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(6*Z*,9*Z*,12*Z*)-6,9,12-Octadecatriene and (3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-icosatetraene, the novel sex pheromones produced by emerald moths

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ABSTRACT

(6*Z*,9*Z*,12*Z*)-6,9,12-Octadecatriene and (3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-icosatetraene, hydrocarbons unsaturated more highly than usual lepidopteran Type II pheromones, were identified from geometrid females of *Hemithea tritonaria* and *Thalassodes immissaria intaminata*, respectively. The triene was synthesized by a double Wittig reaction between hexanal and an ylide derived from (*Z*)-1,6-diiodo-3-hexene, and the tetraene was synthesized by a coupling between (*Z*)-3-undecenal and an ylide derived from (3*Z*,6*Z*)-1-iodo-3,6-nonadiene. Each synthetic compound attracted males of the corresponding emerald moths in a field.

Sex pheromones, which have been identified from about 600 lepidopteran species, are mainly classified into two major groups, that is, unsaturated C_{10}–C_{18} fatty alcohols and their derivatives (Type I) and C_{17}–C_{23} unbranched polyenyl hydrocarbons and their epoxides (Type II).[1] Type II compounds, which are biosynthesized from linolenic and linolic acids included in dietary leaves and universally have double bond(s) and epoxy ring(s) at the 3,6,9- or 6,9-positions, are secreted by female moths in highly evolved families, such as Geometridae, Lymantriidae, and Arctiidae. Since these families are composed of many species, further structurally modified components are necessary to develop diverse species-specific communication systems. Geometridae is classified into several subfamilies, such as Larentiinae, Ennominae, and Geometrinae. Sex pheromones have been exclusively studied in species in the former two subfamilies and not in those in the last subfamily, which includes many species, such as emerald moths with green wings.[2] This context indicates that it is highly possible to find new pheromone compounds from the species in Geometrinae. The Iriomote Islands (latitude 24°19′N), located in the subtropics, are inhabited by numerous insect species. In order to understand Type II pheromones in depth, we collected the male and female moths in this group using a light trap and analyzed their pheromone extracts using GC equipped with an electroantennogram detector (GC-EAD)[3] and GC–MS. Consequently, in addition to known unsaturated hydrocarbons, we could find novel Type II pheromones in the extracts.

In the GC-EAD analysis of pheromone gland extracts of *Hemithea tritonaria* females on a high-polar column,[4] two components (**X_1** and **X_2**) elicited remarkable responses from male antennae of this species (Fig. 1A). While the structure of **X_1** is still unknown, **X_2** was estimated to be a C_{18} triene because its retention time is almost the same as that of authentic (3*Z*,6*Z*,9*Z*)-3,6,9-octadecatriene. The detection of M^+ at *m*/*z* 248 on the GC–MS analysis[4] confirmed this speculation, but the mass spectrum of **X_2** (Fig. 1B) was different from that of the 3,6,9-triene.[1,5] Up to date, the 4,6,9- and the 6,9,11-triene have been known as pheromone components or attractants.[1,2] In the GC–MS analysis with the polar column, the t_R of **X_2** (13.04 min) was shorter than that of the authentic trienes with a conjugated dienyl structure, such as (6*Z*,9*Z*,11*E*)-6,9,11-octadecatriene (13.92 min).[6] These results indicate that **X_2** is an unconjugated C_{18} triene previously unidentified from any other insects. The double-bond positions of **X_2** were determined by diimide reduction of the pheromone extract [10 female equivalent (FE)].[7] GC–MS analysis of the crude products showed two octadecadienes and two octadecenes. The t_R (12.73 min) and mass spectrum of the major diene showed good agreement with those of authentic (6*Z*,9*Z*)-6,9-octadecadiene.[8] While we tried DMDS derivatization[9] of the produced monoenes to confirm the double-bond positions, we were not successful because of the limited amount of pheromone extract available. The monoenes, however, were produced in a ratio of approximately 1:2, and the t_{R}s (12.39 and 12.48 min) were the same as those of monoenes derived by the diimide reduction of (6*Z*,9*Z*)-6,9-octadecadiene, indicating that **X_2** was (6*Z*,9*Z*,12*Z*)-6,9,12-octadecatriene **1** with a symmetrical structure. Finally, the structure was confirmed by synthesis applying the

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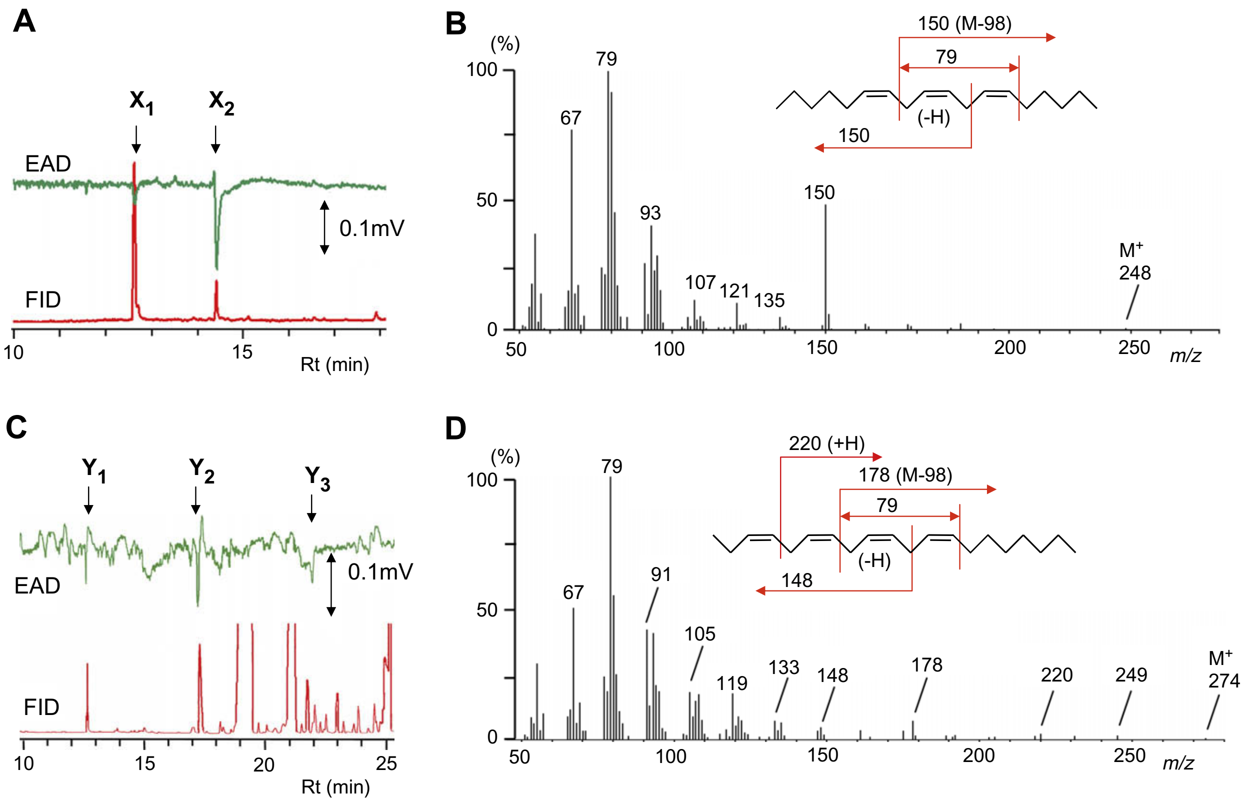

Figure 1. Analyses of the sex pheromone components of two Geometrinae species; (A) GC-EAD analysis of the pheromone gland extract of *Hemithea tritonaria*, indicating two EAG-active components (**X_1**: t_R 12.6 min and **X_2**: t_R 14.4 min), (B) mass spectrum of **X_2**, (C) GC-EAD analysis of the pheromone gland extract of *Thalassodes immissaria intaminata*, indicating three EAG-active components (**Y_1**: t_R 12.6 min, **Y_2**: t_R 17.2 min, and **Y_3**: t_R 21.9 min), and (D) mass spectrum of **Y_2**.

method reported by Pohnert and Boland[10] (Fig. 2A). Starting from 3-hexyne-1,6-diol, 1,6-diiodo-(*Z*)-3-hexene **2** was prepared.[6] With the phosphonium salt of **2**, bis(ylide) **3** was prepared and coupled doubly with hexanal to yield the objective (6*Z*,9*Z*,12*Z*)-triene **1**,[11] which showed the same GC–MS data as the natural pheromone component and strong EAG activity on an antenna of the *H. tritonaria* male.

On the other hand, pheromone gland extracts of *Thalassodes immissaria intaminata* females included many compounds, but its GC-EAD analysis consistently showed three active compounds (**Y_1**–**Y_3**, Fig. 1C). While the structures of **Y_1** and **Y_3** were not determined, M^+ at *m/z* 274 in GC–MS analysis indicated that **Y_2** was a C_{20} tetraene (Fig. 1D). The t_R of **Y_2** (15.50 min) is shorter than those of authentic 1,3,6,9- and 3,6,9,11-tetraenes, such as (*Z*3,*Z*6,*Z*9)-1,3,6,9-icosatetraene (16.66 min).[6] The mass spectrum of **Y_2** showed a characteristic base ion at *m/z* 79 as the compounds with a homoconjugated trienyl structure did. The double-bond positions of **Y_2** were also examined by diimide reduction of the crude pheromone extract (40 FE).[7] The short-time reduction (2 h) produced several products, including (3*Z*,6*Z*,9*Z*)-3,6,9-icosatriene (t_R 15.15 min),[8] and the long-time

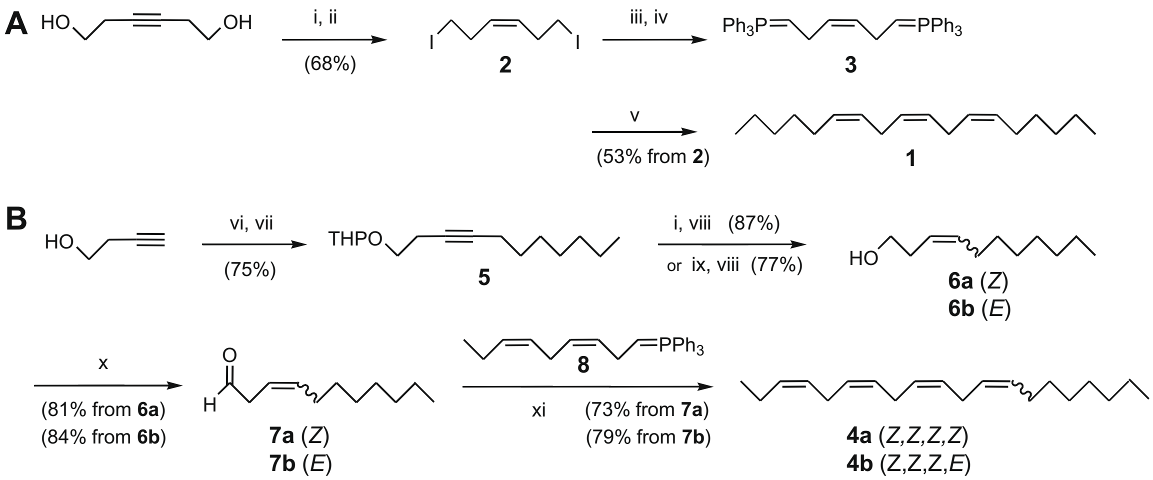

Figure 2. Synthetic routes to polyenyl hydrocarbons; (A) (6*Z*,9*Z*,12*Z*)-6,9,12-octadecatriene (**1**) and (B) (3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-icosatetraene (**4a**) and the (3*Z*,6*Z*,9*Z*,12*E*)-isomer (**4b**). Reagents: (i) H_2/Pd–$BaSO_4$–quinoline/MeOH; (ii) I_2–PPh_3/imidazole/Et_2O–MeCN; (iii) PPh_3/benzene; (iv) $NaN(SiMe_3)_3$/THF; (v) $CH_3(CH_2)_4CHO$; (vi) DHP/PTSA; (vii) (1) BuLi/THF, (2) $CH_3(CH_2)_7Br$/HMPA; (viii) PTSA/EtOH; (ix) Li/$EtNH_2$; (x) IBX/DMSO; (xi) ylide **8**/THF.

reduction (6 h) produced a mixture of icosane and four icosenes. The monones showed three peaks in a ratio of approximately 2:1:1 (14.22, 14.32, and 14.51 min) with the same t_Rs as those of monoenes produced by the reduction of the authentic 3,6,9-triene. We speculated that 9-icosene and 8-icosene (=12-icosene) might have similar chromatographic behaviors and $\mathbf{Y_2}$ might be (3*Z*,6*Z*,9*Z*,12*Z*)-3,6,9,12-icosatetraene **4a** or the (3*Z*,6*Z*,9*Z*,12*E*)-isomer **4b**.

Based on this speculation, we synthesized the two tetraenes starting from 3-butyn-1-ol (Fig. 2B). After protection of the alcohol as THP ether, 1-bromoheptane was coupled with it, giving 3-undecynyl compound **5**. Partial hydrogenation with Pd-$BaSO_4$-quinoline and deprotection of **5** gave (*Z*)-3-undecen-1-ol **6a**, and the Birch reduction and deprotection of **5** gave the (*E*)-isomer **6b**. By oxidation with 1-hydroxy-1,2-benziodoxol-3(1*H*)-one 1-oxide (IBX), these alcohols were converted into the corresponding β,γ-unsaturated aldehydes, **7a** and **7b**. Their NMR data[12] confirmed that the oxidation proceeded without isomerization to a stable (*E*)-2-enal.[13] These aldehydes were separately coupled with the ylide **8**, which was prepared from the phosphonium salt of an iodide derived from (3*Z*,6*Z*)-3,6-nonadien-1-ol,[6] to give the objective (3*Z*,6*Z*,9*Z*,12*Z*)-tetraene **4a** and its (3*Z*,6*Z*,9*Z*,12*E*)-isomer **4b**.[14] GC separation of the two geometrical isomers was not achieved under our conditions, and both isomers showed almost the same GC–MS data as the natural pheromone component. While GC–MS analysis of the diimide reduction of both **4a** and **4b** also showed the same 2:1:1 peaks of monoenes as those of the natural pheromone, the EAG activity of **4a** was higher than that of **4b** against the antennae of the *T. i. intaminata* males.

Although our next objective was to determine the structures of the other EAG-active components, $\mathbf{X_1}$, $\mathbf{Y_1}$, and $\mathbf{Y_3}$, we carried out a field evaluation of each synthetic hydrocarbon as a single component in the coppices of the Iriomote Islands from December 2008 to January, 2009. In the preliminary field trials in a season of low population density, traps baited with **1** and **4a** (each 0.2 mg dose on a white rubber septum) attracted several males of *H. tritonaria* and *T. i. intaminata*, respectively. No males were caught by the **4b** traps and the control one. This result suggests that **1** and **4a** are the principal components of their pheromones. Moreover, we identified **1** from a pheromone gland extract of *Pamphlebia rubrolimbraria rubrolimbraria* and (6*Z*,9*Z*,12*Z*)-6,9,12-icosadecatriene from that of *Maxates versicauda microptera* as an EAG-active component. The mass spectrum of the C_{20} analogue showed the characteristic ions of the 6,9,12-trienes at *m/z* M-98 and 150.[15] These two species are also emerald moths classified into Geometrinae.

Up to date, 1,3,6,9-tetraenyl, 3,6,9,11-tetraenyl, and 4,6,9-trienyl pheromones have been found from species in highly evolved families.[1,2] These components commonly possess a conjugated dienyl structure. Recently, (3*Z*,6*Z*,9*Z*,12*Z*,15*Z*)-3,6,9,12,15-tricosapentaene and pentacosapentaene were identified from three species in Pyralidae,[16] many other species of which solely produce Type I pheromones. Lures baited with these novel pyralid pheromone components only did not trigger male attraction, while they showed a synergistic effect on Type I pheromone compounds. The pentaenes may be biosynthesized from linolenic acid by two additional desaturation steps, such as the biosynthesis of arachidonic acid from linolic acid (desaturation at the ω12- and ω15-positions). The geometrid pheromones found in this study are new natural products, which can be derived from linolic or linolenic acids by only one desaturation at the ω12-position. It would be interesting to determine whether this limited desaturation is characteristic of the pheromones of Geometrinae species. The answer may be found through further research of many lepidopteran pheromones. In addition to analyzing the pheromone extracts of other species caught using a light trap, we are systematically synthesizing (6*Z*,9*Z*,12*Z*)-trienes and (3*Z*,6*Z*,9*Z*,12*Z*)-tetraenes with a C_{17}–C_{23} chain and accumulating their GC–MS data to confirm the characteristic fragmentations, shown in Figure 1. For the identification of natural pheromones, diagnostic ions are very useful. We are also using the synthetic compounds in field surveys to find new male attractants. The male attractants found by random screening tests may yield important information for pheromone studies. The results will be reported in due course.

Acknowledgments

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11. *Spectral data for synthetic (6Z,9Z,12Z)-6,9,12-octadecatriene* **1**: ^{1}H NMR: δ 0.98 (6H, t, $J = 7$ Hz), ~1.3 (12H, m), 2.06 (4H, dt, $J = 7, 7$ Hz), 2.81 (4H, dd, $J = 6, 6$ Hz), 5.37 (6H, m). ^{13}C NMR: δ 14.09, 22.61, 25.65, 27.25, 29.36, 31.56, 127.67, 128.28, 130.41.
12. *Spectral data for 3-undecenal*: (*Z*)-isomer **7a**, ^{1}H NMR: δ 0.88 (3H, t, $J = 7$ Hz), 1.27 (8H, m), 1.37 (2H, m), 2.03 (2H, dt, $J = 7, 7$ Hz), 3.19 (2H, ddd, $J = 7, 2, 2$ Hz), 5.54 (1H, dtt, $J = 11, 7, 2$ Hz), 5.71 (1H, dtt, $J = 11, 7, 2$ Hz), 9.66 (1H, t, $J = 2$ Hz). ^{13}C NMR: δ 14.11, 22.66, 27.65, 29.17, 29.21, 29.33, 31.82, 42.59, 117.95, 135.58, 199.84. IR (neat): 2925, 2854, 2719, 1728, 1466, 1385, 723 cm^{-1}. (*E*)-isomer **7b**, ^{1}H NMR: δ 0.88 (3H, t, $J = 7$ Hz), 1.27 (8H, m), 1.37 (2H, m), 2.06 (2H, dt, $J = 7, 7$ Hz), 3.11 (2H, dd, $J = 7, 2$ Hz), 5.49 (1H, dt, $J = 16, 7$ Hz), 5.62 (1H, dt, $J = 16, 7$ Hz), 9.65 (1H, t, $J = 2$ Hz). ^{13}C NMR: δ 14.10, 22.67, 29.10, 29.15, 29.17, 31.83, 32.74, 47.35, 118.97, 137.06, 200.51. IR (neat): 2925, 2854, 2717, 1728, 1466, 1383, 970 cm^{-1}.
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14. *Spectral data for synthetic 3,6,9,12-icosatetraene*: (3*Z*,6*Z*,9*Z*,12*Z*)-isomer **4a**, ^{1}H NMR δ: 0.88 (3H, d, $J = 7$ Hz), 0.98 (3H, t, $J = 7.5$ Hz), ~1.3 (10H, m), 2.06 (4H, m), 2.82 (6H, m), 5.37 (8H, m). ^{13}C NMR δ: 14.13, 14.28, 20.57, 22.69, 25.56, 25.63, 25.66, 27.28, 29.25, 29.31, 29.69, 31.89, 127.06, 127.57, 127.95, 128.00, 128.49, 128.52, 130.48, 132.02. (3*Z*,6*Z*,9*Z*,12*E*)-isomer **4b**, ^{1}H NMR δ: 0.88 (3H, d, $J = 7$ Hz), 0.97 (3H, t, $J = 7.5$ Hz), ~1.3 (10H, m), 1.98 (2H, dt, $J = 6.5, 6.5$ Hz), 2.08 (2H, dq, $J = 7.5, 7.5$ Hz), 2.76 (2H, m), 2.81 (4H, m), ~5.4 (8H, m). ^{13}C NMR δ: 14.12, 14.29, 20.56, 22.69, 25.54 (×2), 29.17, 29.21, 29.54, 30.47, 31.87, 32.59, 127.05, 127.87, 127.95, 128.00, 128.17, 128.18, 131.05, 131.94.
15. *GC–MS data for (6Z,9Z,12Z)-6,9,12-icosatriene*: t_R 14.66 min, *m/z* (relative intensity), 276 (3%, $[M]^+$), 178 (38%, $[M-98]^+$), 150 (44%), 80 (100%), 79 (98%).
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