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DEACTIVATION OF THE SEX PHEROMONE OF THE DIAMONDBACK MOTH BY ALUMINA[1]

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Yuh-Meei Lin and Y. S. Chow (1982) Deactivation of the sex pheromone of the Diamondback moth by Alumina. *Bull. Inst. Zool., Academia Sinica* **21**(1): 61-66. The sex pheromone of the diamondback moth was deactivated completely when it was chromatographed on active alumina column. It is established that the components of the sex pheromone, (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate, were converted and hydrolyzed respectively to the corresponding alcohol, (Z)-11-hexadecen-1-ol, by active neutral alumina.

The sex pheromone in the female diamondback moth (*Plutella xylostella* (L.)) was first demonstrated and partially purified with a silicic acid column by Chow *et al.*[2] Later Tamaki *et al.*[11] identified the chemical structure of the sex pheromone as a mixture of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate. We also confirmed that these two components were the true sex pheromone of the diamondback moth[3].

During our early work on the isolation and purification of the sex pheromone, it was noticed that the activity of the sex pheromone was lost completely when the crude extract was exposed to alumina column. In order to understand why the alumina column could not be used in the isolation procedure of the diamondback moth sex pheromone, synthetic compounds, (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate were chromatographed on alumina column separately, and the results are reported here.

MATERIALS AND METHODS

Merck neutral aluminium oxide 90 (Activity I) particle size 0.063-0.200 mm (70-230 mesh ASTM) for column chromatography (Art No. 1077) was used. All solvents used, such as methanol and diethyl ether, were guaranteed C. P. for reagent grade and purchased from E. Merck. (Z)-11-hexadecenal was purchased from Farchan. (Z)-11-hexadecenyl acetate was generously donated by Dr. Yoshio Tamaki, National Institute of Agricultural Sciences, Tokyo, Japan. (Z)-11-hexadecen-1-ol was prepared by reduction of (Z)-11-hexadecenal with lithium aluminium hydride in ether solution or alkaline hydrolysis of (Z)-11-hexadecenyl acetate.

Analytical gas-liquid chromatography was performed on a Varian Aerograph Model 2800 chromatograph equipped with FID detector using 5 ft×⅛ in. column packed with 3% SE-30 on 100/120 VARAPORT 30. Oven temperature was programmed from 130°C to 250°C at 8°C/min., carrier gas flow rate was 16 ml/min.. Spectral data were obtained with a Hitachi 215 Infrared Grating Spectrometer and a HP 5985A GC-MS System.

Bioassay was performed both in laboratory and in field attraction experiments according to the methods of Chow *et al.*[2].

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Column chromatography of crude extract of the diamonback moth virgin females:

1000 virgin females were extracted with methylene chloride. After evaporating the methylene chloride, the residue was dissolved in 2 ml of hexane and loaded on the top of a 20 g alumina column (20 mm. in diameter). Fractions eluted stepwise by 50 ml volumes of the solvents, pure hexane, 2%, 4%, 8%, 16%, 32% and 50% diethyl ethyl ether in hexane, and pure diethyl ether were collected as F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8, respectively. Each fraction was subjected to bioassay.

Column chromatography of (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate on alumina column:

Two mg of (Z)-11-hexadecenal was loaded on 2 g of aluminium oxide column (12 mm in diameter), and eluted with 20 ml each of pure hexane, 2%, 4%, 8%, 16%, 32%, 50% diethyl ether in hexane and pure dietyl ether, and collected as fractions F_{1a}, $F_{2a}\cdots F_{8a}$ respectively. Two mg of (Z)-11-hexadecenyl acetate was chromatographed on 2 g of alumina column by the same procedure to obtain eight fractions, labelled F_{1b}, $F_{2b}\cdots F_{8b}$. Each fraction was evaporated to dryness under reduced pressure below 40°C and dissolved in 0.1 ml of hexane before analysis by GC.

Preparation of (Z)-11-hexadecen-1-ol:

Fifty mg of (Z)-11-hexadecenal was dissolved in diethyl ether and was added to the $LiAlH_4$ ether solution, then stirred at room temperature for 30 min. Excess $LiAlH_4$ was destroyed by 10% H_2SO_4, the ppt filtered and the filtrated extracted by diethyl ether. The extract was washed with distilled water and then dried with anhydrous $MgSO_4$. After drying, the solvent was evaporated off and the product was identified by GC-MS and IR as (Z)-11-hexadecen-1-ol.

RESULTS

Deactivation of the crude extract from the virgin females by alumina column:

In laboratory bioassay the crude extract from the virgin females evoked good response from cages males. After chromatographing the crude extract on alumina column, each fraction (F_1-F_8) obtained was subjected to bioassay and no fraction obtained a response from the males nor did a recombination of all the fractions. It seems the crude extract was completely denatured by the alumina column. In a previous report[2], it was shown by the preliminary experiment on saponification (alcoholic sodium hydroxide)-acetylation (acetic anhydride and pyridine) that the sex pheromone probably contains a functional acetate. When acetylation of the fractions (F_1-F_8) was followed by bioassay of each fraction individually or after combining all fractions, biological activity still did not reappear. This revealed that there were more than one component in the sex pheromone and the components could be decomposed by alumina.

TABLE I

The results of GLC analysis of fraction 1a-8a. Column temperature 130-250°C, 8°C/min. flow rate (nitrogen) 16 ml/min. 5 ft×1/8 in. column packed with 3% SE-30 on 100/120 VARAPORT 30

Compounds	Fraction / Tr (min.)	F_{1a}	F_{2a}	F_{3a}	F_{4a}	F_{5a}	F_{6a}	F_{7a}	F_{8a}
unknown	9.2	—	—	—	—	—	—	trace	—
(Z)-11-hexadecenal	9.8	—	—	—	—	ca 30 μg	30 μg	ca 50 μg	—
(Z)-11-hexadecen-1-ol	10.5	—	—	—	—	—	—	700 μg	100 μg
unknown	20	—	—	trace	trace	trace	trace	trace	—

Denature of (Z)-11-hexadecenal by alumina column:

Fractions 1a-8a were analyzed by gas liquid chromatography and the retention times of known compounds and unknowns obtained are shown in Table I.

It will be seen from this table, only small amounts of (Z)-11-hexadecenal were recovered from fractions 5a and 6a. There was a trace amount of unknown compound D (Fig. I peak D) eluted from fractions 3a-6a. Fraction 7a showed a major component accompanied by a

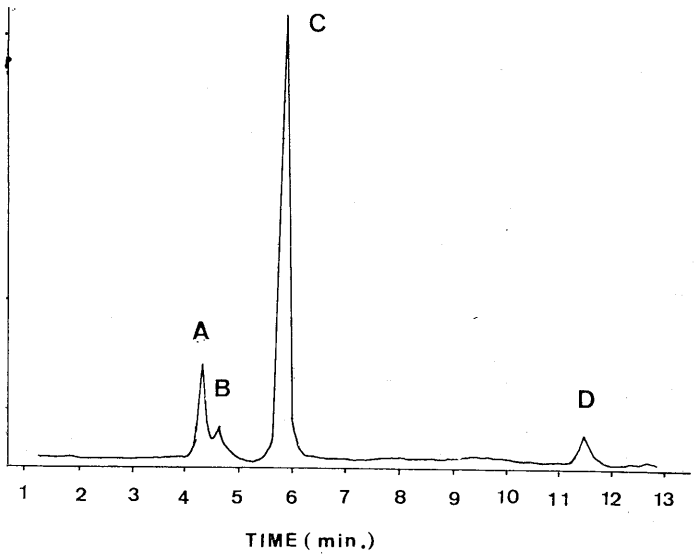

Fig. 1. Gas chromatogram of F_{7a} on 2% OV 101 column. (120-200°C at 5°C/min)

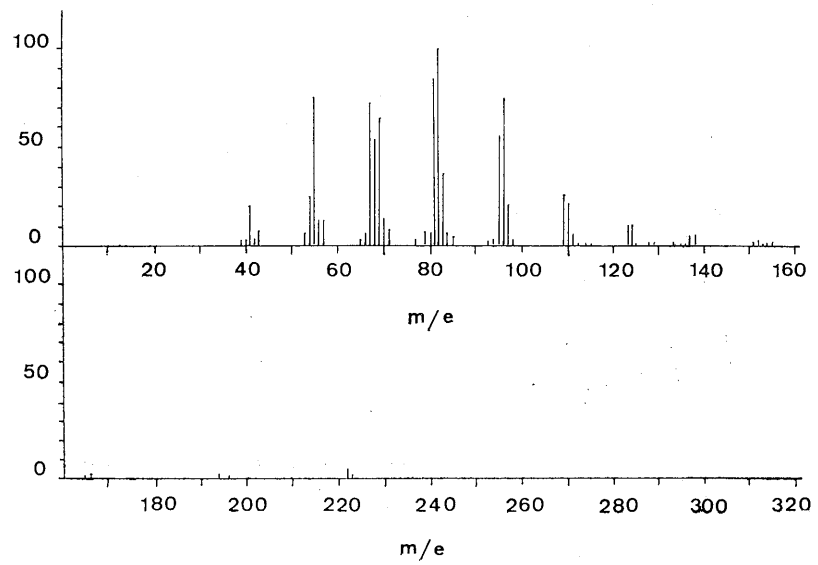

Fig. 2. Mass Spectrum of peak C ((Z)-11-hexadecen-1-ol) in F_{7a}.

TABLE II

The results of GLC analysis of fraction 1b to 8b. Column temperature 130-250°C, 8°C/min., N_2 flow rate 16 ml/min. 5 ft×8 in. column packed with 3% SE-30 on 100/120 VARAPORT 30

Compound	Fracting / Tr (min.)	F_{1b}	F_{2b}	F_{3b}	F_{4b}	F_{5b}	F_{6b}	F_{7b}	F_{8b}
(Z)-11-hexadecenyl acetate	12.4	—	320 μg	430 μg	—	—	—	—	—
(Z)-11-hexadecen-1-ol	10.5	—	—	—	—	30 μg	520 μg	50 μg	25 μg

small amount of original (Z)-11-hexadecenal (Fig. I peak B) and trace of two unknown compounds, A and D (Fig. I peak A and D). The major component of F_{7a} has longer retention time than that of (Z)-11-hexadecenal in the GC chromatogram. It indicates that the compound is more polar or has higher molecular weight than those of (Z)-11-hexadecenal.

Combined gas chromatography-mass spectrometry (GC-MS) analysis of fraction 7a on a OV-101 column (2%, 2 ft×⅛ in, 120-200°C, 5°C/min.) showed one major peak at 5.8 min. The retention time and mass spectrum (Fig. 2) were identical with those of a standard material, (Z)-11-hexadecen-1-ol.

The IR spectrum of compound C showed the absorption of hydroxyl group at 3300 cm^{-1} and 1060 cm^{-1}, identical with that of (Z)-11-hexadecen-1-ol. From the above results it appears that the (Z)-11-hexadecenal was mostly reduced to its corresponding alcohol, (Z)-11-hexadecen-1-ol.

Decomposition of (Z)-11-hexadecenyl acetate by alumina column:

Fractions 1b to 8b were also analyzed by GLC and the results are shown in Table II.

About 0.8 mg of (Z)-11-hexadecenyl acetate was recovered from F_{2b} and F_{3b}. A compound which had shorter retention time than that of (Z)-11-hexadecenyl acetate was collected in fraction 5b-8b. Fraction 7b was subjected to GC-MS analysis. From the retention time and mass spectral data it was confirmed that the compound in F_{7b} was (Z)-11-hexadecen-1-ol. From the above results it appears that (Z)-11-hexadecenyl acetate was partially hydrolyzed to (Z)-11-hexadecen-1-ol when it was chromatographed on an alumina column.

DISCUSSION

Secondary reactions caused by active alumina during chromatography have been summarized by Lederer[7]; for example, the saponification of glycerides and autoxidation of glycerides and autoxidation of fatty acids, the deacetylation of acetylated sugars, elimination of one molecule of acetic acid from xanthinine. Other changes under the conditions of chromatography on alumina, such as hydrolysis of ester, substitution and elimination reaction were also reported[4,5,8,9,10]. In our experiments, it is revealed that (Z)-11-hexadecenyl acetate was 40% recovered and partially (30%) hydrolyzed to (Z)-11-hexadecen-1-ol, whereas (Z)-11-hexadecenal was reduced to its corresponding alcohol, (Z)-11-hexadecen-1-ol, in a higher percentage (about 40%) and only 4% recovered. Since the effective components, (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate were converted to (Z)-11-hexadecen-1-ol, only small amount of (Z)-11-hexadecenal (4%) and (Z)-11-hexadecenyl acetate (40%) remained unchanged.

As we know, in most of the Lepidoptera insects more than one attractant chemical were used as an aid to insure species isolation. The ratio of the components in a multicomponents type of sex pheromone is very important to its ability to attract the opposite sex. Chisholm *et al.*[1] reported that mixtures of (Z)-11-hexadecenal and (Z)-11-hexadecenyl

acetate dispensed from rubber septa in a 7:3 ratio were good lures to males of *Plutella xylostella*. Because alumina chromatography not only changed the main components (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate to large amount of (Z)-11-hexadecen-1-ol, but also modified the ratio of the sex pheromone components from 1:1 to 1:10, so that the crude extract lost its activity completely in laboratory experiments. On the other hand, Koshihara *et al.*[6], Yamada and Kohihara[12] reported that admixture of low concentration of (Z)-11-hexadecen-1-ol to the sex pheromone of the diamondback moth in the ratio 1-10:100 increassed its attractiveness to the male moths. It is clear then the main reason for the crude extracts of the diamondback moth to lose its activity during chromatggraphy on alumina column is because most of its components were converted to (Z)-11-hexadecen-1-ol and only 4% of (Z)-11-hexadecenal and 40% of (Z)-11-hexadecenyl acetate remained. Therefore, alumina column is not recommended as a useful tool for the isolation of some insect sex pheromone which may contain aldehyde or acetates.

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三氧化二鋁對小菜蛾性費洛蒙之不活性化反應

林玉美　周延鑫

處女小菜蛾粗萃取液經三氧化二鋁柱層純化時，其性費洛蒙活性消失。將小菜蛾性費洛蒙之組成成分 (順)-11-十六烯醛與 (順)-11-十六烯醇乙醯酯各別經過三氧化二鋁柱層後，再以紅外線光譜及氣相色析一質譜法分析知前者大部分 (40%) 被還元成其對應之 (順)-11-十六烯醇只有 4%被回收，而後者則有 30%被水解成 (順)-11-十六烯醇，而 40%被回收。