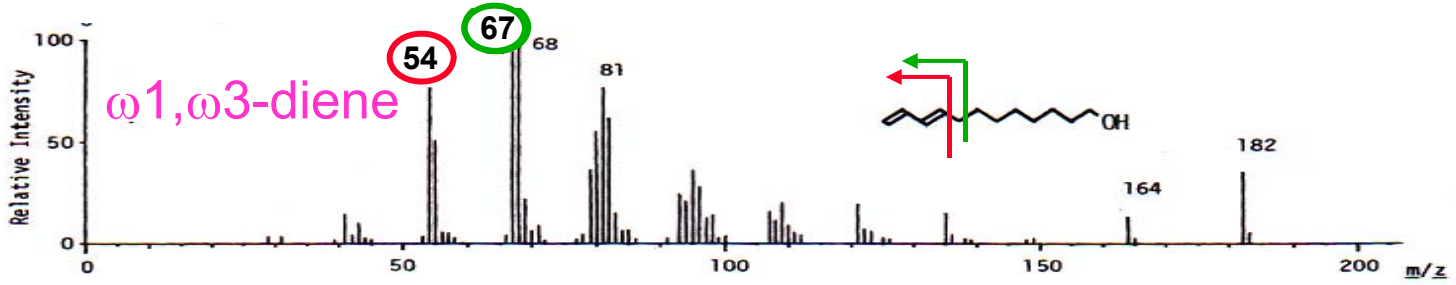
Setothosea asigna (Sasaerila *et al.*, 1997)
Indonesia

E9,11-12:Ald

Darna trima (Sasaerila *et al.*, 2000)
Malaysia



E7,9-10:Acyl derivatives



Identification of ω1,ω3-dienes

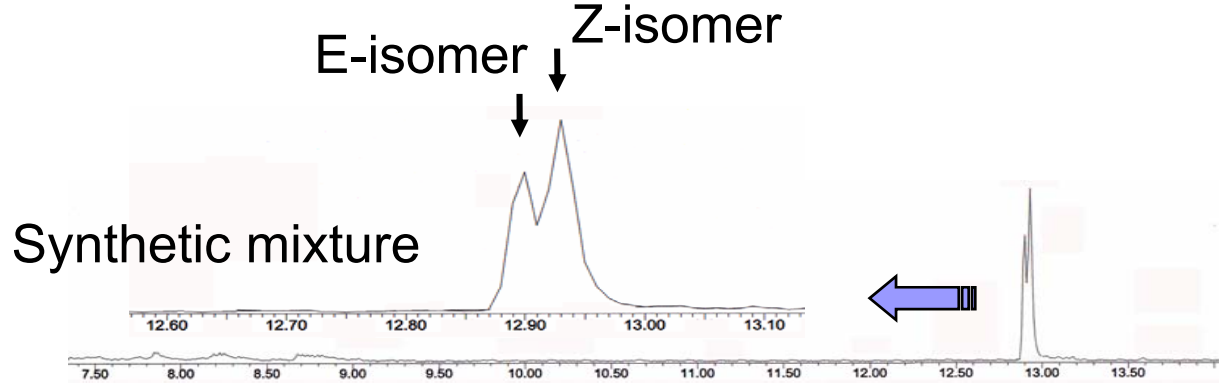
[Limacodidae]

Parasa lepida

Z7,9-10:OH

(A) GC-MS analysis (TIC)

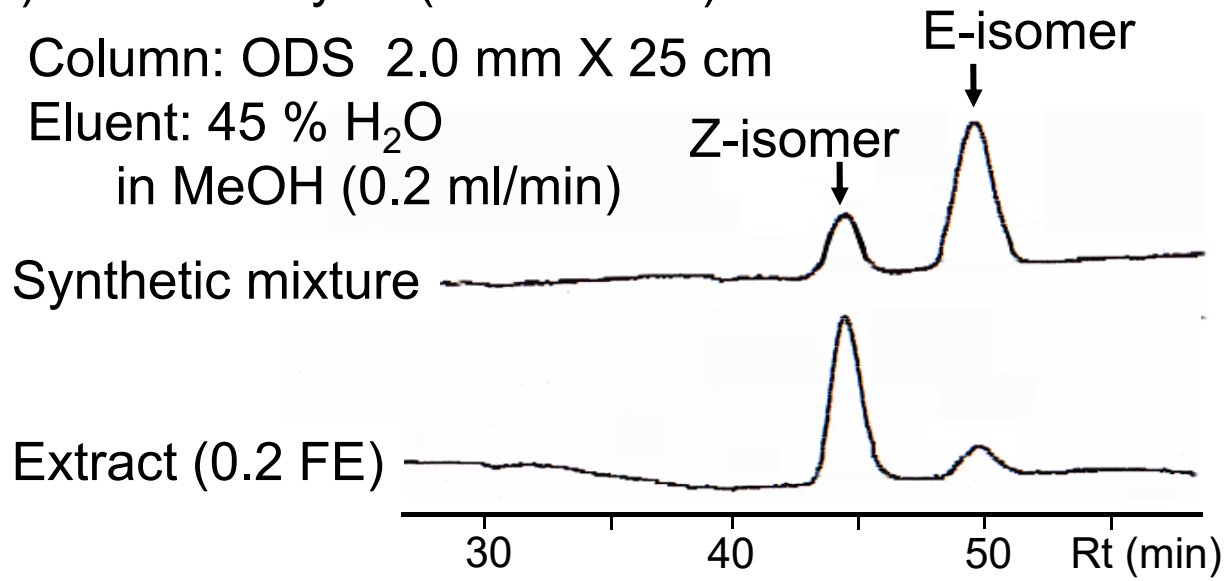
DB-23 column (0.25 mm X 30 m)



(B) HPLC analysis (UV 240 nm)

Column: ODS 2.0 mm X 25 cm

Eluent: 45 % H_2O in MeOH (0.2 ml/min)



Pheromones of Sesiidae species

Do any females produce E13 compounds ?

Z2 compounds ?

Z3 or E3 aldehydes ?

Double bonds		Number of species		
Position	Configuration	OH	OAc	Ald
3,13-Diene	Z3,Z13	4	3	0
	E3,Z13	3	3	0
	Z3,E13	0	1 ?	0
	E3,E13	0	0	0
2,13-Diene	Z2,Z13	0	0	0
	E2,Z13	0	5	3

Macroscelesia spp.

E2,Z13-18:Ald

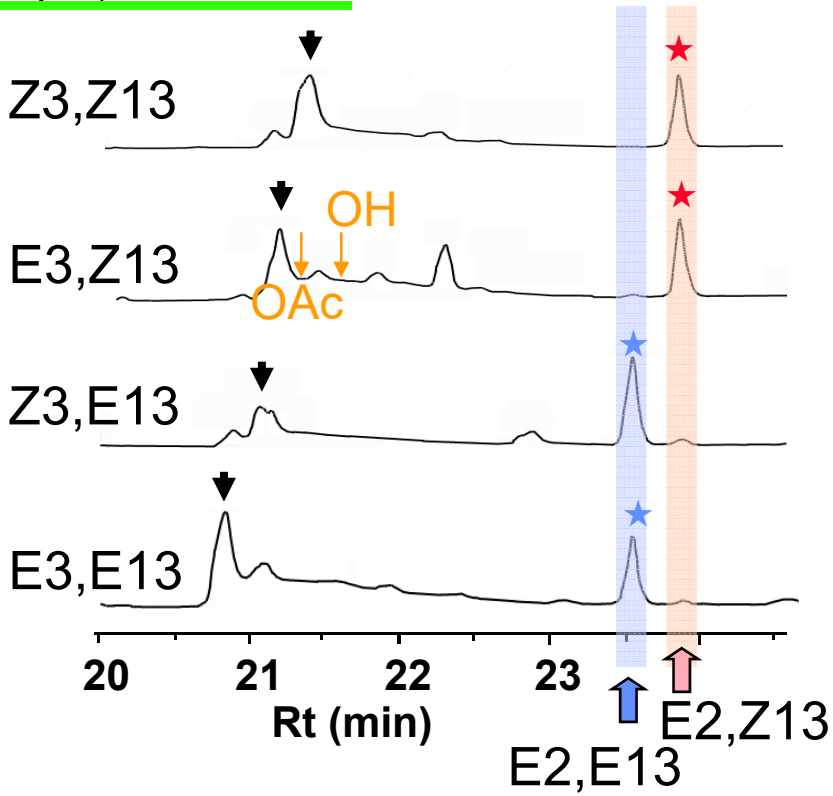
M. japona

M. longipes

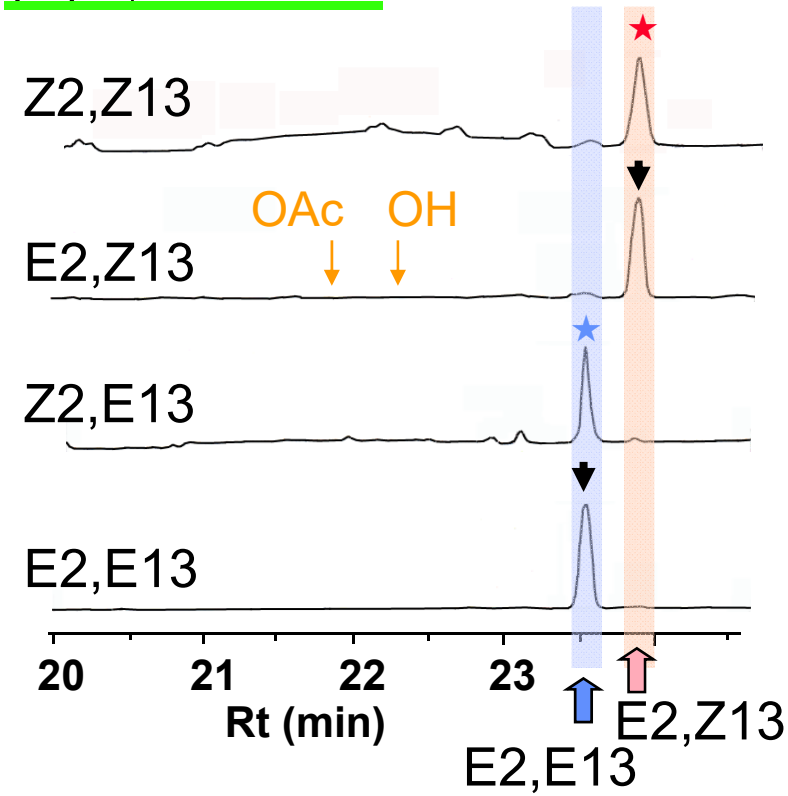
GC analysis of 3,13- and 2,13-dienals

Cool on-column injection, DB-23 column (0.25 mm X 30 m)

(A) 3,13-18:Ald



(B) 2,13-18:Ald



▼ Peaks without any isomerization

★ Isomerized to E2,Z13-18:Ald

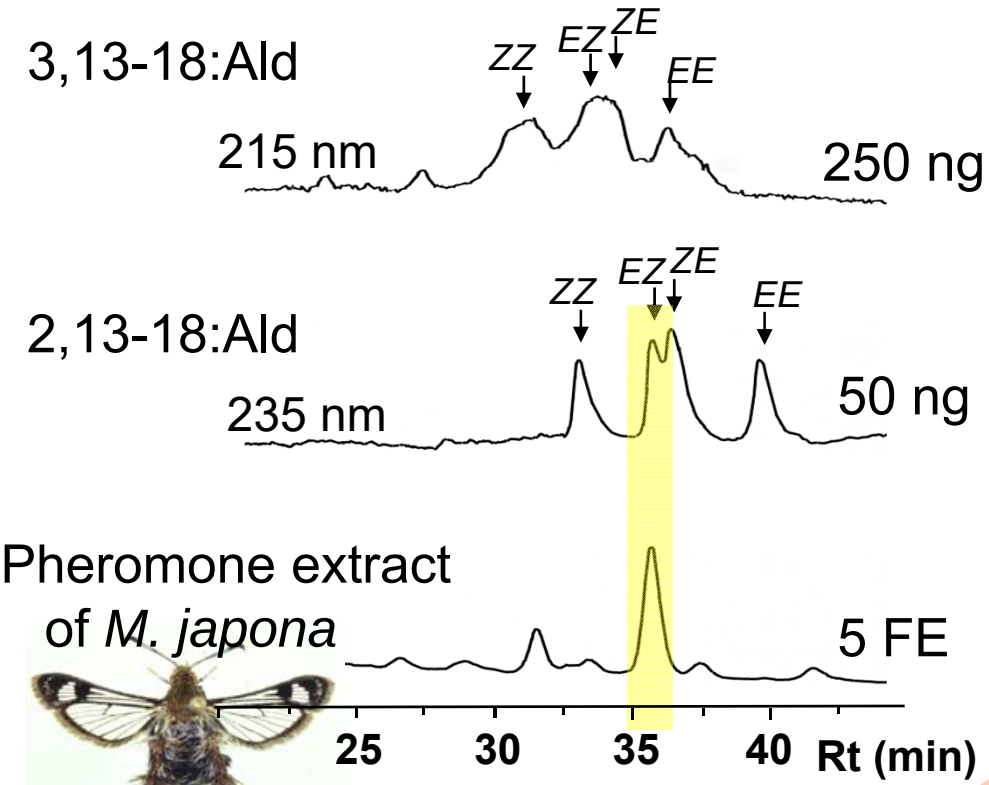
★ Isomerized to E2,E13-18:Ald

GC-MS is not suitable for the analysis of the dienals.

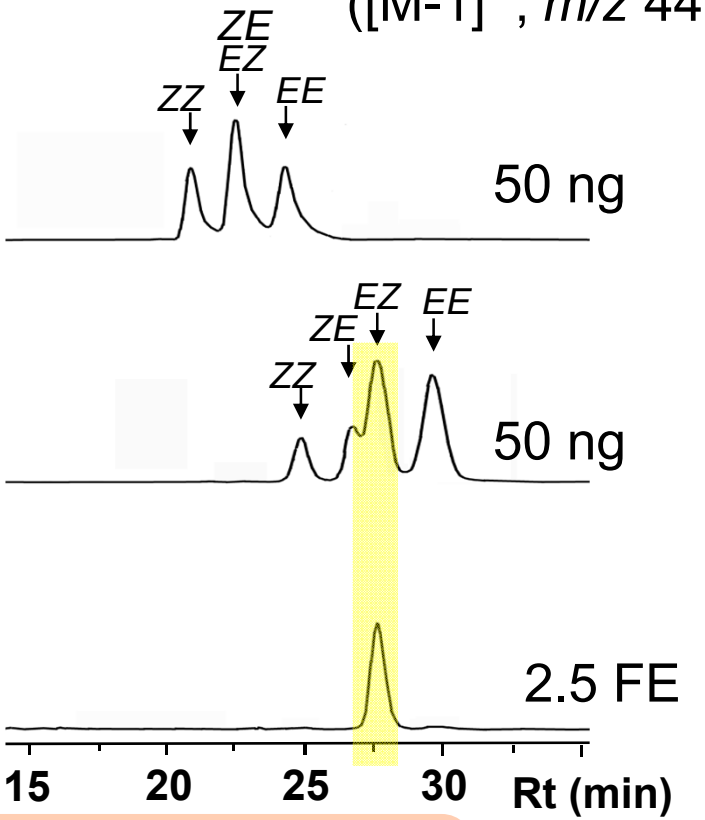
LC and LC-MS analyses of the dienals

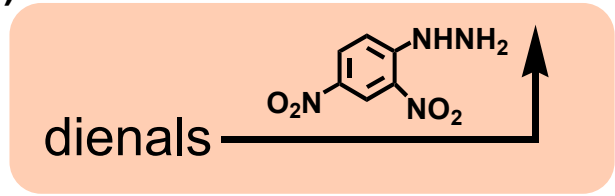
Column: ODS 2.0 mm X 25 cm
Eluent: 16 % H_2O in CH_3CN (0.2 ml/min)

(A) LC analysis



(B) LC-MS (APCI) analysis of DNP derivatives ([M-1]$^-$, *m/z* 443)





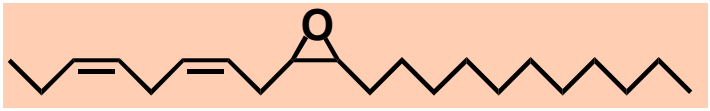
Type II sex pheromones

(A) First Identification

Hill *et al*., (1981) *J. Chem. Ecol.*, **7**: 655

saltmarsh caterpillar moth (*Estigmene acrea*: Arctiidae)

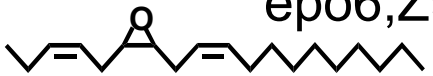
Z3,Z6,epo9-21:H
+ Z9,Z12,Z15-18:Ald
+ Z9,Z12-18:Ald (27:6:1)



(B) Identification in Japan

Biston robustum
(Geometridae)

epo6,Z9-19:H



Z3,epo6,Z9-19:H

Milionia basalis
(Geometridae)

epo3,Z6,Z9-19:H

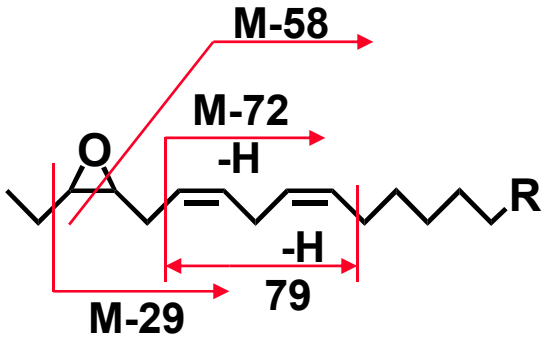
Penina nuda
(Lymantriidae)

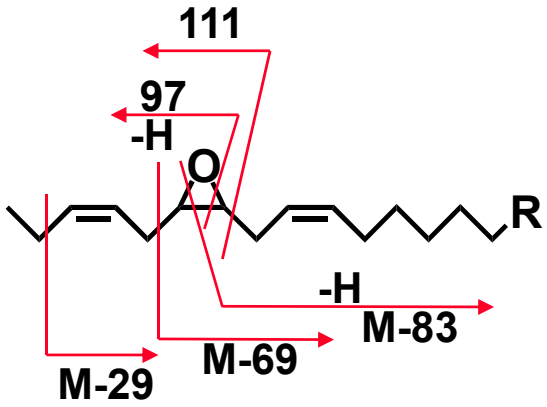
Z3,epo6,Z9-21:H

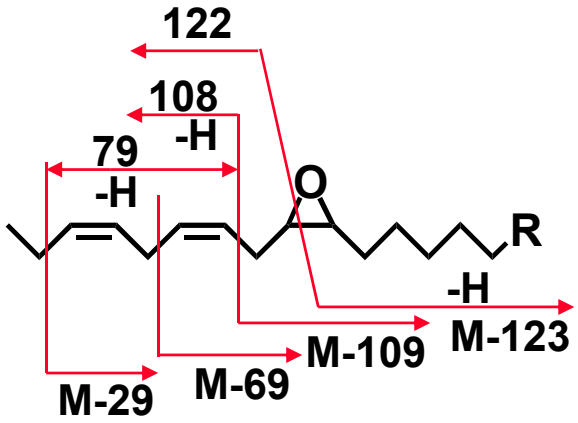
epo3,epo6,Z9-21:H

Diagnostic ions of GC-MS analysis (EI)

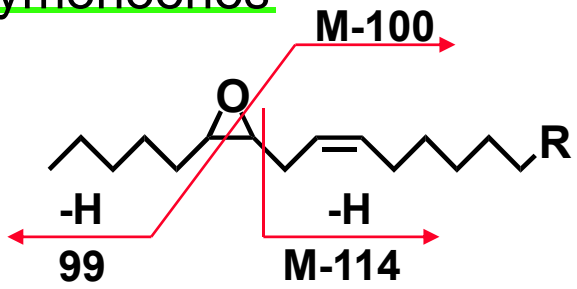
i) Monoepoxydienes

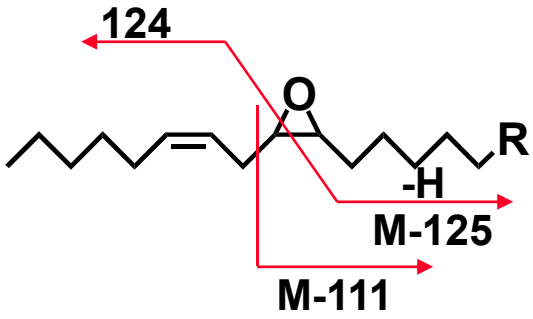




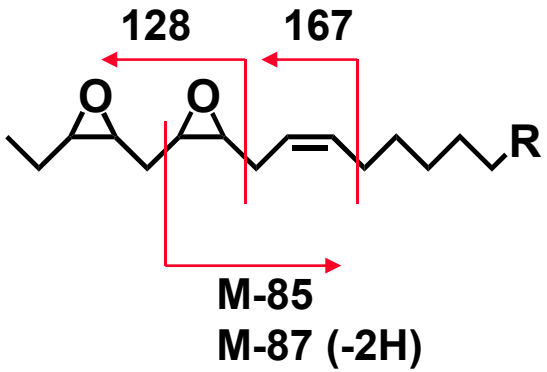


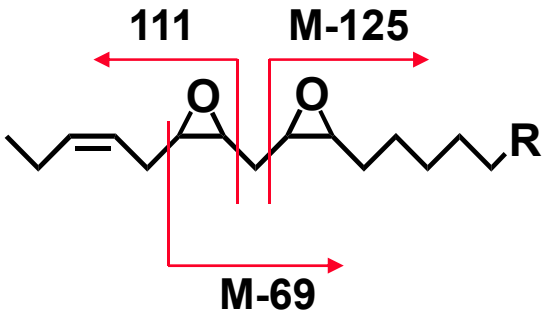
ii) Monoepoxymonoenes

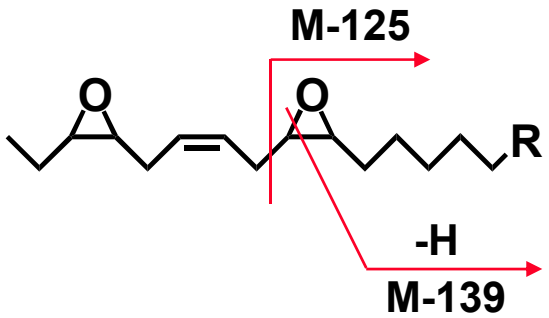




ii) Diepoxymonoenes





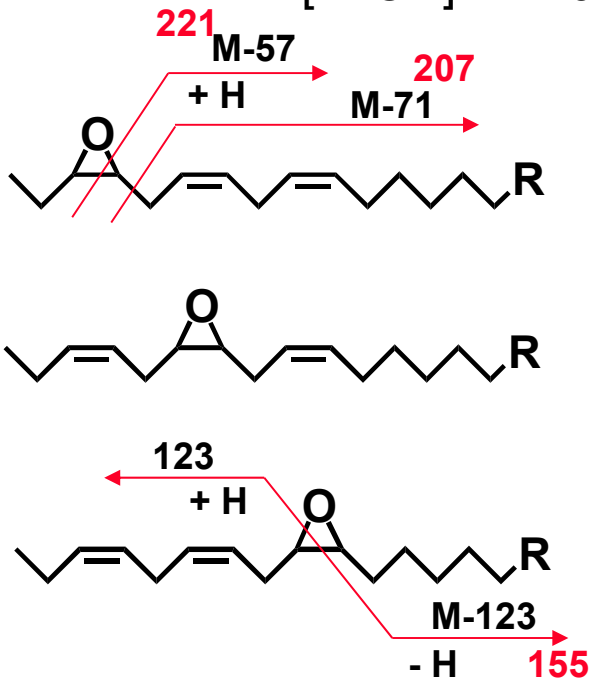


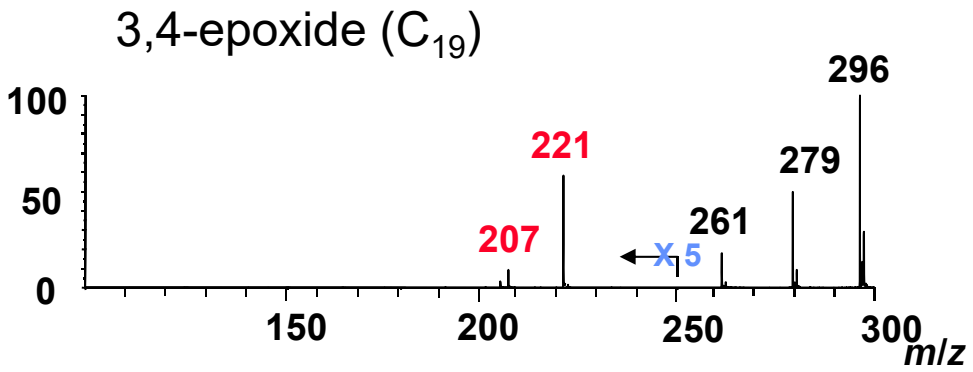
Analysis by LC-TOF MS (ESI)

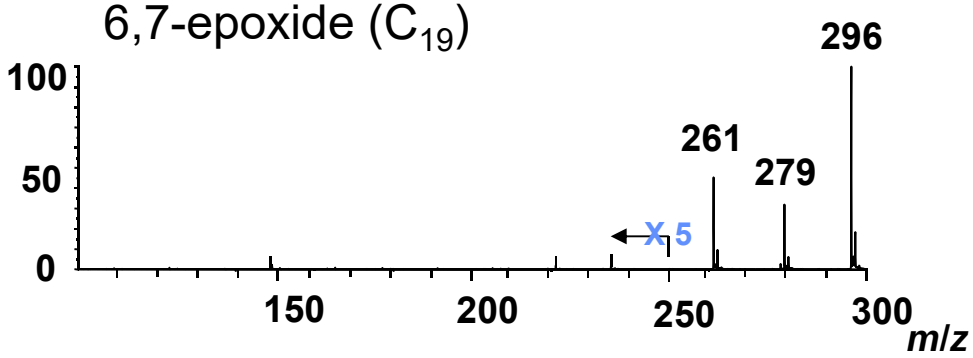
Column: ODS (2.1 mm X 15 cm)
Eluent: 20-5% H_2O in MeOH
Spray tip potential: +3,400 V
Nozzle potential: +120 V

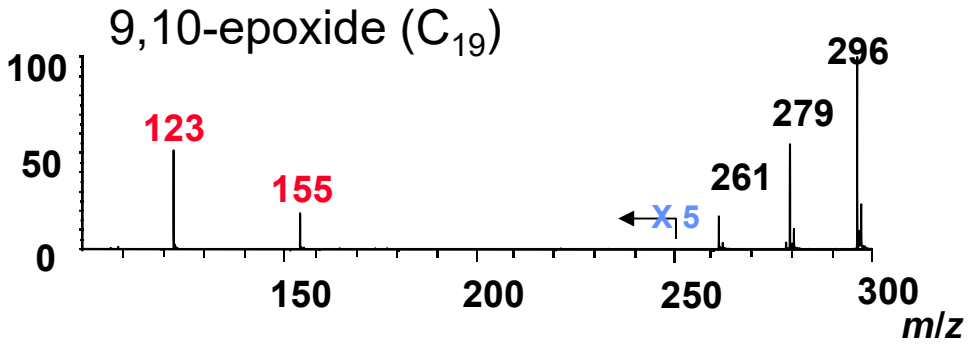
C_{19} compounds
(R=C_4H_9)

$[M+NH_4]^+$	296
$[M+H]^+$	279
$[M-OH]^+$	261







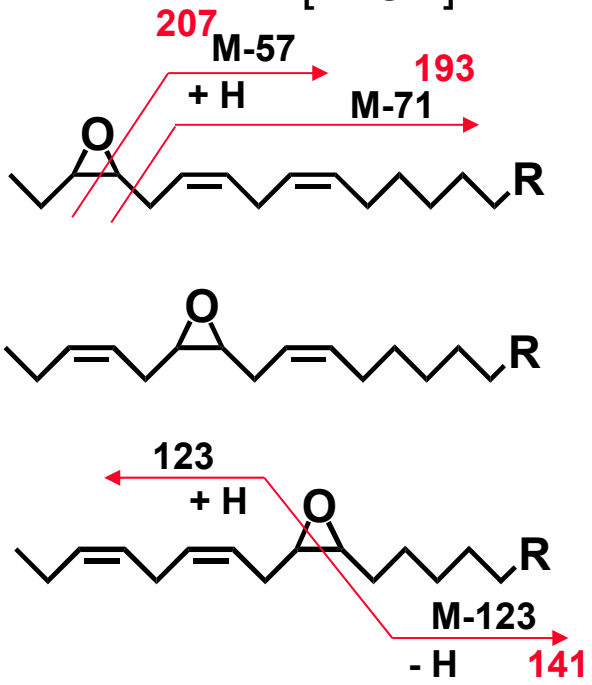


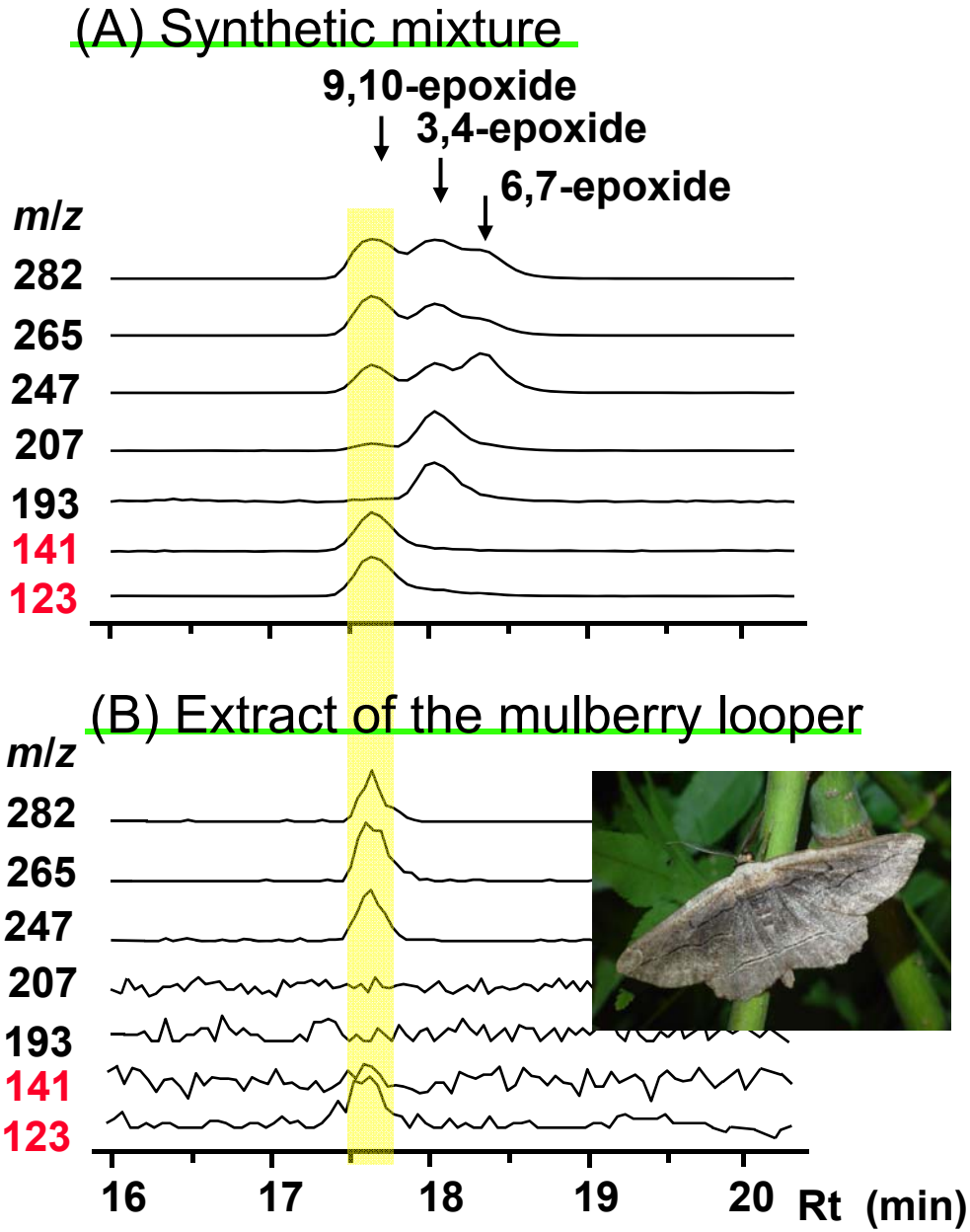
LC-TOF MS (ESI) analysis of pheromones ①

Column: ODS (2.1 mm X 15 cm)
Eluent: 20-5% H_2O in MeOH
Spray tip potential: +3,400 V
Nozzle potential: +120 V

C_{18} compounds
($R=C_3H_7$)

$[M+NH_4]^+$	282
$[M+H]^+$	265
$[M-OH]^+$	247



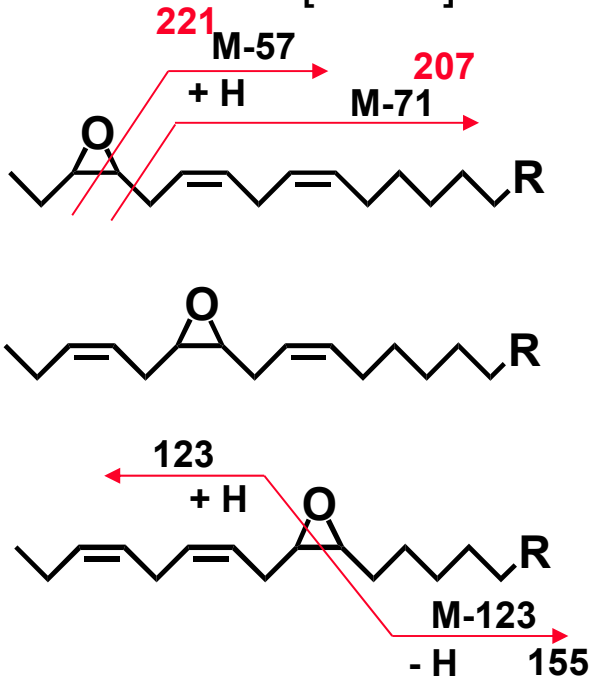


LC-TOF MS (ESI) analysis of pheromones ②

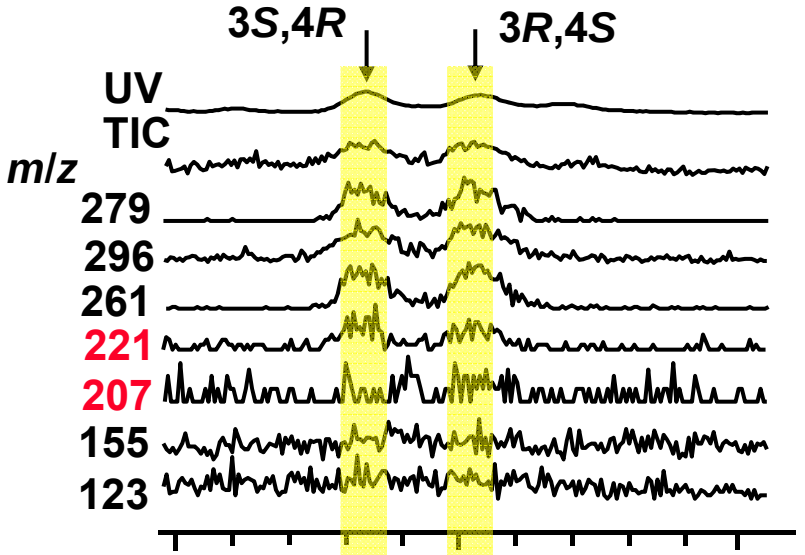
Column: Chiralcel OJ-R
Eluent: 10% H_2O in MeOH
Spray tip potential: +3,400 V
Nozzle potential: +120 V

C_{19} compounds
(R=C_4H_9)

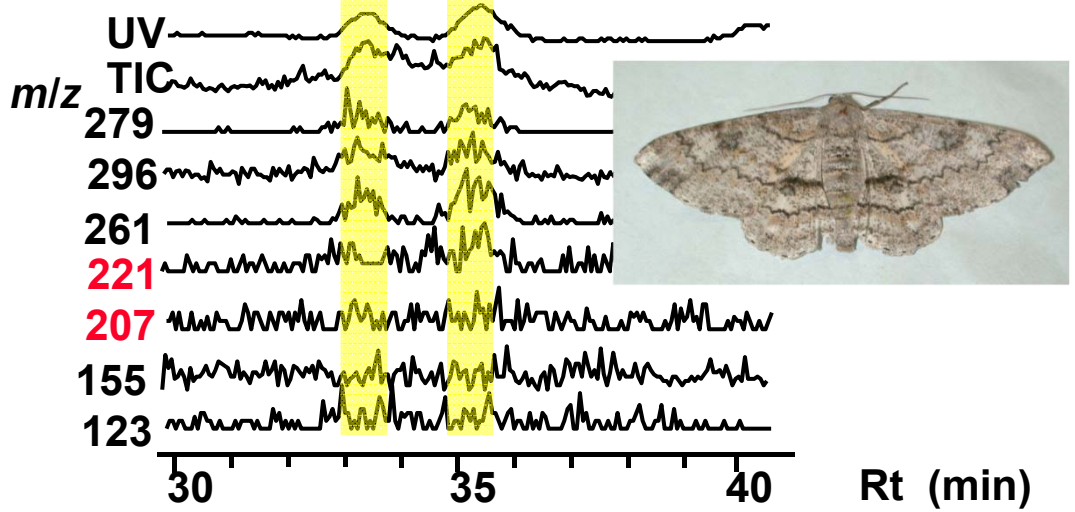
$[M+NH_4]^+$	296
$[M+H]^+$	279
$[M-OH]^+$	261



(A) Racemic mixture of 3,4−epoxide



(B) Extract of the giant looper



Conclusion

EI-Mass spectra of Type I pheromones (conjugated dienes) and Type II pheromones (polyunsaturated hydrocarbons and their epoxy derivatives) showed diagnostic fragment ions for the structure determination.

LC and LC-MS are useful tools for unstable and inapplicable components on GC analysis.

Stereochemistry of epoxy pheromones can be determined by LC-MS with a chiral column.

The diversity of lepidopteran communication systems will be understood in detail using these elegant(?) and smart(?) techniques.

Acknowledgments

Co-workers

Drs. H. Naka, Y. Arita and K. Tsuchida

Identification of the pheromones from Sesiidae species

Drs. F. Komai, M. Kinjo and H. Ishitani

identification of the pheromones from Olethreutinae species

Drs. K. Ohtani, G.-Q. Pu and K. I. Karasawa

Identification of the pheromone from Geometridae species

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Dr. A. Ono
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M. D. A. Islam
N. Numakura
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