Biosci. Biotechnol. Biochem., **70** (2), 508–516, 2006

Synthesis and Characterization of 3,13- and 2,13-Octadecadienyl Compounds for Identification of the Sex Pheromone Secreted by a Clearwing Moth, *Nokona pernix*

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Received September 30, 2005; Accepted October 31, 2005

Several geometrical isomers of 3,13- and 2,13-octadecadien-1-ols and their acetates were synthesized starting from 1,8-octanediol or 1,9-nonanediol utilizing acetylene coupling reactions. In addition to commercially available compounds, all geometrical isomers of each dienyl compound were analyzed by NMR and GC-MS to accumulate chemical data for studies of sex pheromones secreted from clearwing moths classified into the family Sesiidae of Lepidoptera. Although acetoxy derivatives of the 3,13- and 2,13-dienes showed almost the same mass spectra, the alcohols were distinguished by comparing the relative intensities of $[M-18]^+$ at m/z 248, indicating direct differentiation of the two positional isomers without derivatization. Furthermore, each geometrical isomer eluted from a high-polar GC column with a different retention time. Base on these data, a pheromone gland extract of a sesiid moth, *Nokona pernix*, was analyzed by GC-EAD and GC-MS, and two EAG-active components were identified, *viz.*, the (3*E*,13*Z*)- and (3*Z*,13*Z*)-isomers of 3,13-octadecadien-1-ol in a ratio of 9:1. In the field, the synthetic compounds mixed in 9:1 ratio attracted *N. pernix* males well, while a single component scarcely attracted the males. The number of attracted males peaked in the middle of June, and a small second peak was observed in August.

Key words: octadecadien-1-ol; octadecadienyl acetate; sex attractant; Lepidoptera; Sesiidae

Studies on insect mating behaviors have been providing fascinating themes for both evolutionary and behavioral ecologists, because the behaviors affect either premating isolation *via* recognition mechanisms of conspecies or reproductive success depending on the variation of the behaviors.[1)] Sex pheromone as a chemical cue is important in communication between sexes for species copulating in nocturnal conditions lacking light. In addition to the nocturnal species, whereas diurnal insects can possibly find their mating partners by means of visual cues, females of diurnal species in the family of Sesiidae (Lepidoptera), which have clear wings and mimic bees, also secret a species-specific sex pheromone to attract males. The first identification of sesiid sex pheromones was conducted by Tumlinson *et al.* from two pests of a peach tree, *Synanthedon pictipes* and *S. exitiosa*.[2)] Females of the former species secret a diunsaturated compound, (3*E*,13*Z*)-3,13-octadecadienyl acetate (E3, Z13-18:OAc), and those of the latter species secret its (3*Z*,13*Z*)-isomer (Z3,Z13-18:OAc). Referring to this identification, Nielsen *et al.* evaluated the synthetic pheromones and successfully attracted males of eight other sesiid species.[3)] Since then, pheromone and attractants of many sesiid species composed of 3,13- and 2,13-octadecadienyl compounds have been found in studies mainly conducted in the United States and Europe.[4–7)]

In Japan, about 40 sesiid species have been recorded,[8)] and attraction of thirteen species to the lures baited with 3,13-octadecadienyl compounds has been documented.[5–10)] In addition to these attractants, an effective method has been developed to disrupt the mating communication of two harmful pests in Sesiidae, *Synanthedon hector*[11)] and *S. tenuis*[*] as further agricultural application of their lures. Identification of pher-

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omone components in females, however, has never been published for any of the Japanese sesiid species because the larvae burrowing in stems of plants are not found readily. In order to understand the diversity of their communication systems, it is necessary to collect the insects and examine the components actually produced by the females. As a first step in studies on sexual communication systems of the diurnal species, we started to synthesize commercially unavailable isomers of sesiid pheromones and to accumulate analytical data on them to support chemical studies on the Japanese species. Utilizing these data, we tried to identify pheromone components of a sesiid moth, *Nokona pernix*, one of the most common species among the Japanese clearwing moths. While the larvae feeding on *Paederia scandens* (Rubiales: Rubiaceae), an ordinary garden weed, were not agricultural pests, they were rather easily found and could be subjected to future study on the interaction of chemical and visual cues in a mating communication system.

Materials and Methods

Instruments. ^{1}H and ^{13}C NMR spectra were recorded with a Jeol Alpha 500 Fourier transform spectrometer at 500.2 and 125.7 MHz respectively for $CDCl_3$ solutions containing TMS as an internal standard. GC-MS was conducted in the EI mode (70 eV) with an HP5973 mass spectrometer system (Hewlett-Packard, Wilmington, DE, USA) equipped with a DB-23 column (0.25 mm ID × 30 m, 0.25 µm, J & W Scientific, Folsom, CA, USA) or an HP-5 column (0.25 mm × 30 m, 0.25 µm, Hewlett-Packard). The column temperature program for the former column was 100 °C for 2 min, 20 °C/min to 175 °C, and 6 °C/min to 220 °C, and that for the latter column was 50 °C for 2 min, 10 °C/min to 160 °C, and 4 °C/min to 260 °C. The carrier gas was He, and the ion source temperature was 230 °C. The electroantennogram (EAG) activity of natural pheromone components was measured using a gas chromatograph equipped with an electroantennographic detector (GC-EAD).[12] The GC involved an HP-5890 Series II gas chromatograph (Hewlett-Packard) and the same DB-23 capillary column for GC-MS analysis. The column temperature program was also the same as the GC-MS with this column. The effluent from the column was split into two lines, which were led to a flame ionization detector (FID) and EAD at a ratio of 1:1. An antenna was excised at the base from the male moth, and a few distal segments were cut off. Each end of the antenna was attached to a droplet of a saline solution on an electrode of the EAD device such that the sensilla faced the airflow from the GC.

Synthesis of (Z,E)-isomers of 3,13- and 2,13-octadecadienyl compounds (Fig. 1A). By half bromination with HBr and protection of the residual hydroxyl group with 2,3-dihydropyran, 1,8-octanediol (**1a**) was converted into a THP ether of bromohydorin (**2a**), which was coupled with 1-hexyne to prepare a C_{14} acetylene (**3a**). Birch reduction of **3a** using Li metal yielded a monoene compound with an (*E*)-9-double bond (**4a**), and an iodo compound (**5a**) was synthesized by deprotection of **4a** and succeeding iodination of the recovered hydroxyl group by treatment with a complex of iodine and triphenylphosphine. Coupling of **5a** with a THP ether of 3-butyn-1-ol gave a C_{18} enyne (**6a**), which was reduced by catalytic hydrogenation over Pd-$BaSO_4$ poisoned with quinoline and deprotected to accomplish the synthesis of (3*Z*,13*E*)-3,13-octadecadien-1-ol (*Z*3, E13-18:OH). This alcohol was quantitatively acetylated with acetic anhydride in pyridine to yield (3*Z*,13*E*)-3,13-octadecadienyl acetate (Z3,E13-18:OAc). In the same manner, the corresponding 2,13-octadecadienyl compounds, Z2,E13-18:OH and Z2,E13-18:OAc, were synthesized starting from 1,9-nonanediol (**1b**) *via* a coupling reaction between a C_{15} iodo compound (**5b**) and a THP ether of propargyl alcohol.

Synthesis of (E,E)-isomers of 3,13- and 2,13-octadecadienyl compounds (Fig. 1B and C). The enyne compound (**6a**) was reduced to a dienyl compound with 3*E*,13*E* configuration by the Birch reduction, and deprotection of its THP ether produced E3,E13-18:OH. After deprotection, the triple bond at 2-position of the enyne compound (**6b**) was reduced to an (*E*)-double bond by treatment with $LiAlH_4$, and E2,E13-18:OH was obtained. These alcohols were also acetylated with acetic anhydride in pyridine.

Synthesis of (Z,Z)-isomer of 2,13-octadecadienyl compounds (Fig. 1D). The THP group of a C_{15} acetylene (**3b**) was converted to iodine, and the produced iodoacetylene (**7**) was coupled with a THP ether of propargyl alcohol to yield diyne compound (**8**). Both triple bonds were simultaneously reduced to (*Z*)-double bonds by hydrogenation on a Pd catalyst poisoned with quinoline. Removal of the THP group accomplished the synthesis of Z2,Z13-18:OH, which was acetylated to yield Z2,Z13-18:OAc.

Other compounds. E3,Z13-18:OAc, Z3,Z13-18:OAc, and E2,Z13-18:OAc were supplied by Shin-Etsu Chemical Co., Ltd., (Tokyo) with purity levels exceeding 98%. The corresponding alcohols were obtained by hydrolysis with methanolic NaOH.

Insects and pheromone extraction. About 40 overwintering *N. pernix* larvae were collected in Toyota City, Aichi Prefecture, Japan, in December 2003 and vernalized under winter conditions (8 ± 1 °C, dark condition) for one month. After vernalization, they were placed in a plastic cage under laboratory conditions (23 ± 1 °C, 13L:11D) until the emergence of the adults.

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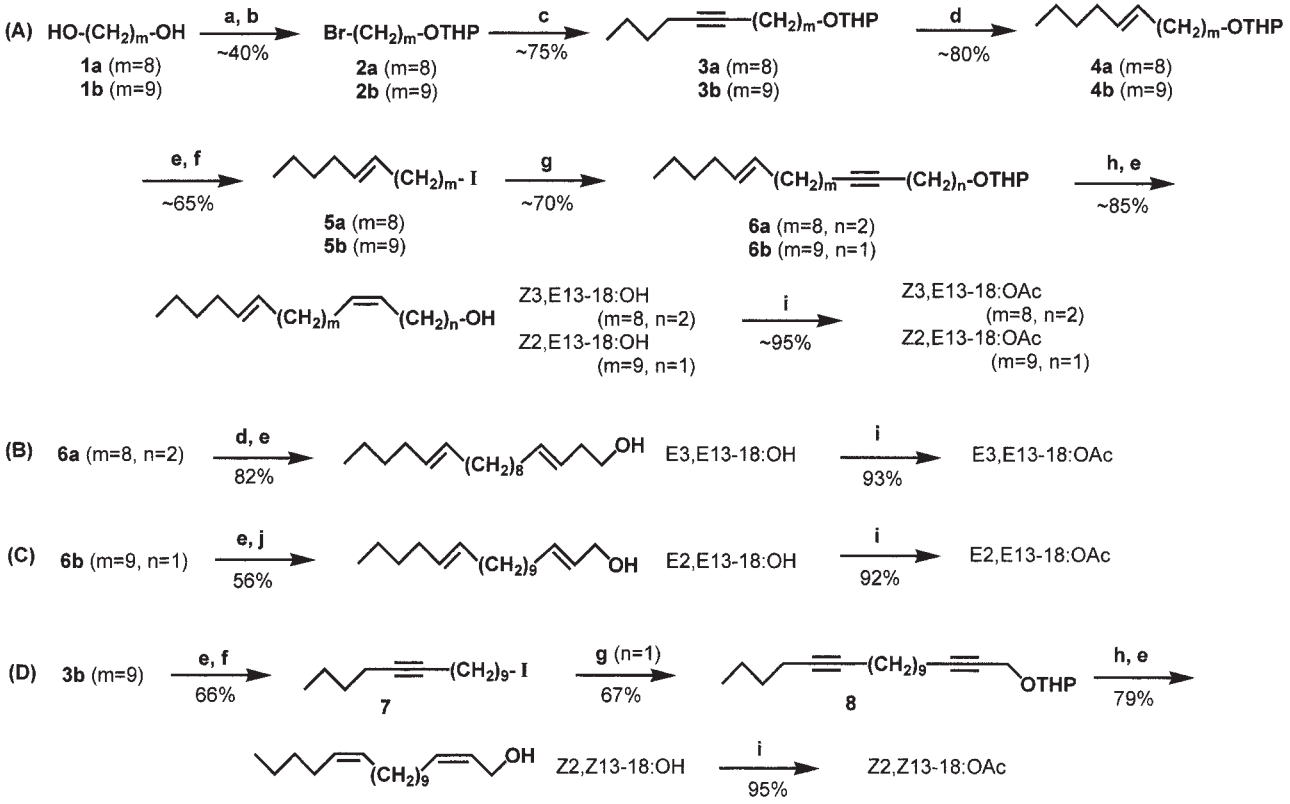

Fig. 1. Synthetic Routes to (A) (3*Z*,13*E*)-3,13- and (2*Z*,13*E*)-2,13-Octadecadien-1-ols and Their Acetates, (B) (3*E*,13*E*)-3,13-Octadecadien-1-ol and Its Acetate, (C) (2*E*,13*E*)-2,13-Octadecadien-1-ol and Its Acetate, and (D) (2*Z*,13*Z*)-2,13-Octadecadien-1-ol and Its Acetate.

(a) HBr (47%)/toluene/18 h, refluxing; (b) 2,3-dihydropyran/3 h, < 45 °C; (c) *n*-$C_4H_9C{\equiv}CLi$ (from *n*-$C_4H_9C{\equiv}CH$ and *n*-C_4H_9Li)/THF-HMPA/1 h, room temp.; (d) Li/$EtNH_2$/1 h, −5 °C; (e) *p*-TsOH/EtOH/3 h, refluxing; (f) I_2-PPh_3-imidazole/Et_2O-MeCN/1 h, 0 °C; (g) $LiC{\equiv}C(CH_2)_n$-OTHP (from $HC{\equiv}C(CH_2)_n$-OTHP and *n*-C_4H_9Li)/THF-HMPA/1 h, room temp.; (h) H_2/Pd (5%)-$BaSO_4$-quinoline/1 h, room temp.; (i) Ac_2O/pyridine/0.5 h, room temp.; (j) $LiAlH_4$/THF/3 h, refluxing.

Their sexes were determined at the adult stage based on their hair tuft.[8] The females showed calling-position when they were placed in direct sunlight; hence the abdominal ends of one- or two-day-old virgin females were excised after irradiation with direct sunlight and separately immersed in hexane (250 µl/female) for 5 min. The crude extracts of two female equivalents (FE) were combined and purified on a micro column packed with Florisil® (100–200 mesh, Floridin Co., 200 mg). Substances were eluted successively with 1 ml each of hexane and 5%, 10%, 20%, and 50% diethyl ether in hexane.

Field tests. The synthetic pheromone was examined in three neighboring fields: Kasugai City, Japan (the Experimental Farm of Meijo University) from May 25 to September 5, 2004 and August 15 to September 19, 2005; Nagoya (the Shiogama-guchi campus of Meijo University) from May 25 to July 30, 2004; and Seto City (a private coppice) from May 25 to July 30, 2004. A rubber septum (white rubber, 8 mm OD, Sigma-Aldrich Co., St. Louis, MO, USA) was used as a dispenser of the synthetic pheromone and placed in the center of a sticky board trap (30 cm × 27 cm bottom plate with a roof, Takeda Chemical Ind. Ltd., Osaka, Japan), which was set 1.5 m above the ground. The positions of the traps were rotated after counting the captured moths to eliminate any positional effect (Latin-square design),[13] and pheromone in the dispensers was replaced at the end of June. The replicated data on numbers of catches (Tables 4 and 5) were analyzed by means of one-way ANOVA; means were log-transformed to ensure the normality and homoscedasticity of the data. The means that had significant differences were separated using the Tukey–Kramer test. The level of significance in all tests was 5%.

Results

Synthesis of 3,13- and 2,13-octadecadieny compounds

There are several reports on the synthesis of sesiid pheromones.[2,14,15] We synthesized several geometrical isomers modifying the method published by Tumlinson *et al.*[2] Namely, the C_{18} 3,13-dienyl skeleton (3,13-18) was constructed with C_8, C_6, and C_4 synthons and the C_{18} 2,13-dienyl skeleton (2,13-18) with C_9, C_6, and C_3 synthons using acetylene coupling reactions, as shown in Fig. 1. Triple bonds were stereoselectively converted

Table 1. ^{1}H NMR Assignments for the Characteristic Signals of 3,13- and 2,13-Octadecadienyl Compounds

Position	Chemical shift (ppm), [coupling constant (Hz)]			
	(*Z*,*Z*)-Isomer	(*E*,*Z*)-Isomer	(*Z*,*E*)-Isomer	(*E*,*E*)-Isomer
3,13-Octadecadien-1-ol (3,13-18:OH)				
1 (2H, t)	3.64 [6.3][a]	3.62 [6.3][a]	3.63 [6.9][a]	3.62 [6.9][a]
2 (2H, dt)	2.32 [7.3][b]	2.26 [6.6][b]	2.32 [7.4][b]	2.25 [6.6][b]
3 (1H, dt)	5.36 [10.6][c]	5.37 [15.2][c]	5.35 [10.9][c]	5.37 [15.2][c]
4 (1H, dt)	5.56	5.55	5.56	5.55
5 (2H, m)	2.06	~2.02	2.06	~2.03
12, 15 (4H, m)	~2.02	~2.02	~1.96	~1.97
13, 14 (2H, m)	5.35	5.35	5.38	5.38
18 (3H, t)	0.90	0.90	0.89	0.89
3,13-Octadecadienyl acetate (3,13-18:OAc)[d]				
1 (2H, t)	4.06 [7.0][a]	4.06 [7.0][a]	4.06 [6.8][a]	4.06 [7.0][a]
2 (2H, dt)	2.37 [7.3][b]	2.31 [6.6][b]	2.37 [7.5][b]	2.31 [6.6][b]
3 (1H, dt)	5.34 [11.2][c]	5.36 [15.2][c]	5.32 [10.9][c]	5.36 [15.2][c]
4 (1H, dt)	5.51	5.51	5.50	5.50
2,13-Octadecadien-1-ol (2,13-18:OH)				
1 (2H, t)	4.18 [5.9][a]	4.08 [4.8][a]	4.18 [5.9][a]	4.07 [4.7][a]
2 (1H, dt)	5.53 [11.0][b]	5.63 [15.4][b]	5.54 [11.2][b]	5.65 [15.4][b]
3 (1H, dt)	5.60 [6.3][c]	5.70 [5.8][c]	5.59 [6.2][c]	5.70 [5.9][c]
4 (2H, dt)	~2.05	~2.02	~2.05	~2.04
2,13-Octadecadienyl acetate (2,13-18:OAc)[e]				
1 (2H, t)	4.61 [6.6][a]	4.50 [6.4][a]	4.62 [6.8][a]	4.50 [6.4][a]
2 (1H, dt)	5.52 [11.0][b]	5.55 [15.2][b]	5.52 [11.0][b]	5.56 [15.4][b]
3 (1H, dt)	5.64 [7.3][c]	5.77 [6.5][c]	5.65 [7.3][c]	5.76 [6.7][c]

[a] J_{1-2}. [b] J_{2-3}. [c] J_{3-4}. [d] CO_2CH_3, 2.04 ppm. [e] CO_2CH_3, 2.05–2.06 ppm.

Table 2. ^{13}C NMR Assignments for the Characteristic Signals of 3,13- and 2,13-Octadecadienyl Compounds

Position	Chemical shift (ppm)			
	(*Z*,*Z*)-Isomer[a]	(*E*,*Z*)-Isomer[a]	(*Z*,*E*)-Isomer[b]	(*E*,*E*)-Isomer[b]
3,13-Octadecadien-1-ol (3,13-18:OH)				
1	62.3	62.0	62.3	62.0
2	30.8	36.0	30.8	36.0
3	124.9	125.7	125.0	125.7
4	133.6	134.3	133.5	134.4
5	27.4	32.7	27.4	32.7
6–11	29.3–29.8	29.2–29.8	29.2–29.7	29.2–29.7
12[c]	26.9	26.9	32.3	32.3
13[d]	129.9	129.86	130.3	130.32
14[d]	129.9	129.88	130.3	130.34
15[c]	27.2	27.2	32.6	32.6
3,13-Octadecadienyl acetate (3,13-18:OAc)[e]				
1	64.0	64.2	64.0	64.2
2	26.8	32.0	26.8	32.0
3	124.2	125.0	124.2	125.0
4	133.0	133.6	133.0	133.6
2,13-Octadecadien-1-ol (2,13-18:OH)				
1	58.5	63.8	58.5	63.8
2	128.3	128.8	128.3	128.9
3	133.2	133.6	133.3	133.5
4	27.5	32.2	27.5	32.3
2,13-Octadecadienyl acetate (2,13-18:OAc)[f]				
1	60.4	65.4	60.4	65.3
2	123.3	123.7	123.2	123.7
3	135.5	136.7	135.5	136.7
4	27.6	32.3	27.5	32.3

[a] C-16, 32.0 ppm; C-17, 22.4 ppm; C-18, 14.0 ppm.
[b] C-16, 31.9 ppm; C-17, 22.2 ppm; C-18, 14.0 ppm.
[c,d] Chemical shift values might be reversed.
[e] CO_2CH_3; 171.1 and 21.0 ppm.
[f] CO_2CH_3; 170.9 and 21.0 ppm.

into (*Z*)-double bonds by hydrogenation over a Pd catalyst poisoned with quinoline, and (*E*)-double bonds by Birch reduction or by reduction with $LiAlH_4$.

The ^{1}H and ^{13}C NMR data for each synthetic isomer are shown in Tables 1 and 2 respectively, in addition to those of commercially available compounds. In these tables, signals from the 5-position to the 18-position of C_{18} 3,13-dienyl acetate (3,13-18:OAc), 2,13-dien-1-ol (2,13-18:OH), and 2,13-dienyl acetate (2,13-18:OAc) are not listed because their chemical shifts were almost same as those of 3,13-dien-1-ol (3,13-18:OH). Not only the coupling constants of olefinic protons and the chemical shifts of allylic protons in Table 1, but also the chemical shifts of olefinic and allylic carbons in Table 2 confirm the configurations of the double bonds. These ^{1}H NMR data coincided well with those of previous papers,[14] but there are some conflicts with published ^{13}C NMR assignments.[14]

GC-MS analysis of the synthetic standards

Table 3 shows retention times (t_R) and Kovats retention indices (KI) of the 3,13- and 2,13-dienlyl compounds on DB-23 and HP-5 capillary columns. Each 3,13-diene eluted faster than the corresponding 2,13-diene with the same functional group and configuration. This result coincides with the behaviors of monoenyl compounds, *viz.*, 3-dodecenyl acetate eluted faster than 2-dodecenyl acetate.[16] On the former polar column, four geometrical isomers of each compound were separable except for the (*Z*,*Z*)- and (*E*,*E*)-isomers of 2,13-18:OAc. It is noteworthy that the elution order of alcohols is different from that of acetates, *viz.*, *E*,*E*→*E*,*Z*→*Z*,*E*→*Z*,*Z* of 3,13-18:OH and *E*,*E*→*Z*,*E*→*E*,*Z*→*Z*,*Z* of 3,13-18:OAc. In the case of 2,13-dienyl compounds, this order is quite different, *viz.*, *E*,*E*→*Z*,*E*→*E*,*Z*→*Z*,*Z* of 2,13-18:OH and *Z*,*E*→*E*,*E* and *Z*,*Z*→*E*,*Z* of 2,13-18:OAc. Dienyl compounds with (*E*)-double bond(s) at the 3 and/or 13-positions eluted faster than those with (*Z*)-double bond(s), but an opposite effect was observed for the configuration at the 2-position of 2,13-18:OAc, as reported for monoenyl compounds.[16] When the (*E*)-13-double bond changed the configuration, KI values measured on the DB-23 column were universally increased by about 20. Conversion of the configuration at the 2- or 3-position caused different effects on the KI values depending on a functional group at the terminal position.

Geometrical isomers represented very similar mass spectra. Furthermore, it is difficult to differentiate the mass spectra of 3,13-18:OAc and 2,13-18:OAc. But 3,13-18:OH were easily distinguished from 2,13-18:OH by examining their mass spectra, particularly the relative intensity of $[M - H_2O]^+$ at m/z 248, *viz.*, 1% of E3,Z13-18:OH and 7% of E2,Z13-18:OH, as shown in Fig. 2.

Table 3. Retention Times (t_R) and Kovats Retention Indices (KI) of Synthetic 3,13- and 2,13-Octadecadienyl Compounds

		DB-23 (0.25 mm ID × 30 m, 0.25 μm)[a]				HP-5 (0.25 mm ID × 30 m, 0.25 μm)[b]	
		Alcohol		Acetate		Alcohol	Acetate
		t_R (min)	KI	t_R (min)	KI	t_R (min)	t_R (min)
3,13-Diene	(*Z*,*Z*)-Isomer	11.39	2684	11.01	2639	27.13[c]	29.80[c]
	(*E*,*Z*)-Isomer	11.09	2648	10.91	2627	26.98	29.86
	(*Z*,*E*)-Isomer	11.20	2661	10.84	2618	27.13	29.80
	(*E*,*E*)-Isomer	10.91	2627	10.74	2606	26.98	29.85
2,13-Diene	(*Z*,*Z*)-Isomer	11.67	2718	11.02	2640	27.40[c]	29.94[c]
	(*E*,*Z*)-Isomer	11.49	2696	11.17	2658	27.39	30.21
	(*Z*,*E*)-Isomer	11.46	2693	10.85	2619	27.39	29.92
	(*E*,*E*)-Isomer	11.30	2673	10.99	2636	27.36	30.19

[a]Column temperature: 100 °C for 2 min, 20 °C/min to 175 °C, and 6 °C/min to 220 °C.
[b]Column temperature: 50 °C for 2 min, 10 °C/min to 160 °C, and 4 °C/min to 260 °C.
[c]KI values of (*Z*,*Z*)-isomer are as follows: Z3,Z13-18:OH 2060, Z3,Z13-18:OAc 2182, Z2,Z13-18:OH 2073, Z2,Z13-18:OAc 2197.

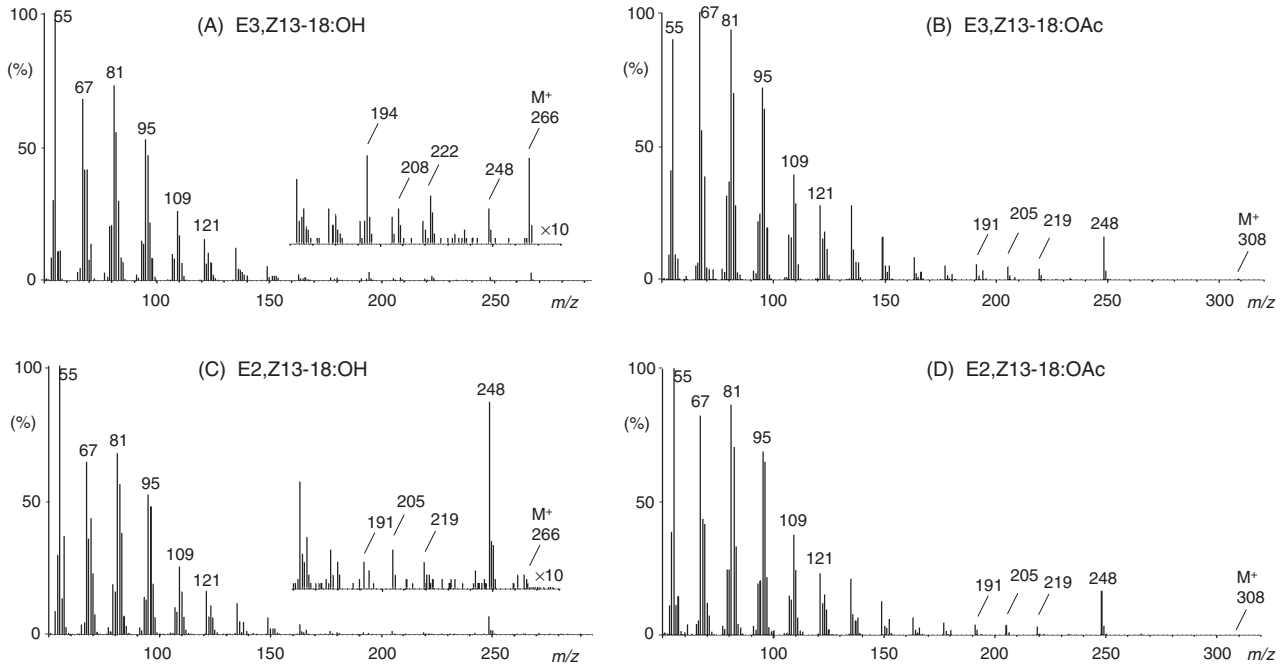

Fig. 2. Mass Spectra of (A) E3,Z13-18:OH, (B) E3,Z13-18:OAc, (C) E2,Z13-18:OH, and (D) E2,Z13-18:OAc.

Dehydroxylation, which creates this fragment ion, was probably accelerated by migration of the double bond at the 2-position. In addition to the different intensity of $[M - H_2O]^+$, fragment ions at m/z 194, 208, and 222 are characteristic of 3,13-18:OH, and those at m/z 191, 205, and 219 are characteristic of 2,13-18:OH.

Identification of pheromone components from Nokona pernix

Figure 3 shows the result of GC-EAD analysis of the crude extract (0.5 FE) on a DB-23 capillary column. The male antennae of *N. pernix* reproducibly responded to two components (**I** and **II**) with the following KI values: component **I**, 2649 (t_R 11.55 min), and component **II**, 2684 (t_R 11.84 min). These values are almost the same as those for synthetic E3,Z13-18:OH and Z3,Z13-18:OH respectively. Both of the EAG-active components were eluted with 10% diethyl ether in hexane from a Florisil column indicating their alcoholic functionality. The authentic alcohols were recovered in a 10% diethyl ether fraction, while the acetate derivatives were eluted with 5% diethyl ether in hexane, a less polar solvent. Their chemical structures were further confirmed by GC-MS analysis of the crude pheromone extract. Their spectra coincided more closely with that of 3,13-18:OH, and the values of t_R revealed their double-bond configuration again. The other eight GC-MS injections (1 FE each) showed their average titers in the pheromone glands

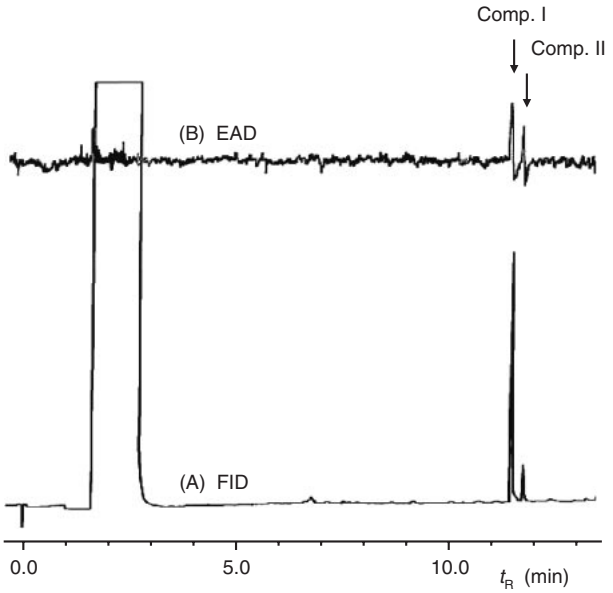

Fig. 3. GC Analysis (DB-23 Column) of a Crude Pheromone Extract of *N. pernix* (0.5 FE) with (A) a Flame Ionization Detector (FID) and (B) an Electroantennographic Detector (EAD).

The male antennae responded to two components, **I** (E3,Z13-18:OH, KI 2649, t_R 11.55 min), and **II** (Z3,Z13-18:OH, KI 2684, t_R 11.84 min).

to be 12.3 ± 10.8 ng/female for E3,Z13-18:OH and 1.4 ± 1.3 ng/female for Z3,Z13-18:OH. While large individual variation was observed for the total amounts of the two components, their mixing ratios were approximately uniformed at 9:1. In these pheromone extracts, no acetate components were detected.

Field attraction of Nokona pernix males

The attraction of the *N. pernix* males to various mixtures of synthetic E3,Z13-18:OH and Z3,Z13-18:OH was tested in Aichi Prefecture, Japan, where 695 males in total were captured with pheromone lures in 2004. Table 4 shows the results of Test I (May 29 to June 15) and Test II (June 15 to July 12) in Kasugai City. In both tests, 9:1 was the optimum ratio among the examined combinations of E3,Z13-18:OH and Z3,Z13-18:OH. Single E3,Z13-18:OH proved slightly attractive, whereas single Z3,Z13-18:OH attracted no males. The seasonal trend of male attraction is shown in Fig. 4. In that year, male flights started on May 29, and the number of captured males increased up to the middle of June. The numbers peaked on June 9 at Nagoya (16.0 males/day), June 14 in Kasugai City (51.0 males/day), and June 17 in Seto City (8.0 males/day). The first flight season ended in early July. In Kasugai City, a small second peak was observed in the middle of August. The activity of the geometrical isomers was examined in Kasugai City (August 15 to September 19, 2005). While the lures including the main natural-type component (E3,Z13-18:OH) and another unnatural-type isomer as a minor component caught males, no males were attracted to the lures including the minor natural-type component (Z3,Z13-18:OH) and another unnatural-type isomer as a major component, as shown in Table 5.

Table 4. Attraction of *N. pernix* Males by Traps Baited with Two Synthetic Pheromone Components, (3*E*,13*Z*)- and (3*Z*,13*Z*)-Isomers of 3,13-Octadecadien-1-ol[a]

Lure (mg/septum)		Captured males/trap[b] (mean ± SD)	
E3,Z13-18:OH	Z3,Z13-18:OH		
Test I (May 29 to June 15, 2004)			
1.00	0	0.5 ± 0.5	c
0.90	0.10	49.7 ± 41.9	a
0.50	0.50	2.7 ± 1.6	b
0.10	0.90	0.0 ± 0.0	c
0	1.00	0.0 ± 0.0	c
0	0	0.0 ± 0.0	c
Test II (June 15 to July 12, 2004)			
1.00	0	0.0 ± 0.0	b
0.99	0.01	0.0 ± 0.0	b
0.95	0.05	8.7 ± 6.5	a
0.90	0.10	20.7 ± 13.4	a
0.70	0.30	7.7 ± 3.8	a
0.50	0.50	1.3 ± 1.5	b
0	0	0.0 ± 0.0	b

[a]Tested at the Experimental Farm of Meijo University (Kasugai City, Japan). [b]Mean number of six traps (Test I) and three traps (Test II). Values followed by a different letter are significantly different at the 5% level by the Tukey–Kramer test.

Discussion

Since pheromone content in a female moth is very low, synthetic standards have played an important role in the analysis of natural components, particularly for the determination of the positions and configurations of double bonds.[17] This study confirms that full sets of the standards, including some commercialized compounds and others synthesized stereospecifically by the method shown in Fig. 1, are also meaningful for the identification of sex pheromones in sesiid moths. Four geometrical isomers of each C_{18} 3,13- or 2,13-dienyl compound (acetate and alcohol), whose structures were confirmed by NMR measurements (Tables 1 and 2), were separable on a polar GC column such as DB-23 (Table 3). Co-chromatography with the standards can estimate configurations of the double bonds in pheromone components, if the unsaturated positions are determined. The unsaturated position in monoene compounds can easily be determined by characteristic fragment ions derived from an adduct with dimethyl disulfide (DMDS),[18] and furthermore it has been reported that mass spectra of DMDS adducts of dieneyl compounds also inform the positions of two double bonds.[19] 3,13-18:OAc and 2,13-18:OAc, however, dominantly produced a mono-DMDS adduct at the common 13-position, and our trial did not succeed in recording the spectrum of a di-DMDS adduct with characteristic ions reflecting the double bond at the 3- or

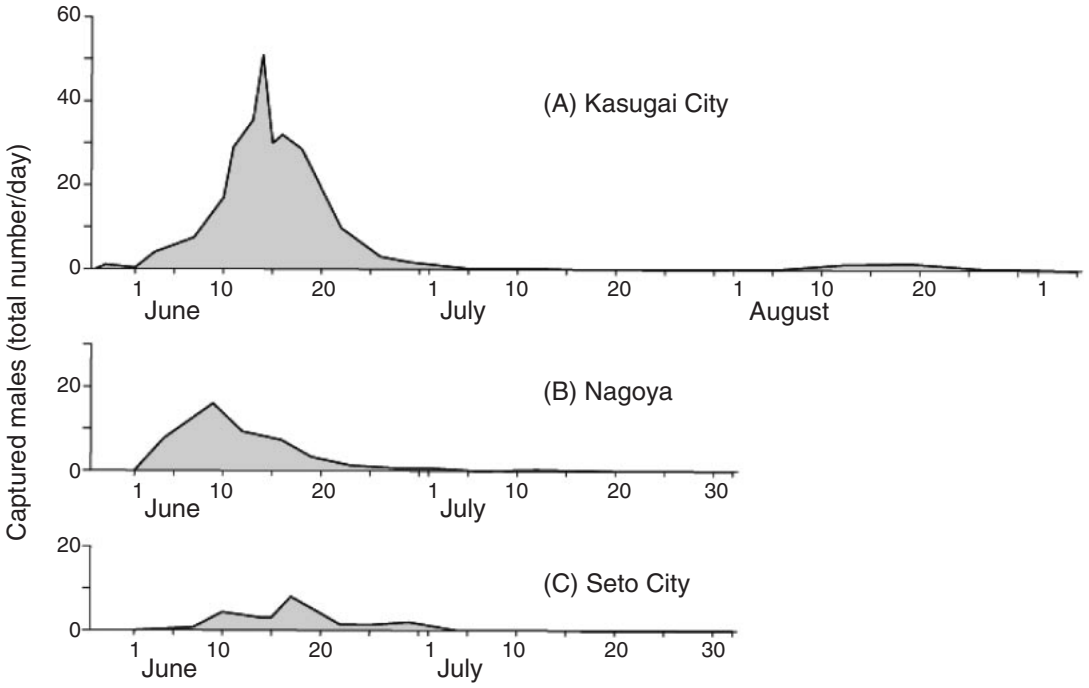

Fig. 4. Numbers of *N. pernix* Males Captured by Pheromone Traps per Day in Three Fields. Tests were conducted at three different fields in Aichi Prefecture in 2004.

Table 5. Attraction of *N. pernix* Males by Traps Baited with a Synthetic Lure Including Two Geometrical Isomers of 3,13-Octadecadien-1-ol[a]

Lure (mg/septum)				Captured males/trap[b] (mean ± SD)	
E3,Z13-18:OH	Z3,Z13-18:OH	E3,E13-18:OH	Z3,E13-18:OH		
0.90	0.10	0	0	4.7 ± 1.5	a
0.90	0	0.10	0	2.0 ± 2.6	ab
0.90	0	0	0.10	1.3 ± 1.2	ab
0	0.10	0.90	0	0.0 ± 0.0	b
0	0.10	0	0.90	0.0 ± 0.0	b
0	0	0	0	0.0 ± 0.0	b

[a]Tested at the Experimental Farm of Meijo University in Kasugai City, Japan from August 15 to September 19, 2005. E3,Z13-18:OH and Z3,Z13-18:OH are natural-type isomers and E3,E13-18:OH and Z3,E13-18:OH are unnatural-type isomers.
[b]Mean number of three traps followed by a different letter are significantly different at the 5% level by the Tukey–Kramer test.

2-position. Fortunately, 3,13-18:OH and 2,13-18:OH showed different mass spectra without any derivatization; thus $[M - H_2O]^+$ at m/z 248 was detected abundantly in that of the latter alcohol (Fig. 2). This result means that differentiation of their acetates can be accomplished by GC-MS measurement after hydrolysis.

Based on these results, pheromone components of a clearwing moth, *Nokona pernix*, were successfully clarified. On GC-EAD analysis, two distinct EAG-active components (**I** and **II**) were found in a crude extract of the pheromone glands (Fig. 3). Their chemical structures and a 9:1 mixing ratio in the pheromone extract were revealed by GC-MS measurements, which showed identical data to those of authentic E3,Z13-18:OH (Component **I**) and Z3,Z13-18:OH (Component **II**). Filed evaluations showed that the male moths were optimally attracted to these synthetic compounds when mixed in the same ratio as detected in the pheromone extracts (Tables 4 and 5). E3,Z13-18:OH and Z3,Z13-18:OH have been identified from sesiid females of two and four other species respectively. Furthermore, these alcohols were composed of attractants of 36 sesiid species, indicating their ordinariness in the mating communication systems of this insect group. But it is noteworthy that *N. pernix* is the first species secreting these two alcohols as pheromone components without any acetates.[5–7] Taxonomists have demonstrated a close relationship between the genera *Nokona* and *Paranthrene* in the family of Sesiidae.[20–22] These alcohols have been identified from some species in *Paranthrene*, and the pheromone of *N. pernix* supports the taxonomical results.

In spite of the large number of species in the family of Sesiidae, the structural diversity known to date for pheromonal compounds is limited; almost all of them have the *Z*,*Z* or *E*,*Z* configuration in the 3,13-18 or 2,13-18 skeleton.[5–7] This identification with *N. pernix* proposed an additional example. No compounds unsaturated at the 13-position with an *E* configuration have been found from sesiid species. Owing to visual cues, diversity of chemical cues might be not necessary for the mating communication of diurnal insects. It would be interesting to determine whether sesiid species have developed an enzyme to introduce the (*E*)-13-double bond, and hence we intend to utilize the synthetic compounds with a *Z*,*E* or *E*,*E* configuration in random screening tests to find new attractors.

Many Sesiid species inhabiting the temperate regions are single-brooded, and adults of *N. pernix* usually appear once a year in June. In a warm year, adults have been found in September, too.[8] Although it has not been confirmed that this species is fully double-brooded, since the numbers encountered in September were very low, field monitoring by pheromone traps has indicated that it might be partially double-brooded (Fig. 4). The mean temperature from March to May in 2004 in the Nagoya region, including our three fields, was 1.3 °C higher than in normal years. This warmer weather might have resulted in their earlier emergence, leading in turn to a small second brood. The pheromone traps brought an ecological finding. Furthermore, we are examining mating behavior with several kinds of dummies of *N. pernix* impregnated with the synthetic pheromone to analyze recognition ability of visual cues.

Acknowledgments

We thank Mr. D. Wakatsuki and Ms. Y. Katayama at Meijo University and Mr. H. Yamazaki at Kagoshima University, who kindly helped prepare cocoons of *N. pernix*, and Dr. F. Mochizuki at Shin-etsu Chemical Co., Ltd., Dr. A. Mochizuki at NIAES, Mr. K. Fukuzumi (in Nagoya) and Mr. H. Shibata of Kyoto University, who provided us with important information. This work was supported in part by research funds from Heart Co., Ltd. (Tokyo).

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