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HOST-RECOGNIZING KAIROMONES FOR PARASITIC WASP, *Anisopteromalus calandrae*, FROM LARVAE OF AZUKI BEAN WEEVIL, *Callosobruchus chinensis*

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Abstract—Host-recognizing kairomones for the stinging behavior of the parasitic wasp, *Anisopteromalus calandrae*, were identified on host azuki bean weevil larvae, *Callosobruchus chinensis* (L.). The kairomones were extracted with acetone from Chinese green beans, from which emerged wasps and host weevils had been removed. The kairomones are a mixture of triacylglycerols and fatty acids, each of which is separately active, and with no observable synergistic effect between them. These compounds are known to be constituents of an oviposition-marking pheromone of host azuki bean weevils. However, they differ from the previously reported saturated hydrocarbons and diacylglycerols of the kairomone that another parasitic wasp, *Dinarmus basalis*, uses for the host recognition of *C. chinensis*. Thus, *A. calandrae* and *D. basalis* selectively utilize different constituents of the oviposition-marking pheromone of *C. chinensis* as host-recognizing kairomones.

Key Words—*Anisopteromalus calandrae*, parasitic wasp, oviposition-marking pheromone, host-recognizing kairomone, azuki bean weevil, *Callosobruchus chinensis*.

INTRODUCTION

The azuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) is a serious pest of stored *Vigna* legumes, such as azuki beans (*V. angularis*), cowpea beans (*V. catiang*), and Chinese green beans (*V. radiata*). The physiology

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and ecology of this multivoltine insect has been widely researched, and it had also been used as a test insect against insecticides. Consequently, the chemical ecology of this weevil has been well studied, including that of oviposition-marking pheromones (Ohshima et al., 1973; Sakai et al., 1986; Honda and Ohsawa, 1990), a copulation-release pheromone (Tanaka et al., 1981), and oviposition stimulants (Ueno et al., 1990; Matsumoto et al., 1994; Tebayashi et al., 1995).

The parasitic wasp, *Anisopteromalus calandrae* (Howard) (Hymenoptera: Pteromalidae), is an important natural parasite of larvae of the maize weevils, *Sitophilus zeamais* Motschulsky, a major pest of stored corn, *Zea mays* L. (Williams and Floyd, 1971; Arbogast and Mullen, 1990; Smith, 1992). This wasp also attacks several other bruchid weevils, including azuki bean weevils (Utida and Nagasawa, 1949; Okamoto, 1971, 1972), rice weevils, *S. oryzae* (L.) (Press et al., 1984; Cline et al., 1985), granary weevils, *S. granarius* (L.) (Ghani and Sweetman, 1955; van den Assem et al., 1984), and Mexican bean weevils, *Zabrotes subfasciatus* Bohman.

Adults of *A. calandrae* are sexually dimorphic; females are entirely black and ca. 2.5 mm in length while males have partly milk-white abdomens and are ca. 2.0 mm in length. When an azuki bean or Chinese green bean containing host larvae is presented to a female, the following sequential behaviors are observed: the female walks and examines the surface of the bean with antennal tapping, bends her abdomen vertically, stings her ovipositor through the surface of the bean (stinging behavior), and lays an egg on the surface of the larva. Stinging behavior is only observed when females locate beans that contain host larvae.

We previously reported that another parasitic wasp, *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae), utilizes the oviposition-marking pheromone of its host azuki bean weevil as a host-recognizing kairomone (Kumazaki et al., 2000). This prompted us to search for similar kairomone(s) in *A. calandrae*, which paratitizes the same larvae. Here, we report that *A. calandrae* utilizes different constituents of the oviposition-marking pheromone of its host azuki bean weevils as host-recognizing kairomones.

METHODS AND MATERIALS

Host and Wasp. Azuki bean weevils and *A. calandrae* were obtained from colonies maintained at Fujii's laboratory, but which had been subsequently reared for several generations on Chinese green beans, *Vigna radiata* (L.) in Suzuki's laboratory. Although Chinese green beans are smaller than azuki beans (ca. 60 mg/bean), azuki bean weevils developed well in this host. The rearing method of the weevil host and *A. calandrae* is reported elsewhere (Kumazaki et al., 2000). Both sexes of wasps were introduced into plastic Petri dishes (9 cm ID $\times$ 20 mm high) containing Chinese green beans in which host larvae were present (ca. 18 days old). Both insect species were reared at 25°C, 60% relative humidity, and 16L:8D

photoregime. Wasps began to emerge 16–18 days after being exposed to infested beans. The newly emerged wasps (not sexed) were either assayed or used for further reproduction.

Prior to the bioassay, 1- to 3-day-old mated, female wasps were individually released into glass Petri dishes (18 mm ID × 13 mm high; mini P, Tsukuba Flat Co.) on an acrylic plate and conditioned overnight. Only females that showed stinging behavior (2 hr) when presented to a bean with host larvae were bioassayed.

Bioassay. In preliminary studies, the active kairomone(s) eliciting the stinging behavior could be extracted with acetone from beans containing host larvae. In addition, the behavior was observed only when the extract was applied to a fresh bean. Based on these observations, the bioassay in this study was conducted as follows. Two fresh green beans were used for each trial; one was treated with 5 μl [1 mg bean equivalent (BE)] of the test sample, and the other was treated with 5 μl of acetone as a control. After evaporation of the solvent, the beans were placed ca. 1 cm apart on a filter paper (3 × 3 cm) inside an 18-mm glass Petri dish. A mated, 2- to 4-day-old female wasp (described above) was released. Her stinging behavior was observed for 15 min, and the number of stings on each bean was recorded. The assay was repeated 15 times for each sample with a different female. Samples were diluted 10-fold with acetone when necessary. Total number of the stinging behaviors for each sample was compared to the control using Wilcoxon's sign test ($P < 0.05$) (Sokal and Rohlf, 1973).

Analytical Methods. Gas chromatography (GC) was performed with three capillary columns: a fused silica CP-Sil 19 CB column (25 m × 0.25 mm, 0.2 μm; Chrompack, Middelburg, The Netherlands), a heat-resisting MP65HT column (25 m × 0.25 mm, 0.1 μm; Quadrex, New Haven, Connecticut, USA) with a Hewlett-Packard 5890 (Palo Alto, California, USA) gas chromatograph, or a bonded-phase FFAP column (25 m × 0.25 mm, 0.25 μm; Quadrex) with a Hitachi 263-30 gas chromatograph (Hitachi, Ltd., Tokyo, Japan), with nitrogen as the carrier gas. The column temperatures were raised from 100 to 230°C at 4°C/min for analyses of fatty acid methyl esters (FFAP and CP-Sil columns; the injector and detector temperature was set at 250°C) and from 300 to 365°C (held for 10 min) at 5°C/min for analyses of triacylglycerols (MP65HT column; the injector and detector temperature was set at 400°C). Mass spectra were measured with a Hitachi M-80B GC mass spectrometer (MS) coupled with a Hitachi 367-0200 gas chromatograph equipped with an Al-clad methylsilicone capillary column (25 m × 0.22 mm, 0.10 μm, Quadrex) (EI, 70 eV). Mass spectra of methyl esters were obtained by GC-MS (column temperature: 120–270°C programmed at 4°C/min) and those of triacylglycerols were measured by the direct inlet method, using helium as the carrier gas. Thin layer chromatography (TLC) was performed with precoated silica gel $60F_{254}$ plates (E. Merck, Darmstadt, Germany). The developing solvent was a mixture of hexane–ether (7:1, v/v) or hexane–ether–acetic acid (70:30:1, v/v). Spots were detected by exposing the sample to iodine vapor or

spraying with 0.02% rhodamine 6G ethanol solution, followed by UV (365 nm; fluorescent spots). The infrared (IR) spectrum was measured with a JASCO IRA-1 spectrometer (Japan Spectroscopic Co. Ltd., Tokyo, Japan) as a liquid film.

Extraction and Fractionation of Kairomone(s). As described above, the kairomone(s) was (were) extracted with acetone only from beans that had contained host larvae. The selection of beans previously infected by host weevils is important as extracts from uninfected beans were not active. The kairomone(s) was (were) extracted from the green beans (2.6 kg, 30 mg/bean) with acetone (2.5 liters $\times$ 2) at room temperature for two days. The extract was dried, and the major part of the residue (40 of 50 g) was partitioned with ether and water. Half of the active layer (14.8 of 30 g) was chromatographed on a silica gel (SiO_2) column (125 g, 3.5 cm ID $\times$ 37 cm, Wako-gel C-200, Wako, Tokyo, Japan) with a stepwise elution of 1.5 liters each of hexane, hexane–ether (90:10 and then 50:50 v/v), ether, and methanol. Only the 10% ether in hexane fraction (10.8 g) was active, and this was checked by TLC (hexane–ether 7:3, v/v) to give three spots (R_fs $= 0.85$, 0.64, and 0.11), in which the last two spots (the R_f 0.11 spot was tailing) were considered to be triacylglycerols (TGs) and fatty acids (FAs), respectively. The fraction was submitted to Florisil column chromatography (WAK Florisil, Wako) to separate the seven fractions of lipids, according to the method of Carroll (1961). The column (150 g, 3.2 cm ID $\times$ 31 cm) was successively eluted with 1.5 liters each of hexane (fraction 1, 0.04 g), hexane–ether [95:5 (fraction 2, 1.0 g), 85:15 (fraction 3), 75:25 (fraction 4), and 50:50 (fraction 5, 1.2 g)], 2% methanol in ether (fraction 6, 1.6 g), and 4% acetic acid in ether (fraction 7, 3.2 g). Fractions 3 and 4 were combined, because they yielded spots with the same R_f value (0.65) in TLC. This combined fraction (named fraction I, 3.2 g,) and fraction 7 were separately active.

Extraction of Triacylglycerols and Fatty Acids from Chinese Green Beans. Because we used previously infected Chinese green beans, it is possible that the source of the TGs and FAs were derived from green beans. To investigate this possibility, 200 g of crushed fresh green beans were extracted with acetone (200 ml $\times$ 2) for 24 hr. After evaporation of the solvent, the residue was partitioned with ether and water. The ether layer (0.47 g) was submitted to SiO_2 column chromatography (5 g, 10 mm ID $\times$ 14 cm) with stepwise elution of hexane, hexane–ether (90:10, 50:50 v/v), ether, and methanol (50 ml each). TGs and FAs were eluted in 10% ether in hexane (390.7 mg) and analyzed with Florisil column chromatography (5 g, 10 mm ID $\times$ 14 cm). The column was successively eluted with 50 ml each of hexane, hexane–ether (95:5, 85:15, 75:25, and 50:50 v/v), 2% methanol in ether, and 4% acetic acid in ether.

Surface Hexane Washing from Previously Infected Green Beans. Approximately 40 g of green beans were placed into each of five 9-cm plastic Petri dishes, and ca. 150 adult host weevils were added to each dish. After eight days, all of the adult weevils had died and were removed with a sieve, but the hatched larvae had invaded the beans. The surfaces of these beans were washed with hexane for

30 sec, and the TGs and FAs were identified with TLC. The same total volume (200 g) of fresh green beans that had not been invaded by weevils were extracted with hexane for 30 sec as a control.

Synthesis of Triacylglycerols. TGs were synthesized by the following two methods. For method A, commercially obtained 1-monoacylglycerols or 1,3-diacylglycerols (Wako) were treated overnight at 60°C with the desired acyl chlorides in the presence of pyridine. The crude TGs were purified by SiO_2 column chromatography and eluted with 10% ether in hexane. The following nine TGs were prepared (acronyms and yields are given in parentheses): 1-oleoyl-2,3-dipalmitoylglycerol (1O2P3P, 26.4 mg, 94.1%), 1,3-dioleoyl-2-palmitoylglycerol (1O2P3O, 36.7 mg, 89.6%), 1,2-dioleoyl-3-palmitoylglycerol (1O2O3P, 22.1 mg, 76.5%), 1,2-dilinoleoyl-3-palmitoylglycerol (1L2L3P, 10.0 mg, 78.1%), 2-linoleoyl-1-oleoyl-3-palmitoylglycerol (1O2L3P, 4.6 mg, 59.6%), 1,3-dioleoyl-2-stearoylglycerol (1O2S3O, 35.3 mg, 50.0%), 2-linoleoyl-1-oleoyl-3-stearoylglycerol (1O2L3S, 10.4 mg, 73.2%), 2-linoleoyl-1,3-dioleoylglycerol (1O2L3O, 6.9 mg, 65.0%), and 1,2-dilinoleoyl-3-oleoylglycerol (1L2L3O, 4.4 mg, 55.2%).

For method B, three TGs were prepared according to Golding et al. (1993). 2,2-Dimethyl-1,3-dioxolane-4-methanol (100 μl, 0.8 mmol, Wako) was dissolved in 1.5 ml pyridine, to which excess acyl chlorides were added. This was stirred overnight at 60°C. The crude monoacylesters of 2,2-dimethyl-1,3-dioxolane-4-methanol were treated by SiO_2 column chromatography and eluted with 10% ether in hexane to yield pure esters. The acetonides of the esters were cleaved by treating with trifluoroacetic acid in 80% THF aqueous solution and stirring at 0°C in (Leblanc et al., 1986). The crude 1-monoacylglycerols were purified by SiO_2 column chromatography and then reacted with acyl chlorides (two molar equivalents) as described in A: 1-palmitoleoyl-2,3-dipalmitoylglycerol (1P'2P3P, 6.1 mg, 13.4%), 1-linoleoyl-2,3-dipalmitoylglycerol (1L2P3P, 30.3 mg, 17.4%), and 3-oleyl-1,2-distearoylglycerol (1S2S3O, 55.9 mg, 6.2%).

Some acyl chlorides were prepared by the reaction of the desired acids with oxalyl chloride. For example, palmitic acid (20 mg, 0.08 mmol) was dissolved in dry benzene (1 ml), to which equimolar oxalyl chloride was added, and the mixture was refluxed for 1 hr. After benzene and oxalyl chloride were removed, palmitoyl chloride (21.7 mg) was obtained. Palmitoleoyl chloride and stearoyl chloride were prepared in the same way.

RESULTS

Chemical Analysis of Active Fraction 7. Kairomonal activity was found only from fraction 7 (active at 10^{-2} BE) and Fraction I (active at 10^{-1} BE). No synergistic effect was observed between the fractions because their mixture did not

TABLE 1. KAIROMONAL ACTIVITY OF FRACTIONS

Sample	Dose (mg BE)	Total stinging behaviors (N)[a] Sample	Control	P	Activity
Acetone extract	10^{-2}	42	18	<0.025	+
	10^{-3}	42	24	NS	−
Ether layer	10^{-2}	51	8	<0.005	+
	10^{-3}	36	12	NS	−
Aqueous layer	10^{-2}	33	28	NS	−
	10^{-3}	27	47	NS	−
Fractions separated by SiO_2 column chromatography					
H fraction	10^{-1}	23	21	NS	−
10% E/H fraction	10^{-1}	27	1	<0.025	+
	10^{-2}	29	11	NS	−
50% E/H fraction	10^{-1}	12	14	NS	−
E fraction	10^{-1}	15	14	NS	−
MeOH fraction	10^{-1}	12	15	NS	−
Fractions separated by Florisil column chromatography					
Fraction 1	10^{0}	35	29	NS	−
	10^{-1}	16	4	NS	−
Fraction 2	10^{0}	13	13	NS	−
	10^{-1}	12	4	NS	−
Fraction 3 + fraction 4 (fraction I)	10^{-1}	36	4	<0.025	+
	10^{-2}	20	8	NS	−
Fraction 5	10^{0}	7	3	NS	−
	10^{-1}	3	4	NS	−
Fraction 6	10^{0}	5	12	NS	−
	10^{-1}	13	20	NS	−
Fraction 7	10^{-1}	26	9	<0.05	+
	10^{-2}	34	5	<0.025	+
	10^{-3}	19	31	NS	−
Fraction I + fraction 7	10^{-2}	36	12	<0.025	+
	10^{-3}	18	15	NS	−

[a] Total number of stinging behaviors for each bean during 15 min ($N = 15$). BE = bean equivalent; NS: not significant; H: hexane; E: ether; MeOH: methanol; AcOH: acetic acid. +: active; −: inactive.

enhance the activity (Table 1). In view of their TLC (hexane–ether 7:3, v/v) and Florisil column chromatographic behaviors, the chemicals were considered to be FAs ($R_f = 0.13$, tailing) and TGs ($R_f = 0.65$), respectively.

Fraction 7 was treated with diazomethane to yield methyl esters, which were analyzed by GC and GC-MS and compared with the authentic samples. Ten peaks were detected and identified (Table 2). The fraction 7 (3.2 g) obtained from 1.04 kg green beans, with emerged weevil removed, had a 10^{-2} BE (active dose) of 30.9 ng FAs. Thus, the absolute amount of each acid in 10^{-2} BE of fraction 7 was calculated on the basis of the GC peaks (Table 2). The most abundant acid was palmitic acid (40.8%). Kairomonal activity of authentic acids or their mixtures was assayed

TABLE 2. FATTY ACID COMPOSITION OF ACTIVE FRACTION

Fatty acid	Composition (%)	Absolute amount (ng)[a]
Lauric acid	0.1	0.03
Myristic acid	0.8	0.25
Pentadecanoic acid	0.1	0.03
Palmitic acid	40.8	12.61
Palmitoleic acid	0.7	0.22
Heptadecanoic acid	0.3	0.09
Stearic acid	6.7	2.07
Oleic acid	33.0	10.20
Linoleic acid	12.7	3.92
Linolenic acid	4.8	1.48

[a] Absolute amount in active dose (10^{-2} mg BE, 30.9 ng). BE = bean equivalent.

at doses of 10^0–10^{-3} BE (Table 3). Individual acids, or a mixture of five major acids, were inactive, but the complete mixture (10 acids) was active at a dose of 10^{-2} BE (natural ratio), whose activity was equal to those of the acetone extract and fraction 7.

TABLE 3. KAIROMONAL ACTIVITY OF AUTHENTIC ACIDS

Sample	Dose (mg BE)	Stinging behaviors (N)[a]		P	Activity
		Sample	Control		
Mixture of 10 acids[b]	10^{-1}	46	10	<0.025	+
	10^{-2}	25	9	<0.05	+
	10^{-3}	18	14	NS	–
Mixture of 5 acids[c]	10^{0}	6	5	NS	–
	10^{-1}	16	10	NS	–
	10^{-2}	4	15	NS	–
Lauric acid	10^{-1}	44	25	NS	–
Myristic acid	10^{-1}	20	11	NS	–
Pentadecanoic acid	10^{-1}	16	11	NS	–
Palmitic acid	10^{-1}	9	12	NS	–
Palmitoleic acid	10^{-1}	22	11	NS	–
Heptadecanoic acid	10^{-1}	26	29	NS	–
Stearic acid	10^{-1}	28	22	NS	–
Oleic acid	10^{-1}	14	13	NS	–
Linoleic acid	10^{-1}	14	11	NS	–
Linolenic acid	10^{-1}	24	16	NS	–

[a] Total no. of stinging behaviors for each sample during 15 min ($N = 15$). NS: not significant; +: active; –: inactive. BE = bean equivalent.
[b] A mixture of 10 acids (natural ratio).
[c] A mixture of palmitic, stearic, oleic, linoleic, and linolenic acids (natural ratio).

Chemical Analysis of Active Fraction I. Another active fraction (Fraction I) was comprised of TGs. Its IR spectrum showed the following frequencies: 2940, 2860, 1750 (ester C=O), 1470, and 1160 cm^{-1}. No OH stretching frequency was observed. In TLC, fraction I gave a spot with $R_f = 0.64$ (trioleoylglycerol, $R_f = 0.64$) (hexane–ether 7:3, v/v).

Fraction I showed the activity at a dose of 10^{-1} BE, which contained 309 ng of TGs. An aliquot of fraction I was hydrolyzed with KOH in ethanol–water overnight at room temperature. The acidic fraction was analyzed by GC and GC-MS, after methylation with diazomethane. The GC profile was very similar to that of the methyl esters of fraction 7. The aqueous layer, upon evaporation of ethanol and water after neutralization, was heated with potassium hydrogen sulfate ($KHSO_4$) to confirm the presence of glycerol, using the acrolein test (Hayashi, 1991). The characteristic pungent odor of acrolein confirmed the presence of glycerol.

The MS spectrum of the TGs (fraction I) was measured with the direct inlet method, and the following fragments were detected; *m/z* 57 (base peak), 237, 239, 261, 263, 265, 267 (fatty acid residues), 336, 338, 340, 549, 551, 571, 573, 575, 579, 601, 603, and 605 (residues leaving FAs from TGs) (Murata and Takahashi, 1973).

Direct GC analysis of the TGs involved in fraction I was conducted using an MP65HT capillary column. The column temperature was 300–360°C programmed at 5°C/min. A total of 13 peaks were observed from the gas chromatogram (Figure 1), which were separated on the basis of total number of carbons that constituted the FAs of the TGs. They were C_{48} (2.5%), C_{50} (33.1%), C_{52} (50.0%), and C_{54} (14.4%), compared with those of the synthetic TGs (Table 4). The major components of the TGs were PPO (24.2%) and POO (26.3%). In this column, TGs with the same total number of carbons as those of FAs, but with different carbon positions, could not be distinguished. For example, 1,2-dipalmitoyl-3-oleoylglycerol (1P2P3O), and 1,3-dipalmitoyl-2-oleoylglycerol (1P2O3P) yielded one peak.

TGs and FAs from Chinese Green Beans. Florisil column chromatography separated 153 mg TGs and 124.1 mg FAs from 5 and 15% ether in hexane and 4% acetic acid in ether fractions, respectively. These fractions were obtained from 200 g (ca. 60 mg/bean) of crushed green beans and were calculated as 45.9 μg TGs and 37.3 μg FAs per bean, or 765 μg TGs and 620.5 μg FAs per g beans.

TGs and FAs in Surface Hexane Washings of Green Beans from Which Weevils Were Removed. The hexane washing from the beans oviposited by weevils produced 72.5 mg or 38.5 μg/bean of hydrocarbons (the main component), TGs and FAs, which were identified with TLC (hexane–ether 7:3, v/v). Conversely, the hexane washing from the fresh bean control (no weevil oviposition) produced almost no residue (0.3 mg).

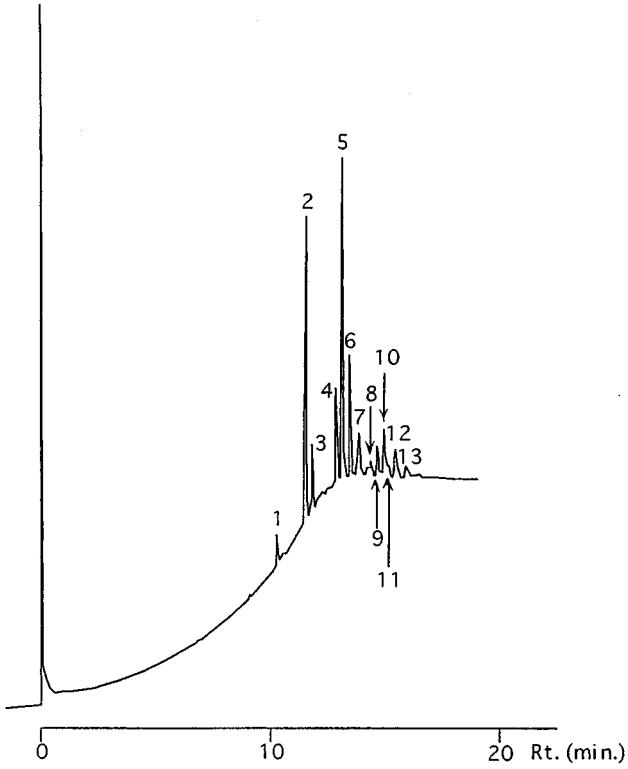

FIG. 1. Gas chromatogram of the active triacylglycerols (fraction I). Condition: MP65HT capillary column; 300–365°C (10 min) at 5°C/min.

DISCUSSION

The host-recognizing kairomones for the parasitic wasp, *A. calandrae*, were demonstrated to be comprised of fatty acids (FAs) and triacylglycerols (TGs). Both fractions separately elicited kairomonal activity, but no synergistic effect was observed. The activity of the FAs (fraction 7) was more than 10^{-2} mg BE (30.9 ng), whereas for the TGs (Fraction I) it was more than 10^{-1} mg BE (309 ng). Thus, fraction 7 was ten times more active than Fraction I. Fraction 7 was composed of 10 carboxylic acids, of which palmitic acid was main component (40.8%). Individual acids or a mixture of five major acids were inactive, but the complete acid mixture was active. This suggests that minor acids were required for activity.

The FAs constituting the TGs in fraction I were characterized, although their attaching positions to glycerol were not determined. The carbon numbers

TABLE 4. TOTAL CARBON NUMBERS OF FATTY ACIDS AND THEIR CONSTITUENTS, AND COMPOSITION OF EACH TRIACYLGLYCEROL INVOLVED IN FRACTION I

GC peak no.[a]	Total carbon no. of acid constituents	Acid constituent	Composition (%)
1	C_{48}	PPP'	2.5
		Subtotal	2.5
2	C_{50}	PPO	24.2
3		PPL	8.9
		Subtotal	33.1
4	C_{52}	PSO	10.6
5		POO	26.3
6		POL	10.1
7		PLL	3.0
		Subtotal	50.0
8	C_{54}	SSO	1.5
9		SOO	2.7
10		OOO	4.2
11		SOL	0.9
12		OOL	3.5
13		OLL	1.6
		Subtotal	14.4

[a] GC peak no. corresponds that of Figure 1. P: palmitic; P': palmitoleic; S: stearic; O: oleic; L: linoleic acids.

constituting the FA component of the TGs in this fraction ranged from C_{48} to C_{54}, in which C_{52} (50.0%) was the major components.

Azuki bean weevils secrete an oviposition-marking pheromone to prevent multiple oviposition for a bean, which consists of FAs, hydrocarbons, and TGs (Oshima et al., 1973; Sakai et al., 1986; Honda and Ohsawa, 1990) in a ratio of ca. 1:4.7:2. Of these three constituents, the FA fraction is composed of palmitic (21.6%), stearic (11.5%), oleic (45.5%), linoleic (10.7%), and linolenic (4.1%) acids and 7.4% other acids; the TG fraction comprises a mixture of C_{48} (1.0%), C_{50} (18.9%), C_{52} (47.7%), C_{54} (31.7%), and C_{56} (0.4%) (Honda and Ohsawa, 1990). In the present study, the active kairomones for *A. calandrae* were also comprised of FAs and TGs and are almost the same constituents as for those of the host pheromone. Although TGs and FAs were components of the control fresh green beans (765 and 620.5 μg/bean, respectively), the major fractions of the separated kairomones are thought to be insect-derived, because the amount of TGs and FAs obtained from previously infected green beans was calculated to be 3120 μg/g

beans, i.e., ca. 4–5 times more than those obtained from the control beans. In addition, hexane washing of surface green beans yielded almost no residue. Thus, the major part of the kairomones must be derived from the host pheromone.

We previously reported that other pteromalid wasp, *Dinarmus basalis*, utilizes the saturated hydrocarbons (and diacylglycerols) of the oviposition-marking pheromone of azuki bean weevils as a host-recognizing kairomone (Kumazaki et al., 2000). Thus, the two pteromalid wasps selectively utilize different constituents of the oviposition-marking pheromone of azuki bean weevils as host-recognizing kairomones. For *D. basalis*, TGs as well as diacylglycerols were active. Moreover, both saturated hydrocarbons and diacylglycerols (triacylglycerols) were required for activity in this wasp, whereas in *A. calandrae*, FAs and TGs were separately active, and no synergistic effect was observed between them. *D. basalis* mainly parasitizes azuki bean weevils, whereas *A. calandrae* also attacks other bruchid species. The narrower host range of *D. basalis* compared to *A. calandrae* might be because both constituents of its host pheromone are necessary to search for, or recognize, its host larvae. It would be interesting to know whether FAs and TGs can independently promote stinging behavior.

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