Interspecific Difference in *Venturia* Kairomones in Larval Feces of Four Stored Phycitid Moths

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Kairomone components of a parasitic wasp, *Venturia canescens* present in larval feces of four host phycitid moths, *Cadra cautella*, *Plodia interpunctella*, *Anagasta kuehniella* and *Ephestia ellutella* were purified to compare them. All components of the kairomones obtained were classifiable to seven groups of 2-acylcyclohexane-1,3-diones based on carbon numbers (from C₁₆ to C₂₀) and unsaturation. Although most compounds were common among species, quantitative differences of each component were noticed and were characteristic to species. *Cadra* and *Plodia* contained 2-palmitoylcyclohexane-1,3-dione as the major component. *Cadra* was noticed to possess 2-arachidoylcyclohexane-1,3-dione as an extra component. The other two species characteristically contained an unsaturated 2-acyl side chain as the main component, that is, *Anagasta* possessed 2-hexadecenoyl- and conjugated 2-hexadecadienoyl-cyclohexane-1,3-dione as the main components and *Ephestia* possessed 2-octadecenoylcyclohexane-1,3-dione.

INTRODUCTION

The ichneumon parasite *Venturia* (= *Nemeritis*) *canescens* GRAVENHOST searches for its host at close range in response to kairomone(s) present in the mandibular glands of host larvae *Anagasta kuehniella* ZELLAR (CORBET, 1971). Presence of the same or similar kairomone(s) is also suggested in other related phycitid moths: *Ephestia elutella* HÜBNER, *Cadra* (= *Ephestia*) *cautella* WALKER, and *Plodia interpunctella* HÜBNER (MUDD and CORBET, 1973). A total of 17 kairomone components, eight 2-acylcyclohexane-1,3-diones (hereafter abbreviated such as 2-acyl-CHDs) and nine 4-hydroxy-2-acyl-CHDs, are identified in the gland of *Anagasta* (MUDD, 1978, 1981, 1983). The presence of 4-hydroxy-2-oleoyl-CHD and 2-palmitoyl-CHD are suggested from their molecular formulae derived from mass spectrometry (MOSSADEGH, 1980).

Two major kairomone components were isolated from feces of *Plodia* larvae and identified as 2-palmitoyl-CHD and 2-oleoyl-CHD (KUWAHARA et al., 1983). 2-Palmitoyl- and 2-stearoyl-CHD were identified as the common kairomone components of *Plodia* and *Cadra*, and 2-arachidoyl-CHD was additionally identified in *Cadra* (NEMOTO et al., 1987). These results suggest that qualitative and quantitative differences of the kairomone components exist among the four species of host larvae. In the present work, kairomone components were compared among the four species including the two species partially reported previously.

MATERIALS AND METHODS

Insect and bioassay. Three of the four species of phycitid moths, except *Ephestia*, were reared with rice bran and *Ephestia* with corn flour mixed with dried yeast (8%), at 25°C and at ca. 60% RH under 16L–8D photo regimen. *V. canescens* were also fed available phycitid larva under the same conditions. All feces derived from each phycitid moth were soaked in *n*-hexane separately and kept in a cool place until workable amounts were collected.

The kairomonal activity was monitored as reported previously (NEMOTO et al., 1987). When wasps displayed so-called “cocking” and “jubbing” behaviour to the filter paper with test materials, this activity was evaluated as (+) and (++) respectively, and the impregnated test material was concluded to be “active”.

Purification of kairomone component. Silicic acid (SiO₂, Wako-Gel C-200) was used for all column chromatographies at each indicated solvent system. Kairomone components were purified from feces by the following procedure. Hexane extracts (1.4 kg) of *Anagasta* feces (ca. 21 l) was first separated by solubility in *n*-hexane at –20°C. The soluble fraction after evaporation of the solvent was again dissolved in methanol at the same condition to obtain the soluble fraction (825 g). The resultant oil was fractionally distilled under reduced pressure to give the distillate (516 g, below 176°C/1 mm Hg). The distillate was then repeatedly chromatographed (3 times) on SiO₂ columns, eluting with a hexane-ether mixture (once) and with a benzene-ethylacetate mixture (99:1) (twice). The active fraction (41 mg, active at 100 ppm) showed a single spot (*R_f*: 0.45, UV and I₂ vapour) on TLC (Merck Silicagel 60 F₂₅₄; 5 × 20 cm, solvent; benzene and ethylacetate (19:1)) and used for analyses.

The *Plodia* kairomone (55.1 mg, active at 100 ppm) from the feces (40 l) was similarly obtained. Partially purified kairomone (410 mg) was obtained from the same volume of *C. cautella* feces. A portion of the fraction was further purified by preparative TLC to give the pure kairomone (1 mg, active at 100 ppm).

Hexane extract (ca. 60 g) from *Ephestia* feces (8 l) was directly applied to a SiO₂ column and the active eluate was repeatedly chromatographed using the same mixture of solvent to obtain the active fraction (550 mg). A portion of the fraction was further purified by preparative TLC as mentioned above to give pure kairomone (1 mg active at 100 ppm).

GLC and GC-MS analysis. Four synthetic 2-acyl-CHDs (NEMOTO et al., 1987) were used as the internal standards: 2-lauroyl-, 2-myristoyl-, 2-palmitoyl-, 2-stearoyl- and 2-arachidoyl-CHDs. Gas liquid chromatography (GLC) was carried out by a Yanaco G-180FT gas chromatograph using a short glass column (3 mm id × 12.4 cm in length) packed with 10% FFAP on Chromosorb W (AW, HMDS, 80–100 mesh), at temperature programmed conditions (150–270°C or 200–250°C, 2°C/min or 3°C/min, as indicated in each chromatogram). Mass spectra (MS) at 70 eV were measured using a Hitachi RMU-6MG mass spectrometer by a direct-probe method. MS of all 2-acyl-CHDs give two groups of characteristic ions, one at *m/z* 139, 154 and 167 (base ion peak) commonly due to the molecular skeleton, and the other as a set of M⁺ and M⁺–H₂O innate for each corresponding compound. Therefore, each compound of 2-acyl-CHDs is assignable or at least classifiable without isolation, although ion-intensities are not always proportional to its abundance. Mass chromatograms (MS-Chro) using both groups of ions were obtained by a JOEL D-300 mass spectrometer coupled with a

Fig. 1. Gas-liquid chromatogram of purified kairomone. See the text for peak identification.

Yokogawa-Hewlett-Packard gas chromatograph 5880A at cyclic scanning (3 sec) equipped with a short glass column (4 mm id $\times$ 16 cm in length) packed with 10% FFAP coated on Chromosorb W (AW, HMDS, 80-100 mesh).

RESULTS

All kairomone fractions from the four species of phycitid moths indicated the same level of activity (active at 100 ppm) to the wasps. These kairomones consisted of two major and broad peaks (peaks 1 and 2) with a few minor peaks as shown in Fig. 1. The t_R values of the two main peaks were co-chromatographically identical to those of synthesized 2-palmitoyl- (as peak 1), and 2-oleoyl- and 2-stearoyl-CHD (as peak 2). In *Cadra* the t_R value of a third broad peak (peak 3) was identical to that of 2-arachidoyl-CHD.

Fig. 2. EI-MS spectrum of the diagnostic ion region for purified kairomone at 70 eV.

Although kairomone components of the four species consisted of the same peaks 1 and 2 by GLC, the quantitative ratios of these peaks and the feature of shoulder peaks were characteristic to each species. Peak 1 from *Ephestia* and *Anagasta* were broader than the corresponding peaks from *Cadra* and *Plodia*. Peak height of peak 1 with a distinct shoulder was almost the same as peak 2 in *Plodia*. Peak 1 was only half of peak 2 in *Cadra* which also had peak 3. The ratio of peak 1 to 2 in *Anagasta* was 2 to 5 and that of *Ephestia* was 1 to 3. Therefore, the proportion of peak 2 in kairomones decreased in the following order: *Ephestia* > *Anagasta* > *Cadra* > *Plodia*.

MS of the purified *Cadra* kairomone (Fig. 2) gave the following 6 sets of ions (m/z); 406–388, 378–360, 376–358, 350–332, 348–330 and 346–328. Each set corresponded to the respective ion sets of 2-acyl-CHD: arachidoyl-, stearoyl-, octadecenoyl-(possibly oleoyl-), palmitoyl-, hexadecenoyl-, and hexadecadienoyl-. Kairomones of the other three species (Fig. 2) consisted of almost the same ion-sets, except m/z 406–388. In *Anagasta* kairomone the most prominent ion m/z 328 was not accompanied by m/z 346, indicative of 2-hexadecadienoyl-CHD, and also in three species of kairomones m/z 356 was not accompanied by m/z 374, suggesting 2-octadecadienoyl-CHD. Percentages of each ion within a species were calculated as a criteria for comparison and are summarized in Table 1. Each species possessed its own characteristic mixture of 2-acyl-CHDs. *Cadra*'s kairomone was composed mainly of saturated 2-acyl-CHDs, judging from 406, 388, 378, 360, 350 and 332. In *Plodia* m/z 350 was the most prominent ion as in *Cadra*, however, 2-octadecenoyl- (2-oleoyl-) CHD (m/z 376–358) was the major component among 2-C₁₈-CHDs. Kairomones of *Anagasta* and *Ephestia* were composed

Fig. 3. MS-chromatogram of each ion-set (M^+ and M^+-H_2O). *C. cautella* (above) and *P. interpunctella* (below).

Fig. 4. MS-chromatogram of each ion-set (M^+ and M^+-H_2O). *A. kuehniella* (above) and *E. elutella* (below).

of olefinic 2-acyl-CHDs, the former of 2-hexadecadienoyl- and the latter of 2-octadecenoyl-CHD.

MS-Chro monitored by innate ion sets of each 2-acyl-CHD, as shown in Figs. 3 and 4, indicated reasonable t_R regions for each component. Peak 1 consisted of 2-palmitoyl- (m/z 350–332) and 2-hexadecenoyl-CHD (m/z 348–330). Nonconjugated 2-hexadecadienoyl-CHD (m/z 346–328) appeared by GLC as a distinct shoulder of peak 1, as noticed in *Plodia* and *Cadra* kairomones. The same ion set at m/z 346–328 found in peak 2 of *Ephestia*, *Anagasta* and *Cadra* was concluded as conjugated 2-hexadecadienoyl-CHD from the nature of the liquid phase used for GLC. *Anagasta*'s kairomone contained the largest amount of conjugated CHD, judging from the ion intensity. On the other hand, the 2-hexadecadienoyl-CHD of *Plodia* was nonconjugated. The other two species, *Cadra* and *Ephestia* contained both types of 2-hexadecadienoyl-CHDs: conjugated dienes as main components and nonconjugated diene as a minor.

Peak 2 of all kairomones except *Plodia* commonly consisted of 2-stearoyl- (m/z 378–360), 2-octadecenoyl- (m/z 376–358), and nonconjugated 2-octadecadienoyl-CHD (m/z 374–356) along with conjugated 2-hexadecadienoyl-CHD (m/z 346–328). Conjugated 2-octadienoyl-CHD was not detected in MS-Chro of any of the kairomones. The result agreed in all respects with those obtained from MS, and furthermore, missing

Table 1. Relative abundance of M^+ and $M^+ - H_2O$ ions by direct-probe mass spectrometry

Insect species	Side-chain structure						
	C_{16}			C_{18}			C_{20}
	Saturated	Monoene	Diene	Saturated	Monoene	Diene	Saturated
<i>A. kuehniella</i>	17 (8)	60 (23)	0 (34)	12 (13)	11 (22)	+ (+)	—
<i>E. elutella</i>	19 (15)	26 (18)	2 (11)	12 (9)	41 (33)	0 (14)	—
<i>P. interpunctella</i>	48 (35)	21 (14)	6 (14)	3 (0)	22 (18)	0 (19)	—
<i>C. cautella</i>	43 (22)	15 (6)	5 (17)	18 (12)	15 (18)	0 (20)	4 (5)

Numbers in parenthesis indicate $M^+ - H_2O$. + : Detected by MS-Chro.

C_{18} -diene consists of nonconjugated diene exclusively. Content of C_{16} -diene is mixture of conjugated and nonconjugated diene; for each species see the text.

ions such as m/z 346 of *Anagasta* and m/z 374 of *Cadra*, *Plodia* and *Ephestia* were detected clearly in reasonable t_R regions. 2-Arachidoyl-CHD (m/z 406–388) of *Cadra*, on the contrary, was not detectable in MS-Chro, possibly due to its low intensity. MS and MS-Chro revealed that the kairomone of each species was composed of 7 groups of compounds as listed in Table 1. Side chain consisted of saturated, monoenoic or dienoic acyl group with carbon numbers varying from 16 to 20, though most components were found among all four species. *Cadra* and *Plodia* characteristically contained 2-palmitoyl-CHD as a major component. On the other hand, the other two species possessed olefinic 2-acyl-CHDs as a major component, that is, *Anagasta* contained 2-hexadecenoyl- and conjugated 2-hexadecadienoyl-CHD and *Ephestia* 2-octadecenoyl-CHD.

Based on MS-Chro, the peak 2 of *Anagasta* by GLC was concluded mainly to be a conjugated 2-hexadecadienoyl-CHD, of *Ephestia* to be 2-octadecenoyl-CHD, of *Cadra* to be an equal-amount mixture of 2-stearoyl- and 2-octadecenoyl-CHD, and of *Plodia* to be 2-octadecenoyl-CHD. Presence of 2-arachidoyl-CHD was a characteristic component in *Cadra*, and *Plodia* possessed no detectable amount of conjugated 2-hexadecadienoyl-CHD. These points distinguish the kairomones between the two native species. The difference between the two invaded (or possibly invading this country) species was noticed in the composition of olefinic 2-acyl-CHDs; *Anagasta* contained the largest amount of 2-hexadecenoyl- and conjugated 2-hexadecadienoyl-CHD, and *Ephestia* the largest quantity of 2-octadecenoyl-CHD. Thus the kairomone composition was also found to be different among the four species of moths, judging from the ion-intensities of MS and also the results of GLC. The presence of nonconjugated 2-hexadecenoyl-CHD in two species of the phycitid moths was newly detected.

DISCUSSION

From the mandibular glands of host larvae of *Anagasta* 8 2-acyl-CHDs have been identified along with 9 4-hydroxy-2-acyl-CHD: 5 2-hexadecenoyl-CHDs (7-Z-, 9-Z-, 10-E-, 11-Z-, 14-E-), one 2-hexadecadienoyl-CHD (12-Z-, 14-E-), one 2-octadecenoyl-CHD (Z-9-) and one 2-octadecadienoyl-CHD (9-Z-, 12-Z-) (Mudd, 1978, 1981, 1983). The present and previous studies demonstrated presence of similar components of the kairomone in all larval feces of host species. These facts do not contradict each other, because feces may have a chance to be contaminated with mandibular secretions, when

larval behaviour of tunnel cleaning is considered. Feces may also be contaminated with silken webs which are known to be kairomonally active.

These compounds all belong to 2-acyl-CHDs. However, none of the 4-hydroxy-2-acyl-CHDs have been detected in fecal extracts. This may be attributable to the purification procedure, especially to fractional distillation because high boiling 4-hydroxy-2-acyl-CHDs might easily be wiped out by the distillation process. In fact, when kairomone of *Ephestia* was purified without using the fractional distillation procedure, a SiO₂ column eluate of the more polar fraction was also noticed to be active. Therefore, the results in this study again are not contradictory to reported results on *Anagasta* (MUDD, 1978, 1981, 1983).

Although kairomone components of the four phycitid moths differed quantitatively among each other, they were roughly classifiable into two groups. That is, the native phycitid moths in Japan, *Cadra* and *Plodia* possess similar components with the most abundant being 2-palmitoyl-CHD. But *Cadra* was also noticed to possess 2-arachidoyl-CHD as an extra component. On the other hand, the other two invaded (or supposedly invaded) species possess unsaturated 2-acyl-CHDs as a major component.

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