

# GC/FT-IR Analysis of Novel 4,6,9-Triene and 2,4,6,9-Tetraene Occurring in a Female Pheromone Gland of *Arctia plantaginis* (Erebidae: Arctiinae)

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## Abstract

Fifteen subspecies of the wood tiger moth, *Arctia plantaginis* (Lepidoptera: Erebidae: Arctiinae), have been recorded in the Northern Hemisphere. An analysis of crude pheromone extracts by GC equipped with an electroantennographic (EAG) detector showed four EAG-active components (Comps. I-IV) that were commonly involved in the pheromone glands of two subspecies inhabiting Japan and Finland. Comp. I is a major component (>75%) and the others are minor components (3% - 15%). Their mass spectra, measured by GC/MS, revealed the chemical structures of C<sub>21</sub> unsaturated hydrocarbons as follows: 3,6,9-triene for Comp. I, 4,6,9-triene for Comp. II, 1,3,6,9-tetraene for Comp. III, and 2,4,6,9-tetraene for Comp. IV. Comps. I and III are known Type II pheromone compounds, and their retention times coincide with those of the authentic standards with all *Z* configurations. As a next step, the extract was analyzed by GC/FT-IR to determine the configuration of Comps. II and IV. Their IR spectra showed two characteristic C-H bending absorptions around 990 and 945 cm<sup>-1</sup> due to the conjugated dienyl moieties; thus, *Z* and *E* configurations were assigned to the double bonds at the 2- and 4-positions, respectively. Their *Z* double bonds at the 6- and 9-positions are indicated by no absorptions around 970 cm<sup>-1</sup>, due to the isolated double bonds with *E* configurations. Finally, the structures of Comps. II and IV were confirmed by synthesis using a double Wittig reaction. The synthetic (4*E*,6*Z*,9*Z*)-4,6,9-triene and (2*Z*,4*E*,6*Z*,9*Z*)-2,4,6,9-tetraene showed strong EAG activity, and their

chemical data coincided well with those of the natural Comps. II and IV, indicating the correctness of the structure determination by GC/FT-IR analysis and its usefulness for Type II pheromone compounds.

### Keywords

Natural Products, Insect Sex Pheromone, GC/FT-IR, Wood Tiger Moth, (4*E*,6*Z*,9*Z*)-4,6,9-Henicosatriene, (2*Z*,4*E*,6*Z*,9*Z*)-2,4,6,9-Henicosatetraene

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## 1. Introduction

Lepidopteran sex pheromones, which have been identified from more than 670 species, are mainly sorted into two types according to their chemical structures [1] [2] [3]. Type I pheromones are composed of unsaturated fatty alcohols, acetates, and aldehydes, and are most commonly found from female moths in many families. Type II pheromones are composed of polyunsaturated hydrocarbons and their epoxides and are mostly produced by females in highly evolved groups: Erebiidae (including former Arctiidae and Lymantriidae [4]) and Geometridae [5] [6]. Species-specific pheromones are created by the blending of some compounds with different structures, such as chain length, unsaturated degree, and positions of the double bond and epoxy ring. Arctiinae (former Arctiidae) is divided into three tribes: Lithosiini, Synthomini, and Arctiini. To date, sex pheromones of 23 species in the tribe Arctiini have been reported, and the pheromones of 16 of these species are composed of Type II compounds [5]. Only three species inhabiting Japan are included, while more than 100 Arctiini species have been recorded in Japan. To increase the information about the Japanese species, we started to investigate a sex pheromone of the wood tiger moth, *Arctia plantaginis* (former *Parasemia plantaginis* [7]), which belongs to Arctiini. Large distribution area of this species and great geographic variation in their phenotypes gives a unique possibility to study whether this species is its early steps of divergence. It is noteworthy that 15 subspecies of *A. plantaginis* have been recorded in the Northern Hemisphere, and four of these subspecies are distributed in Japan. It should be interesting to know whether differences in sex pheromones are one of the most important candidates driving pre-zygotic isolation.

Lepidopteran pheromones have usually been investigated by gas chromatography (GC) with an electroantennographic (EAG) detector (GC-EAD) and GC combined with mass spectrometry (GC/MS) [2]. GC-EAD can differentiate biologically active compounds from others in the crude extract, and then chemical structures of the pheromone candidates are identified by GC/MS [8]. The sensitivity of both instruments is so high that a pheromone gland extract of one female sometimes provides enough information to clarify the chemical cues when compared with published data of known compounds. However, the configuration of a double bond is scarcely determined by only GC/MS analysis because

geometrical isomers show similar mass spectra. Thus, for proper determination, it is necessary to compare chromatographic behaviors of a natural component with synthetic standards. On the other hand, geometrical isomers of alkenyl compounds with a straight chain usually show different infrared (IR) spectra, and their configurations can be determined by examining the absorptions of C-H stretching and bending at the double bond. We have utilized GC combined with Fourier transform infrared spectrometry (GC/FT-IR) to reveal configurations of Type I pheromone compounds produced by the nettle moth [9]. Our previous research provided a good opportunity to experience the high sensitivity of GC/FT-IR that was comparable to that of GC/MS. In this study, we successfully applied the same instrument to determine the structure of novel Type II compounds occurring in the pheromone glands of the wood tiger moth.

## 2. Experimental

### 2.1. Analytical Instruments

For an analysis with gas chromatography (GC) with an electroantennographic (EAG) detector (GC-EAD), an HP-5890 Series II gas chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) was equipped with a DB-23 capillary column (0.25 mm ID  $\times$  30 m, 0.25  $\mu$ m film; J & W Scientific, Folsom, CA, USA). The effluent from the column was split into two lines, which led to a flame ionization detector (FID) and EAD at a ratio of 1:1 [9]. The oven temperature was maintained at 80°C for 1 min and then programmed at 8°C·min<sup>-1</sup> to 210°C. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded by a Jeol Delta 2 Fourier transform spectrometer (JEOL Ltd., Tokyo, Japan) at 399.8 and 100.5 MHz, respectively, for CDCl<sub>3</sub> solutions containing TMS as an internal standard. <sup>1</sup>H-<sup>1</sup>H COSY, HMQC, and HMBC spectra were also measured with the same spectrometer, using the usual pulse sequences and parameters. GC/MS was conducted in EI mode (70 eV) with an HP5973 mass spectrometer system (Agilent Technologies) equipped with a split-splitless or a cool on-column injector and the same DB-23 column in the analysis by GC-EAD. The column temperature program was 50°C for 2 min, 10°C·min<sup>-1</sup> to 160°C, and 4°C·min<sup>-1</sup> to 220°C. The carrier gas was helium. IR spectra were recorded using FT-IR (Discover IR; Spectra Analytics, Marlborough, MA) coupled to a GC (GC 7980C; Agilent Technologies), which was equipped with an HP-5 capillary column (0.25 mm ID  $\times$  30 m, 0.25  $\mu$ m film; Agilent Technologies, Santa Clara, CA). A liquid nitrogen-cooled photoconductive mercury-cadmium-telluride detector was used with a FT-IR resolution of 8 cm<sup>-1</sup>. Compounds eluting from the capillary column were solidified on a zinc selenide disk at -30°C and rotated at 3 mm·min<sup>-1</sup>. The distance between the column end and disk was 5 mm. The oven temperature for all GC analyses was set initially at 50°C for 2 min. and then programmed at 10°C·min<sup>-1</sup> to 160°C and 4°C·min<sup>-1</sup> to 220°C. The flow rate of the carrier gas (He) was 1.0 mL·min<sup>-1</sup>, and the GC inlet temperature was 220°C.



HMPA at  $-80^{\circ}\text{C}$  under Ar gas to make bis(ylide), which was further treated with a mixture of dodecanal and (*E*)-2-hexenal (RCHO in **Figure 1**). After warming to room temperature, the crude products were extracted with hexane and cleaned by the usual workup. While this reaction produces byproducts, such as a tetraene with a short chain and a diene with a long chain, the desired E4,Z6,Z9-21:H was obtained in 18% yield, as calculated from the phosphonium salt, after the crude products were chromatographed on a silica gel column impregnated with  $\text{AgNO}_3$  using hexane and mixtures of hexane and benzene as eluents.  $^1\text{H}$  NMR  $\delta$  ppm: 0.88 (3H, t,  $J = 7$  Hz,  $\text{CH}_3$ ), 0.91 (3H, t,  $J = 7.5$  Hz,  $\text{CH}_3$ ), 1.22 - 1.38 (18H, m,  $\text{CH}_2 \times 9$ ), 1.42 (2H, tq,  $J = 7.5, 7.5$  Hz,  $\text{CH}_2\text{CH}_3$ ), 2.06 (2H, dt,  $J = 7.5, 7$  Hz,  $\text{CH}_2\text{CH}_2\text{CH} = \text{CH}$ ), 2.08 (2H, dt,  $J = 7.5, 7$  Hz,  $\text{CH}_2\text{CH}_2\text{CH} = \text{CH}$ ), 2.90 (2H, dd,  $J = 7, 7$  Hz,  $\text{CH} = \text{CHCH}_2\text{CH} = \text{CH}$ ), 5.26 (1H, dt,  $J = 10.5, 7.5$  Hz,  $\text{CH} = \text{CHCH} = \text{CH}$ ),  $\sim 5.35$  (2H, m,  $\text{CH}_2\text{CH} = \text{CHCH}_2$ ), 5.68 (1H, dt,  $J = 15, 7$  Hz,  $\text{CH} = \text{CHCH} = \text{CH}$ ), 5.95 (1H, dd,  $J = 11, 10.5$  Hz,  $\text{CH} = \text{CHCH} = \text{CH}$ ), 6.32 (1H, dd,  $J = 15, 11$  Hz,  $\text{CH} = \text{CHCH} = \text{CH}$ );  $^{13}\text{C}$  NMR  $\delta$  ppm: 13.8 ( $\text{C}^1$ ), 14.1 ( $\text{C}^{21}$ ), 22.5 ( $\text{C}^2$ ), 22.7 ( $\text{C}^{20}$ ), 26.0 ( $\text{C}^8$ ), 27.3 ( $\text{C}^{11}$ ), 29.3 ( $\times 2$ ), 29.6, 29.7 ( $\times 4$ ), 31.9 ( $\text{C}^{19}$ ), 35.0 ( $\text{C}^3$ ), 125.5 ( $\text{C}^5$ ), 127.4 ( $\text{C}^9$ ), 127.8 ( $\text{C}^7$ ), 128.7 ( $\text{C}^6$ ), 130.5 ( $\text{C}^{10}$ ), 135.0 ( $\text{C}^4$ ).

### 2.5. Synthesis of 2,4,6,9-Tetraene (E2,E4,Z6,Z9-21:H and Z2,E4,Z6,Z9-21:H)

E2,E4,Z6,Z9-21:H was prepared in a manner and at a yield similar to those of the synthesis of 4,6,9-triene using (2*E*,4*E*)-2,4-hexadienal (Sigma-Aldrich Corp., Milwaukee, WI, USA) instead of (*E*)-2-hexenal.  $^1\text{H}$  NMR  $\delta$  ppm: 0.88 (3H, t,  $J = 7$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.22 - 1.38 (18H, m,  $\text{CH}_2 \times 9$ ), 1.78 (3H, d,  $J = 6.5$  Hz,  $\text{CH}_3\text{CH} = \text{CH}$ ), 2.06 (2H, dt,  $J = 7, 7$  Hz,  $\text{CH} = \text{CHCH}_2\text{CH}_2$ ), 2.93 (2H, dd,  $J = 7, 7$  Hz,  $\text{CH}_2\text{CH} = \text{CH}$ ),  $\sim 5.35$  (3H, m,  $\text{CH} = \text{CHCH}_2\text{CH} = \text{CHCH}_2$ ), 5.72 (1H, dq,  $\text{CH}_3\text{CH} = \text{CH}$ ,  $J = 14, 6.5$  Hz), 6.00 (1H, dd,  $\text{CH} = \text{CHCH} = \text{CHCH} = \text{CH}$ ,  $J = 11, 11$  Hz), 6.15 (2H, m,  $\text{CH} = \text{CHCH} = \text{CHCH} = \text{CH}$ ), 6.39 (1H, dd,  $\text{CH} = \text{CHCH} = \text{CHCH} = \text{CH}$ ,  $J = 15, 11$  Hz);  $^{13}\text{C}$  NMR  $\delta$  ppm: 14.2 ( $\text{C}^{21}$ ), 18.4 ( $\text{C}^1$ ), 22.7 ( $\text{C}^{20}$ ), 26.2 ( $\text{C}^8$ ), 27.3 ( $\text{C}^{11}$ ), 29.4 ( $\times 2$ ), 29.6, 29.7 ( $\times 4$ ), 32.0 ( $\text{C}^{19}$ ), 125.5 ( $\text{C}^5$ ), 127.3 ( $\text{C}^9$ ), 128.7 ( $\text{C}^6$ ), 129.7 ( $\text{C}^7$ ), 129.9 ( $\text{C}^2$ ), 130.7 ( $\text{C}^{10}$ ), 131.9 ( $\text{C}^3$ ), 133.1 ( $\text{C}^4$ ).

Z2,E4,Z6,Z9-21:H was synthesized using 2,4-hexadienal, which was a mixture of (2*E*,4*E*)- and (2*Z*,4*E*)-isomers (ca. 85:15) (Wako Pure Chemical Industries Ltd., Osaka, Japan). The produced 2,4,6,9-tetraene, a mixture of (2*E*,4*E*,6*Z*,9*Z*)- and (2*Z*,4*E*,6*Z*,9*Z*)-isomers, was treated with tetracyanoethylene and the unreacted (2*Z*,4*E*,6*Z*,9*Z*)-isomer was recovered.  $^1\text{H}$  NMR  $\delta$  ppm: 0.88 (3H, t,  $J = 7$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.22 - 1.38 (18H, m,  $\text{CH}_2 \times 9$ ), 1.78 (3H, d,  $J = 7$  Hz,  $\text{CH}_3\text{CH} = \text{CH}$ ), 2.06 (2H, dt,  $J = 7, 7$  Hz,  $\text{CH} = \text{CHCH}_2\text{CH}_2$ ), 2.94 (2H, dd,  $J = 7, 7$  Hz,  $\text{CH} = \text{CHCH}_2\text{CH} = \text{CH}$ ),  $\sim 5.4$  (3H, m,  $\text{CH} = \text{CHCH}_2\text{CH} = \text{CHCH}_2$ ), 5.53 (1H, dq,  $\text{CH}_3\text{CH} = \text{CH}$ ,  $J = 10.5, 7$  Hz), 6.08 (2H, m,  $\text{CH} = \text{CHCH} = \text{CHCH} = \text{CH}$ ), 6.50 (2H, m,  $\text{CH} = \text{CHCH} = \text{CHCH} = \text{CH}$ );  $^{13}\text{C}$  NMR  $\delta$  ppm: 13.5 ( $\text{C}^1$ ), 14.1 ( $\text{C}^{21}$ ), 22.7 ( $\text{C}^{20}$ ), 26.2 ( $\text{C}^8$ ), 27.3 ( $\text{C}^{11}$ ), 29.3, 29.4, 29.6, 29.7 ( $\times 4$ ), 31.9 ( $\text{C}^{19}$ ), 126.8 ( $\text{C}^2$ ), 127.2 ( $\text{C}^9$ ), 127.5 ( $\text{C}^4$ ), 128.1 ( $\text{C}^5$ ), 128.9 ( $\text{C}^6$ ), 129.7 ( $\text{C}^3$ ), 130.4 ( $\text{C}^{10}$ ), 130.8 ( $\text{C}^7$ ).

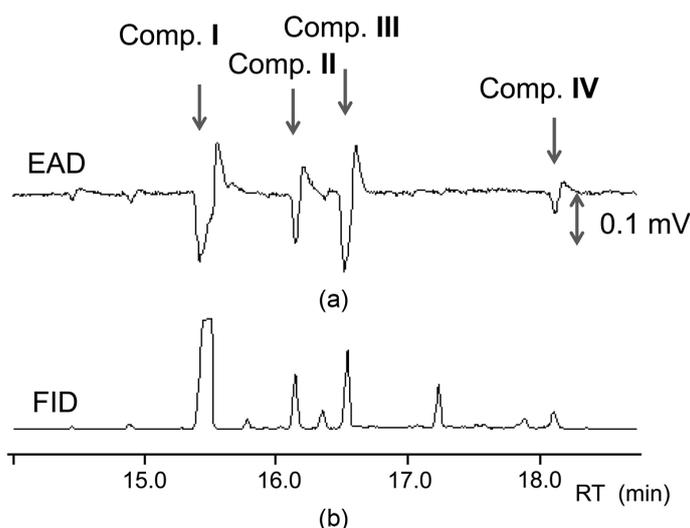
### 3. Results and Discussion

#### 3.1. GC-EAD Analysis of a Pheromone Extract

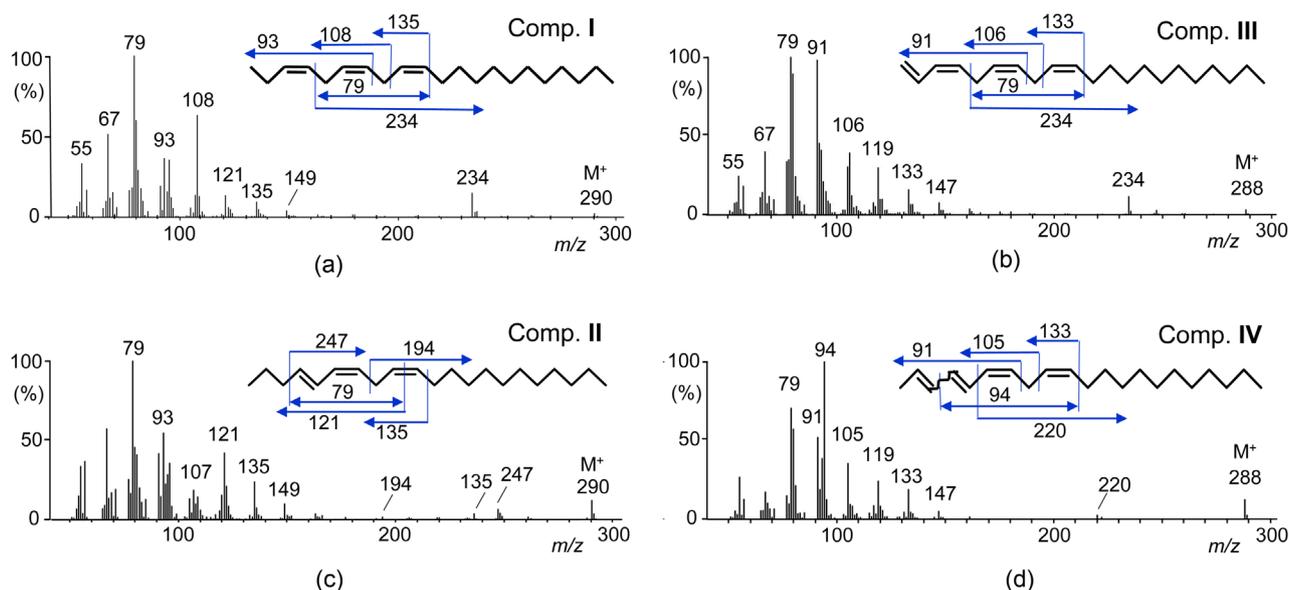
A pheromone gland extract of one *A. plantaginis* female, which was derived from a larva collected in the fields of Japan, showed four EAG-active components on the GC-EAD analysis with a DB-23 column (Comp. I, RT 15.4 min; Comp. II, RT 16.2 min; Comp. III, RT 16.5 min; Comp. IV, RT 18.2 min) (Figure 2). These pheromone candidates reproducibly elicited a response from male antennae of the Japanese subspecies in repeated analyses of both extracts from Japanese and Finnish subspecies. Peak areas recorded by FID indicated that Comp. I is a major (>75%) and others are minor components (3% - 15%).

#### 3.2. GC/MS Analysis of a Pheromone Extract

A crude pheromone extract of one female was analyzed with a DB-23 column, and Figure 3 shows the mass spectra of Comps. I-IV. The molecular ion  $M^+$  of Comps. I and II at  $m/z$  290 indicates trienes with a  $C_{21}$  chain. The mass spectrum of Comp. I with a base peak at  $m/z$  79 and its RT (16.75 min) coincide with those of authentic Z3,Z6,Z9-21:H [10]. The base peak of Comp. II, detected also at  $m/z$  79, indicates a 6,9-dienyl structure commonly involved in many Type II pheromone components [8], and that its RT (17.40 min) is longer than that of the 3,6,9-triene suggests that another double bond is conjugated to the homo-conjugated dienyl moiety. Since its mass spectrum is different from that of a 6,9,11-triene synthesized previously [11], we estimated the possibility of a



**Figure 2.** GC analysis of a crude pheromone extract of a *Arctia plantaginis* female (DB-23 capillary column) (a) by a flame ionization detector (FID) and (b) by an electroantennographic detector (EAD). The antenna of a *A. plantaginis* male collected in Japan responded reproducibly to four components (Comps. I-IV) in a pheromone gland extract of Japanese moths. Peak areas recorded by FID indicate their approximate mixing ratio of 30:3:5:1.

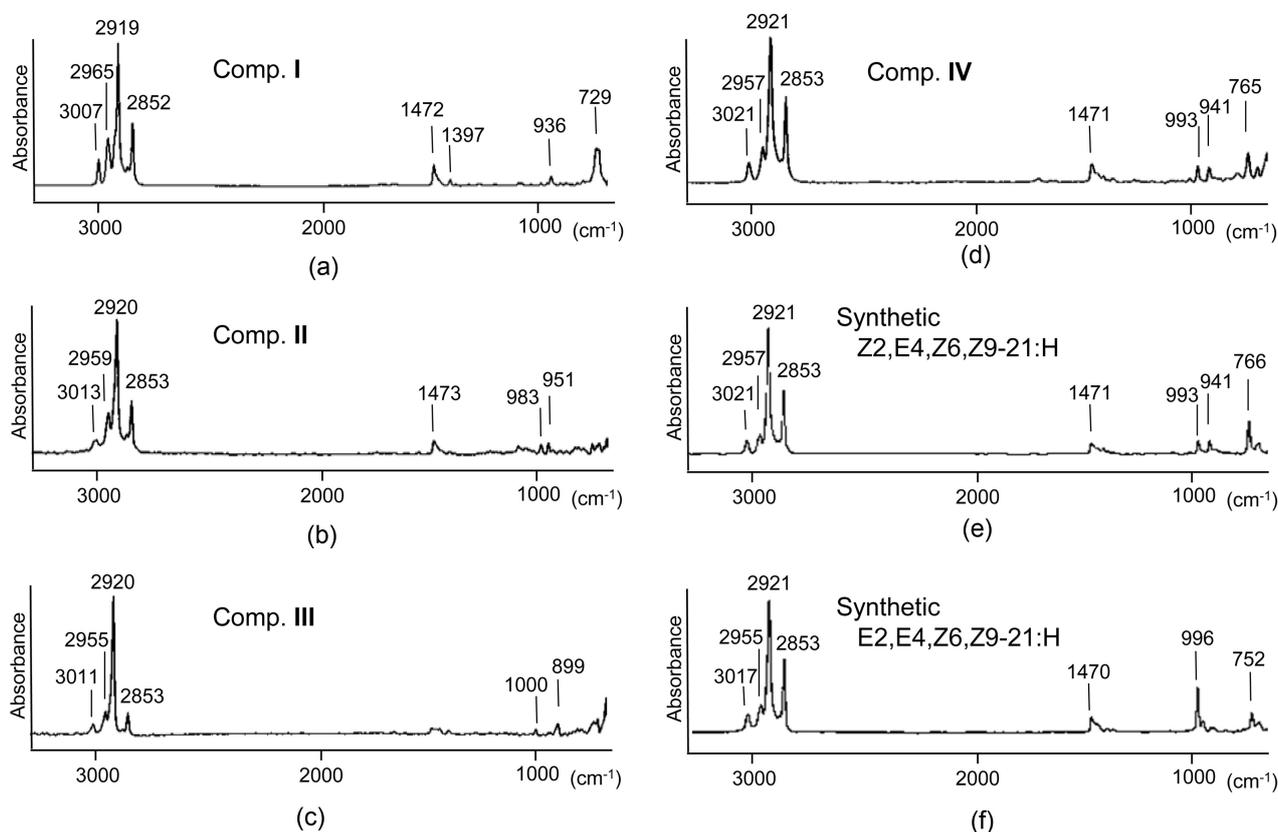


**Figure 3.** Mass spectra recorded by GC/MS analysis of a crude pheromone extract of a *Arctia plantaginis* female collected in Japan; (a) Comp. I, (b) Comp. II, (c) Comp. III, and (d) Comp. IV.

4,6,9-triene for Comp. II. On the other hand, the  $M^+$  of Comps. III and IV at  $m/z$  288 indicates  $C_{21}$  tetraenes. The spectrum of Comp. III with a base peak at  $m/z$  79 and its RT (17.71 min) coincide with those of authentic 1,Z3,Z6,Z9-21:H. Although the base peak of Comp. IV is recorded at  $m/z$  94, the spectral similarity of these two tetraenes suggests their common moiety, such as the 6,9-dienyl structure. The GC/MS data of Comp. IV are different from those of 3,6,9,11-tetraene synthesized previously [11]. The intensity of its  $M^+$  is higher than that of 1,Z3,Z6,Z9-21:H, and its RT (19.70 min) on the strong polar column is much longer than that of the tetraene that includes only one conjugated diene system. Therefore, we conceived a 2,4,6,9-tetraene that included a conjugated triene system as one possibility for Comp. IV.

### 3.3. GC/FT-IR Analysis of a Pheromone Extract

Figure 4 shows the IR spectra of Comps. I - IV, which were recorded from a crude pheromone extract of four females by using GC/FT-IR with an HP-5 column. The spectra of Comps. I (RT 19.01 min) and III (RT 19.57 min) coincide well with those of the corresponding synthetic Z3,Z6,Z9-21:H and 1,Z3,Z6,Z9-21:H, respectively; thus, all *Z* configurations of their three double bonds are confirmed by the absorption at around  $3010\text{ cm}^{-1}$  of C-H stretching at a 1,2-disubstituted double bond and the absence of absorption at around  $970\text{ cm}^{-1}$  of the C-H bending with an *E* configuration. In the case of Comp. III, additional absorptions were detected at  $1000$  and  $899\text{ cm}^{-1}$ , caused by C-H bending of geminal hydrogen atoms at the terminal double bond [9]. On the other hand, Comp. II (RT 19.71 min) showed two absorptions at  $983$  and  $951\text{ cm}^{-1}$ , which are also detected for Z6,Z9,E11-21:H. These absorptions, which are characteristic for conjugated dienes with an *E,Z* configuration, revealed an *E* configuration



**Figure 4.** Infrared spectra recorded by GC/FT-IR analysis of a crude pheromone extract of a *Arctia plantaginis* female collected in Japan and synthetic tetraenes; (a) Comp. I, (b) Comp. II, (c) Comp. III, (d) Comp. IV, (e) synthetic Z2,E4,Z6,Z9-21:H, and (f) synthetic E2,E4,Z6,Z9-21:H.

at the 4-position of Comp. II. Similar absorption at 993 and 941  $\text{cm}^{-1}$  of Comp. IV (21.66 min) suggests that it includes an *E,Z* configuration. A conjugated diene with a *Z,Z* configuration is characterized by a C-H stretching absorption at around 3040  $\text{cm}^{-1}$ ; however, Comp. IV showed absorption at 3021  $\text{cm}^{-1}$ , indicating absence of a conjugated dienyl moiety with a *Z,Z* configuration. Therefore, we assigned *Z* and *E* configurations at the 2- and 4-positions of Comp. IV, respectively. Their *Z* double bonds at the 6- and 9-positions are indicated by no absorptions around 970  $\text{cm}^{-1}$  of isolated double bonds with an *E* configuration; thus, Comps. II and IV are identified as E4,Z6,Z9-21:H and Z2,E4,Z6,Z9-21:H, respectively.

### 3.4. Synthesis and Analysis of 4,6,9-Triene and 2,4,6,9-Tetraene

In order to confirm the structure determination of two new unsaturated hydrocarbons based on the instrumental analyses, their authentic samples were synthesized using a one-pot double-Wittig reaction with a ylide derived from 1,3-dibromopropane (Figure 1). Their yields were not practical, but the desired compounds were easily prepared with commercialized aldehydes with a few steps, and configurations of their double bonds could be verified by NMR analysis. Synthetic E4,Z6,Z9-21:H shows four isolated olefinic proton signals from the

4- to 7-positions and an overlapped multiple signal of two protons at the 9- and 10-positions. Their signal assignments could be accomplished by the COSY spectrum, and the *4E,6Z* configuration was confirmed by coupling constants between two vicinal olefinic protons, *i.e.*,  $J_{4,5}$  (15 Hz) and  $J_{6,7}$  (10.5 Hz). The  $^{13}\text{C}$  signals were analyzed by HMQC and HMBC measurements, and chemical shifts of the allylic carbons at the 3-position (35.0 ppm) and 11-position (27.3 ppm) confirmed the *4E,9Z* configuration. Chemical shifts of three olefinic carbons ( $\text{C}^4\text{-C}^6$ ) in the conjugated dienyl moiety are similar to those of the corresponding carbons ( $\text{C}^{\omega 4}\text{-C}^{\omega 6}$ , namely  $\text{C}^9\text{-C}^7$ ) in (*6Z,8E*)-6,8-dodecadien-1-ol [13].

E2,E4,Z6,Z9-21:H showed three analyzable olefinic proton signals at the 2-, 5-, and 6-positions, which were assigned by COSY measurements. Its *2E,4E,6Z* configuration was confirmed by coupling constants between two vicinal olefinic protons, *i.e.*,  $J_{2,3}$  (14 Hz),  $J_{4,5}$  (15 Hz), and  $J_{6,7}$  (11 Hz). The chemical shift of the allylic methyl carbon signal (18.4 ppm) also disclosed the *2E* configuration. Furthermore, the chemical shifts of five olefinic carbons ( $\text{C}^2\text{-C}^6$ ) in the conjugated trienyl moiety are similar to those of the corresponding carbons ( $\text{C}^{\omega 2}\text{-C}^{\omega 6}$ , namely  $\text{C}^{15}\text{-C}^{11}$ ) in (*10Z,12E,14E*)-10,12,14-hexadecatrienyl acetate [14]. In the case of Z2,E4,Z6,Z9-21:H, its trienyl moiety has a symmetric structure, and almost all of the olefinic proton signals are overlapped, except at the 2-position. This signal was analyzable, and  $J_{2,3}$  (10.5 Hz) revealed the *2Z* configuration, which was also indicated by the chemical shift of the allylic methyl carbon (13.5 ppm). Similar chemical shifts are observed for a (*10Z,12E,14Z*)-isomer of the hexadecatrienyl acetate [14]. The observed chemical shifts of allylic and olefinic carbons in the trienyl moiety of Z2,E4,Z6,Z9-21:H coincide well with calculated values from the data of E2,E4,Z6,Z9-21:H, using an empirical rule found in the  $^{13}\text{C}$  NMR analysis of the trienyl acetates.

Synthetic E4,Z6,Z9-21:H and Z2,E4,Z6,Z9-21:H showed the same GC/MS and GC/FT-IR data as natural Comps. **II** and **IV**, respectively. On the contrary, E2,E4,Z6,Z9-21:H eluted faster than Comp. **IV** from the polar column of GC/MS analysis (RT 19.47 min) and also from the less-polar column of GC/FT-IR analysis (RT 21.34 min). In addition to these differences, the IR spectrum of E2,E4,Z6,Z9-21:H, including a characteristic absorption of C-H bending at  $996\text{ cm}^{-1}$  (Figure 4(f)), is clearly different from that of Comp. **IV**.

## 4. Conclusions

Four EAG-active components (**I** - **IV**) were found in the pheromone gland of Japanese and Finnish subspecies of the wood tiger moth. GC/MS and GC/FT-IR analyses revealed the following structures: Z3,Z6,Z9-21:H for Comp. **I**, E4,Z6,Z9-21:H for Comp. **II**, 1,Z3,Z6,Z9-21:H for Comp. **III**, and Z2,E4,Z6,Z9-21:H for Comp. **IV**. GC/FT-IR played an important role in determining the configuration of Comps. **II** and **IV**, novel bioactive unsaturated hydrocarbons of insects. IR spectra have not often been utilized for pheromone researches because species-specific pheromones are composed of multiple com-

ponents, and even major components are produced only at  $\mu\text{g}$  levels, at most. However, this study successfully measured IR spectra of even minor components in a crude pheromone extract using a new style of GC/FT-IR equipped with a turning zinc selenide disk cooled at  $-30^\circ\text{C}$ . Compounds eluted from a capillary column are fixed on the disk separately, and IR spectra are measured continuously. The most beneficial points are its sensitivity comparable to that of GC/MS and the fact that spectral data can be measured in a solid phase. The IR data are similar to familiarized spectra measured by KBr or liquid-film methods. In addition to the determination of a double-bond configuration, GC/FT-IR seems to be actively used for determining the functional groups in pheromone compounds [9] [15].

We carried out field tests in Japan and Finland; however, synthetic lures could not attract any males. While the subspecies-specific communication system has not been clarified, we expect that the four components found in the pheromone gland are important pheromone candidates because of their strong EAG activities. In order to attract the male by a synthetic lure in the fields, we will conduct further field tests and reexamine additional minor components in the pheromone extract.

Comps. **I - IV** are classified into Type II pheromone compounds, unsaturated hydrocarbons and their epoxy derivatives with a  $\text{C}_{17}$ - $\text{C}_{25}$  straight chain, which are biosynthesized from linoleic and linolenic acids included in dietary plants [2]. Z3,Z6,Z9-21:H has been identified from many moth species in the families of Erebiidae, Geometridae, and Crambidae, and 1,Z3,Z6,Z9-21:H has been identified from four Arctiinae species and one Geometridae species [5] [6]. This tetraene is proposed to be produced in oenocytes *via* the chain elongation of unsaturated fatty acids and additional desaturation [16] [17] [18]. In addition to the 1,3,6,9-tetraene, 6,9,12-triene [19] [20], 3,6,9,12-tetraene [19] [20], 3,6,9,11-tetraene [21], and an epoxy derivative of 6,9,11-triene [22] have been found as moth sex pheromones. Furthermore, this study proved the production of 4,6,9-triene and 2,4,6,9-tetraene by an Arctiinae species. The number of known Type II pheromone compounds is still quite limited. Considering the diversity of species in the above families, many novel components might be used for their mating communication. On the basis of this study, GC/FT-IR will strongly supplement the determination of structure using GC/MS analysis.

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