モモシンクイガ(Carposina niponensis Walsingham)の性フェロモン

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Sex Pheromone of the Peach Fruit Moth, *Carposina niponensis* WALSINGHAM (Lepidoptera : Carposinidae) : Isolation, Identification and Synthesis

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Two compounds were isolated as active components of the female sex pheromone of the peach fruit moth, *Carposina niponensis* WALSHINGHAM. The major components was identified as (*Z*)-7-eicosen-11-one, and the minor one was suggested to be (*Z*)- or (*E*)-7-nonadecen-11-one. (*Z*)-7-Tricosen-11-one was also isolated but this compound was not biologically active. (*Z*)- and (*E*)-isomers of 7-alken-11-ones of carbon numbers ranging from 17 to 23 were synthesized. Biological activity of synthetic (*Z*)-7-eicosen-11-one (compound A) and (*Z*)-7-nonadecen-11-one (compound B) as attractants for male moths was verified in the field. The activity was synergistically enhanced by mixing the two compounds in the ratio of 20 to 1 for compound A to compound B.

INTRODUCTION

The peach fruit moth, *Carposina niponensis* WALSINGHAM, a major economic pest of apple, peach, and other fruits in Japan, has been gradually increasing during recent years. Monitoring of populations of this species has depended entirely on field observations on injured fruits, since the moths are not captured by light traps. Therefore the establishment of a convenient monitoring system for this species by using a trap baited with a synthetic sex pheromone is desired. Observation on mating behavior and preliminary trials to determine the chemical properties of the female sex pheromone of the peach fruit moth were started in 1974 by one of us (K. H.). The presence of a sex pheromone in virgin females was confirmed and a method of laboratory bioassay for the sex pheromone was reported (HONMA, 1975).

MATERIALS AND METHODS

Insects. Most of the insects used as pheromone sources were obtained from injured apples collected in the field. Pupae were held in continuous light. Moths were segregated by sex within 24 hr after emergence and 2- or 3-day-old females were immersed in dichloromethane for lipid extraction. Male moths used for biological assay were held at 20°C under 16 hr photoperiodic regime for 2 to 3 days after emergence. Insects were also reared on young apple fruits at 25°C for a continuous supply of male moths for biological assay.

Biological assay. At the initial stage of this investigation, a behavioral response

assay based on wing vibration and short-range orientation of the male was conducted to monitor the activity of fractions (HONMA, 1975).

Electroantennograms (EAGs) were principally used for assaying various fractions and gas-chromatographic collections during the course of isolation. A male moth was held in a glass tube with his head extruded from the end of the tube and fixed to its edge with beeswax. A glass capillary electrode filled with an insect Ringer solution (NaCl, 0.75 g; KCl, 0.035 g; $CaCl_2$, 0.021 g; water, 100 ml) was inserted into the base of one antenna as an indifferent electrode, and the tip of the antenna was put into another glass capillary filled with the Ringer solution. Stimulation was made by blowing 1 ml of air containing the test substance into a continuous airflow (3 *l*/min) over the antenna. The responses appearing on an oscilloscope were photographically recorded.

Biological activity of the isolated substances and synthetic compounds was also examined in the field at the Fruit-Tree Research Station in Morioka during the flight season of the peach fruit moth. The sample was applied as a hexane solution on a piece of cotton twine (7 mm × 15 mm), which was held in a Takeda sticky trap (25 × 30 cm sticky board with a transparent plastic roof and two port entrances).

Column chromatography. Less than 500 mg of lipid materials was loaded on 30 g of Florisil (100 mesh) packed in a glass column of 15-mm diameter (i. d.) (CARROLL, 1961). Seven fractions were collected by stepwise elution with the following solvents: hexane, 50 ml; 5% ether in hexane, 120 ml; 15% ether in hexane, 150 ml; 25% ether in hexane, 150 ml; 50% ether in hexane, 150 ml; 2% methanol in ether, 150 ml; 4% glacial acetic acid in methanol, 150 ml.

Silicic acid impregnated with silver nitrate was used in column chromatography for further purification of active substances. Silicic acid, (100 g, Mallinckrodt, 100 mesh) was mixed with 20 g of $AgNO_3$ dissolved in about 100 ml of water, and activated at 120°C for 24 hr. The $AgNO_3$-silicic acid was mixed with half its weight of Celite-545 (Johns-Manville), and packed in a glass column of 15-mm diameter as a slurry with pentane. Less than 200 mg of lipid materials was loaded on 10-g $AgNO_3$-silicic acid. Three fractions were collected by stepwise elution with the following solvents: 1% ether in pentane, 50% ether in pentane, and ether.

Thin-layer chromatography. Thin-layer plates 20-cm square and 0.25-mm thick prepared with Kieselgel G (Merck) were used for checking separation of each fraction in Florisil column chromatography. The solvent system employed was hexane-ether-glacial acetic acid (70 : 30 : 1).

Thin-layer plates impregnated with silver nitrate, prepared by using a slurry composed of 25 g of Kieselgel and 50 ml of 10% aqueous $AgNO_3$ solution, were used for purifying synthetic materials. The solvent for development was benzene.

Lipids on thin-layer plates were detected as yellowish-brown spots which appeared in the presence of iodine vapor, or as yellow spots under UV-light after spraying with 0.05% dichlorofluoresein in ethanol.

Gas chromatography. An F & M 402 gas chromatograph equipped with a hydrogen flame ionization detector and an effluent splitter was mostly used. Glass columns of 1 to 1.5-m length and 3-mm i.d. were packed with 15% PEG–20M or 1% OV–1 on HMDS-treated Chromosorb W–AW (60—80 mesh).

For the collection of active fractions, about 5% of the column effluent was directed to the flame ionization detector and the remainder was introduced into a glass capil-

lary of 300-mm length and 1.8-mm i.d. The basal tip of the capillary was preheated to the oven temperature to produce a thermal gradient on the basal part of the glass tube. Preliminary trials by injecting 10-μg tetradecyl acetate indicated that more than 80% of the column effluent was collected on the inside wall of the capillary by this technique.

A Shimazu GC–5A gas chromatograph was used for analysing isomeric mixtures of sythetic compounds and for co-chromatography of isolated compounds to determine their geometry. A glass capillary column (0.24-mm i.d. × 30 m) coated with PEG–20M was used at 170°C.

Mass spectrometry. A Shimazu-LKB 9000 gas chromatograph-mass spectrometer interfaced with a computer data system was used. A spiral glass column of 2-m length and 3-mm i.d. was packed with 5% PEG–20M on HMDS-treated Chromosorb W–AW or 1% OV–1 on the same Chromosorb. Carrier gas (He) flow rate was 30 ml/min; separator temperature, 240—250°C; ion source temperature, 270—290°C; ionization voltage, 70 eV.

Mass fragmentography was conducted with the computer data system (Shimazu GC–MSPACK 300). Scan interval was 6 sec. All the data were plotted out on a digital plotter (Iwatsu, DPL–602).

Saponification, hydrogenation and ozonolysis. Saponification was conducted by 5% or 2.5% KOH in methanol containing a small amount of water. Lipid materials were refluxed for 25 to 30 min at 80°C. Unsaponifiable matter was extracted with ether by the routine method.

For hydrogenation, platinum black (Tokyo Kasei Co.,) was used as a catalyst. Hydrogen gas was bubbled into the sample solutions for 5 to 10 min at room temperature. Freshly distilled hexane was used as a solvent without further drying.

For micro-ozonolysis, ozone was bubbled into hexane solutions of samples held in a dry-ice-acetone bath. The hexane solutions of the resultant ozonides were immediately introduced into the GC–MS. Reductive cleavage of ozonides occurred in the injection port of the GC–MS instrument which was held at 240°C, and ozonolysis products were mass-spectrometrically analyzed.

RESULTS AND DISCUSSION

Isolation

Preliminary trials to determine the chemical properties of the sex pheromone showed that the active substance(s) was resistant to alkali hydrolysis. Activity was lost by treating samples with 2,4-dinitrophenylhydrazine, $LiAlH_4$ or $NaBH_4$, suggesting a carbonyl property of the active substance(s). Therefore, virgin female extract (ca. 3,000 female equivalents(FE)) was saponified with 5% KOH in methanol, and unsaponifiable matter was fractionated by a Florisil column. Behaviorally active substance(s) appeared only in the fraction of 5% ether in hexane. This fraction was purified by a column of $AgNO_3$-silicic acid. Behavioral response assay indicated the elution of active substance(s) by 50% ether in pentane. The active fraction was concentrated and injected onto a gas-chromatographic column of 15% PEG–20M at 185°. EAG analysis of collected effluents showed activity at two separate peaks in the chromatogram (Fig. 1A). Retention time relative to hexadecyl acetate was 1.30 for the earlier active peak and 1.83 for the later active peak. The two fractions corresponding to these two

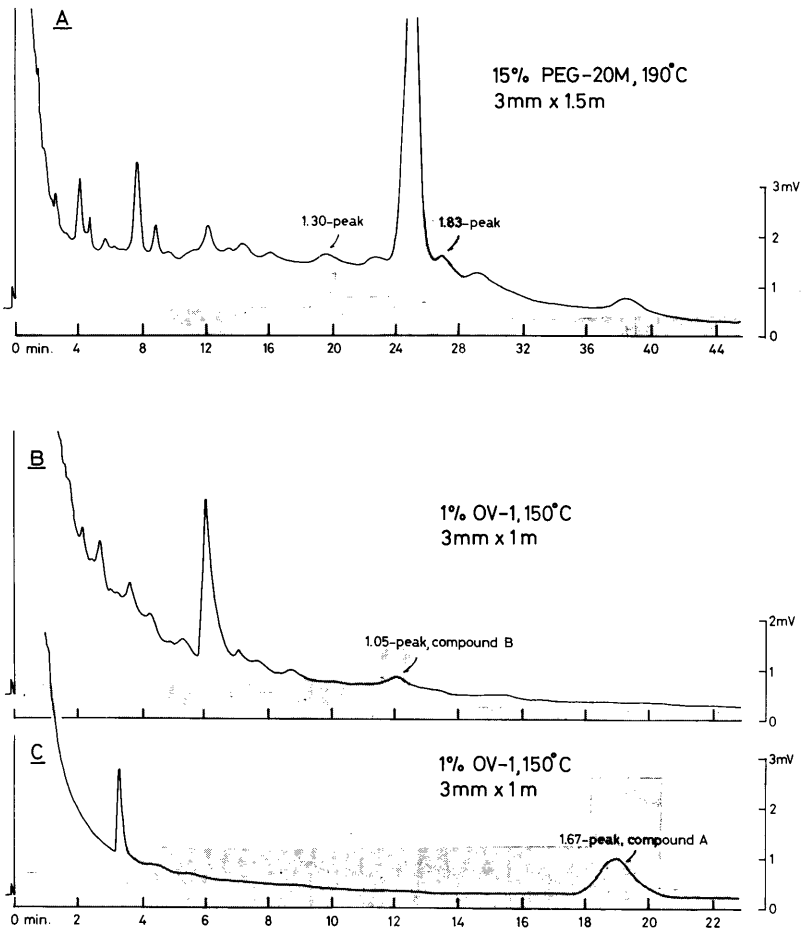

Fig. 1. Gas chromatograms of active fractions, and EAG monitorings of biological activity shown in shaded areas. A: 50% ether/pentane fraction analyzed by 15% PEG-20M column. B: Earlier active peak in the chromatogram A was further fractionated by 1% OV-1 column. C: Later active peak in the chromatogram A was also fractionated by 1% OV-1 column.

peaks were separately pooled, concentrated, and then injected onto a column of 1% OV–1 at 150°C. The 1.30-peak of the PEG–20M column was separated into at least 10 peaks by the OV–1 column, and an EGA active peak was found at retention time 1.05 (compound B) relative to hexadecyl acetate (Fig. 1B). The 1.83-peak of the PEG–20M column was separated into two peaks by the OV–1 column, and an active peak appeared at retention time 1.67 (compound A) relative to hexadecyl acetate (Fig. 1C).

A large proportion of the isolated compounds, A and B, was stored for structural analysis, and the remainder was used in a field assay to determine their activity as attractants. A few micrograms of compound A (exact amount not determined) loaded on a small piece of cotton twine attracted 10.8 males of the peach fruit moth per trap per night. Compound B was not active in the field, probably because of the very small

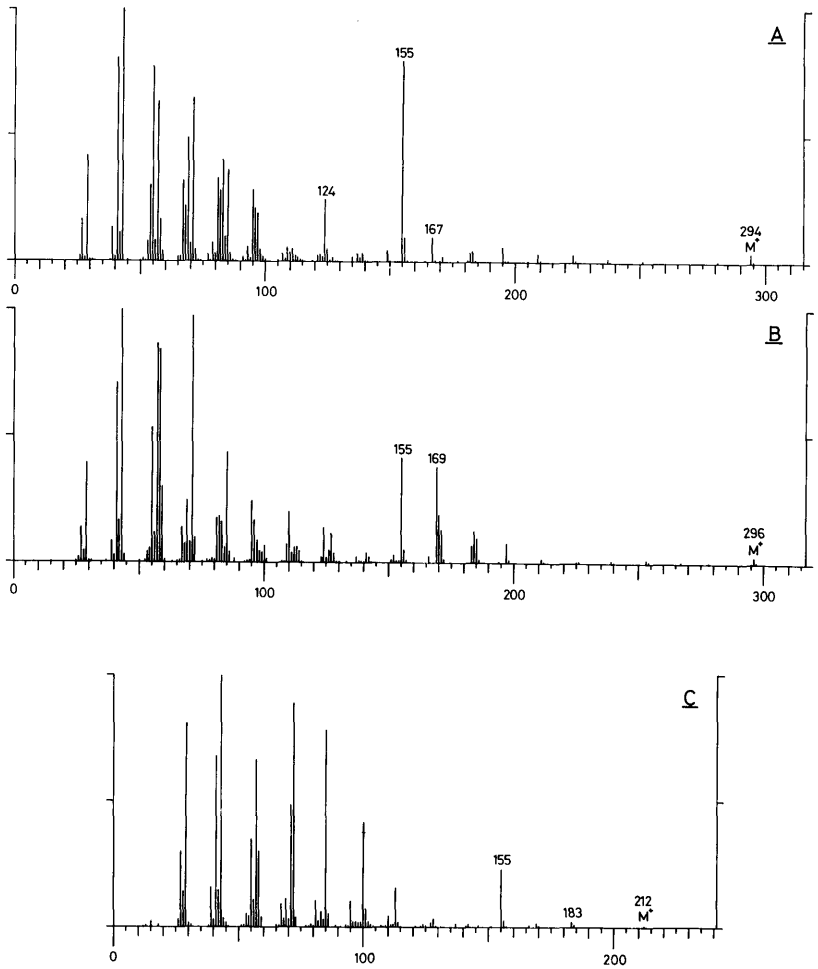

Fig. 2. Mass spectra of isolated compound A (A) and products of catalytic hydrogenation (B) and micro-ozonolysis (C). About 18 μg of compound A was isolated from the extract of 12,000 virgin females.

amount for the field assay.

Another batch of female extract (ca. 12,000 FE) was also saponified with 5% KOH, and unsaponifiable matter was chromatographed over a Florisil column. The fraction of 5% ether in hexane was subjected to a column of $AgNO_3$-silicic acid, and the eluate with 50% ether in pentane which contained the active substances was analysed by gas chromatography. Since the active fraction still contained a large amount of methyl esters of fatty acids, the fraction was again saponified with 2.5% KOH in methanol for 25 min at 80°C. Unsaponifiable matters were purified through a column of Florisil and active substances were collected in 5% ether in hexane. A part of this fraction was analyzed by mass fragmentography, and the remainder was further purified with a 1% OV-1 column at 190°C and then with a 15% PEG-20M column at 200°C.

The amount of compounds A and B isolated from the extract of 12,000 females was gas-chromatographically estimated as 18 μg and 2 μg, respectively.

Identification

The mass spectrum of compound A is shown in Fig. 2A. The highest mass was found at 294. The mass spectrum of hydrogenated compound A showed important ions at m/e (relative abundance) 296 (2), 169 (38), and 155 (42) (Fig. 2B). This spectrum was identical with that of synthetic *n*-eicosan-11-one, indicating compound A to be a monounsaturated eicosan-11-one.

Micro-ozonolysis of compound A produced two fragments. The smaller one coincided in its mass spectrum with *n*-heptanal, showing ions at m/e 96 (8), 86 (10), 81 (11), and 70 (44). The mass spectrum of the larger fragment (Fig. 2C) showed a M^+ ion at 212 (1) and a prominent ion at m/e 155 (23), suggesting the structure of 4-oxotridecanal. The 5-oxopentadecanal prepared by ozonolysis of (*Z*)-6-heneicosen-11-one indicated a M^+ ion at m/e 240 (1) and a prominent ion at 169 (34). Thus compound A was concluded to be (*Z*)- or (*E*)-7-eicosen-11-one.

The mass spectrum of compound B was not clear and suggested the presence of some impurities in this sample. However, careful inspection of several spectra of this sample showed prominent ions at m/e 195 (3—4), 167 (4—5), 141 (28—34), 124 (8—10), and 57 (100). Though no M^+ ion was detected ,these spectra suggested the structure of a homologue of compound A. The prominent ion at m/e 141 would indicate the ion $C_8H_{17}C{\equiv}O^+$ produced by an alpha-cleavage of a ketone. Presence of the ions at m/e 167 and 124 and their relative abundance supported the idea that the partial structure of compound B is the same as that of compound A.

Due to the small amount of compound B and the contamination with impurities, micro-ozonolysis was not conducted on this sample. After several runs for GC–MS analysis, the remainder of the sample was hydrogenated. The mass spectrum of the hydrogenated product showed prominent ions at m/e 197 (5), 184 (7), 169 (30), 156 (14), 141 (29), 124 (12), 96 (21), 85 (17), and 71 (100). This spectrum coincided with that of synthetic *n*-nonadecan-11-one with the exception of the M^+ ion at m/e 282 which was not detected in the sample.

These data suggested that compound B is a 19-carbon homologue of compound A.

Presence of a 23-carbon homologue of compound A

During the course of isolation of compounds A and B, a relatively large amount of a compound showing a mass spectrum of a higher homologue of compound A was detected. A mass fragmentograph of partially purified fractions indicated the presence of a compound (compound C) giving an ion at m/e 197. This compound was gas-chromatographically isolated (yield: 62 μg from 12,000 FE). Its mass spectrum showed pominent fragment ions at m/e 336 (3), 197 (46), 167 (12), 124 (27), and 43 (100). The mass spectrum of hydrogenated compound C showed the structure of tricosan-11-one: i.e., prominent ions at m/e 338 (3), 197 (31), 169 (30), and 43 (100). After micro-ozonolysis, compound C produced two fragments: *n*-heptanal and 4-oxohexadecanal. The mass spectrum of the latter product showed important ions at m/e 254 (1), 225 (3), 197 (19), and 72 (100). Thus, compound C is (*Z*)- or (*E*)-7-tricosen-11-one. Biological activity of this compound was not verified during the isolation.

Step No.

1 $Br(CH_2)_3OH \xrightarrow[H^+]{} Br(CH_2)_3O-$ 

2 $\xrightarrow[NH_3]{HC{\equiv}CNa} HC{\equiv}C(CH_2)_3O-$ 

3 $\xrightarrow[2.\ CH_3(CH_2)_5Br]{1.\ NaNH_2} CH_3(CH_2)_5C{\equiv}C(CH_2)_3O-$ 

4 $\xrightarrow{H^+/MeOH} CH_3(CH_2)_5C{\equiv}C(CH_2)_3OH$

5 $\xrightarrow{CrO_3/pyridine} CH_3(CH_2)_5C{\equiv}C(CH_2)_2CHO$---- (I)

6 $CH_3(CH_2)_nBr \xrightarrow[2.\ (I)]{1.\ Mg/ether} CH_3(CH_2)_nCHOH(CH_2)_2C{\equiv}C(CH_2)_5CH_3$

7 $\xrightarrow{CrO_3/pyridine} CH_3(CH_2)_nC(=O)(CH_2)_2C{\equiv}C(CH_2)_5CH_3$

8 $\xrightarrow{Lind.,\ H_2} CH_3(CH_2)_nC(=O)(CH_2)_2CH{=}CH(CH_2)_5CH_3$ (Z)

Fig. 3. Synthetic steps for (*Z*)-7-alken-11-ones (n=5,6,7,8,9, and 11).

Table 1. PROMINENT FRAGMENT IONS IN THE MASS SPECTRA OF SYNTHETIC (*Z*)-7-ALKEN-11-ONES

Compound	m/e (Relative abundance)
7-Heptadecen-11-one	252 (3), 195 (4), 167 (10), 124 (17), 113 (100), 43 (93)
7-Octadecen-11-one	266 (6), 195 (10), 167 (11), 127 (100), 124 (26), 57 (84)
7-Nonadecen-11-one	280 (4), 195 (10), 167 (9), 141 (100), 124 (25), 57 (92)
7-Eicosen-11-one	294 (5), 195 (7), 167 (10), 155 (100), 124 (28), 43 (91)
7-Heneicosen-11-one	308 (6), 195 (7), 169 (100), 167 (12), 124 (31), 43 (89)
7-Tricosen-11-one	336 (4), 197 (67), 195 (7), 167 (12), 124 (33), 43 (100)

Synthesis

Candidate compounds were synthesized through eight steps from 3-bromopropan-1-ol as a starting material (Fig. 3).

Grignard reagents of 1-bromoalkane of carbon numbers 6, 7, 8, 9, 10, and 12 were separately reacted with 4-undecynal; and 11-hydroxy-4-alkynes were obtained. These secondary alcohols were oxidized to corresponding ketones of carbon number 17 to 21 and 23, and the resultant 11-oxoalk-7-ynes were reduced by using Linder's catalyst to obtain (*Z*)-7-alken-11-ones. All the products after the step No. 6 were purified by Florisil columns. The yield in each step was as follows: 1, 78.0%; 2, 67.5%; 3, 61.5%; 4, 91.8%; 5, 17.0%; each of 6 to 8, about 80%.

A small amount of each (*Z*)-isomer was converted to the corresponding (*E*)-isomer by using azobisisobutylnitril and thiophenol (SGOUTAS and KUMMEROW, 1969). Geometrical isomers were purified by thin-layer chromatography on $AgNO_3$-Kieselgel. The final amount of each compound from 25 g of the starting material, 3-bromopropan-1-ol, was 50 to 120 mg for (*Z*)-isomers and 5 to 50 mg for (*E*)-isomers.

Prominent ions in the mass spectra of the six synthetic ketones of (*Z*)-configuration are shown in Table 1.

Geometry of compounds A and C

Synthetic (*Z*)- and (*E*)-7-eicosen-11-ones appeared at 17.8 min and 18.2 min, respectively, after the solvent front in chromatograms on a capillary column of 30-m PEG–20M at 170°C. Under the same conditions, (*Z*)- and (*E*)-7-tricosen-11-ones appeared at 28.2 min and 28.9 min, respectively. When compound A was co-chromatographed with an equimolar mixture of (*Z*)- and (*E*)-7-eicosen-11-ones, the peak height of a compound corresponding to the (*Z*)-isomer was increased. Similarly, compound C was co-chromatographed with a mixture of (*Z*)- and (*E*)-7-tricosen-11-ones, and the peak height of (*Z*)-isomer was found to be increased.

Thus, compound A and compound C are (*Z*)-isomers of corresponding ketones. The geometry of compound B is not known because no sample was available for this analysis.

Biological activity of synthetic compounds

EAG activity of 0.1 μg of each synthetic (*Z*)-isomer decreased in the following order (amplitude in mV): 7-eicosen-11-one (1.4), 7-nonadecen-11-one (1.2), 7-octadecen-11-one (0.9), 7-heptadecen-11-one (0.7), 7-heneicosen-11-one (0.6), and 7-tricosen-11-one (0.3). For (*E*)-isomers, the maximum response of 0.6 mV at 0.1 μg was obtained with 7-eicosen-11-one. The other five (*E*)-isomers were in the range from 0.2 to 0.3 mV at the same dose.

Field tests on the individual synthetic compounds indicated that (*Z*)-7-eicosen-11-one and (*Z*)-7-nonadecen-11-one were biologically active as attractants for males of the peach fruit moth. The mean numbers of male moths caught by traps baited with 50 μg each of (*Z*)-7-eicosen-11-one and (*Z*)-7-nonadecen-11-one on four successive nights were 10.0 and 6.5, respectively. (*Z*)-7-heptadecen-11-one and (*Z*)-7-octadecen-11-one also showed weak attractiveness for males. Other compounds, (*Z*)-7-heneicosen-11-one, (*Z*)-7-tricosen-11-one, and the six (*E*)-isomers, were not attractive in the field when individually tested. Another field trial indicated that a mixture of (*Z*)-7-eicosen-11-one and (*Z*)-7-nonadecen-11-one in the ratio of 20 : 1 showed about five-fold increase in attractiveness. The attractiveness of 50 μg of the mixture competed well with 10 virgin females of the peach fruit moth. Addition of (*Z*)-7-tricosen-11-one did not improve the activity of the mixture.

These results clearly indicate that (*Z*)-7-eicosen-11-one is the major component and (*Z*)-7-nonadecen-11-one is a minor component of the female sex pheromone of the peach fruit moth. Detailed results of examinations of biological activity of synthetic compounds will be reported elsewhere. The synthetic attractant pheromone, a mixture of the two unsaturated ketones, is potentially useful for monitoring and controlling the population of the peach fruit moth, which is economically important in fruit production in Japan.

Isolation and identification of the unsaturated ketones as the female sex pheromone of the peach fruit moth is particularly interesting, because most of the lepidopterous sex pheromones are esters, alcohols, and aldehydes. The only other example of a ketone is 6-heneicosen-11-one in the douglas-fir tussock moth, *Orgyia pseudotsugata* (McDonnough) (Smith et al., 1975). In this moth, both (*Z*)- and (*E*)-isomers were found attractive to males in a laboratory bioassay.

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