Effect of Kairomones in the Host Searching Behavior of *Apanteles kariyai* WATANABE (Hymenoptera: Braconidae), a Parasitoid of the Common Armyworm, *Pseudaletia separata* WALKER (Lepidoptera: Noctuidae). II. Isolation and Identification of Arrestants Produced by the Host Larvae

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Arrestants which keep *A. kariyai* in the host habitat were isolated from the exuviae of the host larvae and their structures were elucidated by a combination of MS, PMR and CMR spectra. The chemical structures of the arrestants were found to be a series of 2,5-dialkyl-tetrahydrofurans. Impregnation of 0.1 μ g of each arrestant onto a paper disc elicited 70–90% response from *A. kariyai*.

INTRODUCTION

Host searching behavior of the braconid wasp *Apanteles kariyai* WATANABE is induced by chemical stimuli present in the buccal secretion, frass and body surface of the host larvae, *Pseudaletia separata* WALKER. The kairomone functions as an arrestant which keeps the wasp in the host habitat. The arrestant from the three different sources above mentioned was found to consist of the same compounds in a series of homologs (TAKABAYASHI et al., 1985).

In this paper, we report the isolation and identification of these arrestants from the exuviae of *P. separata* host larvae.

MATERIALS AND METHODS

Insect. *P. separata* was reared on either corn leaves or an artificial diet under the condition of $26 \pm 2^\circ\text{C}$ and 17L–7D. The 4th- to 5th- instar larvae were offered to *A. kariyai* for their oviposition and the emerged wasps were kept in a test tube (22 mm diameter, 200 mm long) with a small piece of paper towel moistened with a 50% aqueous honey solution as food.

Bioassay. A female *A. kariyai* was confined to a petri dish (50 mm diameter) with the test material and a filter paper disc impregnated with a test solution was offered to her. The criterion of the bioassay was the characteristic antennal contact to the paper disc impregnated with sample. The number of arresting responses following antennal contact with the paper disc was recorded.

Fig. 1. Purification procedure of arrestants for *A. kariyai* from hexane extracts of exuvium of *P. separata* larvae. 1): (+): characteristic antennal contact to a paper disc at an impregnation of 0.1 μ g per disc. (-): no response.

Purification procedure. The purification procedure of the arrestants from the exuvium was discussed in the previous report (TAKABAYASHI et al., 1985). Arrestants gave a single peak on high performance liquid chromatography (HPLC, Nucleosil; 0.2% ether in hexane) but were revealed to be a mixture consisting of a series of homologs on gas liquid chromatography (GLC, 1% OV-1). The sample was further purified on HPLC (ODS; 2 mm ϕ , 200 mm long) with 20% ethyl acetate in methanol. The isolation process is summarized in Fig. 1.

Instrumental analysis. Mass spectra were measured by a Hitachi model M-80 spectrometer; ionization voltage was 70 eV. PMR and CMR spectra were measured by a JEOL model FX-90Q (90MHz) spectrometer with TMS as an internal standard in CDCl_3 .

RESULTS AND DISCUSSION

Isolation and identification of the arrestants

The results of the analysis of the arrestants by HPLC (ODS; 20% AcOEt in MeOH) and GLC (1% OV-1) is shown in Fig. 2. Each peak on GLC in Fig. 2-A was split into two peaks on ODS as shown in Fig. 2-B; peak V on GLC, for example, split into peaks 8 and 9. Therefore, the arrestants isolated by HPLC (Nucleosil; 0.2% ether in hexane) were further fractionated into 14 fractions by HPLC (ODS). MS spectra of the isolated fractions 8 and 9 showed a very similar fragmentation pattern indicating m/z 506 as the molecular ions.

Major components of the arrestants, fractions 5, 8 and 11 on ODS gave a similar fragmentation pattern, each showing the molecular ions, m/z 478, 506 and 534. EI-MS of fraction 8 is shown in Fig. 3. The high resolution mass spectrum, M^+ 506.5415

Fig. 2. GLC (1% OV-1; 250–300°C) (A) and HPLC (ODS; 20% AcOEt in MeOH) (B) analyses of isolated arrestants.

Fig. 3. EI-mass spectrum of fraction 8 on ODS.

corresponded to the molecular formula $C_{35}H_{70}O_1$ (MW. 506.5423). The characteristic fragment ions m/z 351, 323, 295, 281, 253 and 225 were estimated to be $C_{24}H_{47}O_1$, $C_{22}H_{43}O_1$, $C_{20}H_{39}O_1$, $C_{19}H_{37}O_1$, $C_{17}H_{33}O_1$ and $C_{15}H_{29}O_1$, respectively. From the MS data, fraction 8 was supposed to be an oxygen-containing compound with long alkyl chains, and the index of hydrogen deficiency was calculated as 1. The index of the fragment ions mentioned above, however, was calculated to be 1.5. Fraction 8 was not reduced catalytically with hydrogen nor with sodium borohydride; the compound, therefore, was shown to have an oxygen-heterocyclic ring. Fraction 8 was considered to be a mixture of three structural isomers, each with a pair of fragment ions: m/z 225 and 351, 253 and 323, and, 281 and 295. The sum of each pair is m/z 576, corresponding to $(M^+ + 70)$. The moiety m/z 70 was assumed to be a tetrahydrofuran ring as a common structural unit.

Fig. 4. Assignment of NMR chemical shifts and mass fragments of fraction 8 on ODS.

Table 1. Response of *A. kariyai* to arrestants purified by HPLC (ODS; 20% AcOEt in MeOH)

Fraction on ODS	M^+	m^a	n^b	% response of <i>A. kariyai</i> ^c					
				10	1	10^{-1}	10^{-2}	10^{-4}	10^{-6} ($\mu\text{g}/\text{disc}$)
5	478	{10 12}	{17 15}	70	80	75	60	30	0
8	506	{10 12 14}	{19 17 15}	65	70	90	55	25	0
11	534	{10 12 14 16}	{21 19 17 15}	50	70	70	60	25	0

^{a,b} Number of methylene groups in each side chain. ^c Average value of 20 females.

Presence of a tetrahydrofuran ring (THF) was confirmed by PMR data. The chemical shifts of methine protons (4.0 ppm, *m*) and methylene protons (1.4–2.4 ppm, *m*) were attributed to 2,5-dimethyl-tetrahydrofuran (the Aldrich Library of NMR Spectra, 1974). PMR of fraction 8 gave the following chemical shifts (ppm): 0.86 (6H, *t*, each), 1.25 (58H, *m*), 1.5–2.0 (4H, *m*) and 3.8 (2H, *m*). The multiplet at 3.8 ppm was assigned to the methine protons (2-H and 5-H) and the multiplet at 1.5–2.0 ppm to methylene protons (3-H and 4-H) of the THF ring. CMR spectrum of the fraction gave a total of 9 lines of chemical shifts (ppm) as follows: 14.109 (methyls), 22.680, 26.219, 29.349, 29.671, 31.923, 32.099, 36.106 and 78.667 (C-2 and C-5). From a combination of the MS, PMR and CMR data, we concluded that fraction 8 was a mixture of 2,5-dialkyltetrahydrofuran: 2-undecyl-5-eicosyl- (I), 2-tridecyl-5-octadecyl- (II), 2-pentadecyl-5-hexadecyl- (III) (Fig. 4). The ratio of the three isomers is supposed to be 1.2:0.8:1 from the intensity of the m/z 351, 323 and 295 of the MS spectrum. The assignments of NMR chemical shift and mass fragmentation are

also shown in the figure. Structures of other fractions were estimated mainly by mass spectra (Table 1). Each fraction 5 and 11 on ODS was found to be a mixture of two and four isomers, respectively. All the compounds identified in the extract from the exuviae were found in the buccal secretion and frass of the host larvae. There are four possible stereoisomers from two asymmetric carbons in 2,5-dialkyltetrahydrofuran. Stereochemistry and biological activity of the isomers will be discussed elsewhere.

Bioassay

Bioassay of the fractions on ODS with 0.1 μg per disk showed that all fractions from 2 to 14 had an activity of 70–80% (Fig. 1); results of the major fractions 5, 8, 11 on ODS are shown in Table 1. The activity appeared when 0.1 ng of a sample was impregnated on the disc and increased to 70%–90% at 0.1 μg impregnation.

There are not many 2,5-disubstituted tetrahydrofurans found in natural products. Recently, 2-alkoxy-5-pentyltetrahydrofuran was identified as a flavor component of watermelon essential oil (IWAMOTO et al., 1983). 2,5-Dialkyltetrahydrofurans were found in the cuticular wax of the *P. separata* larvae as a minor component and the major component in the wax was a mixture of saturated hydrocarbons. Although the amounts of the arrestant 2,5-dialkyltetrahydrofurans in the cuticular wax are minimal, *A. kariyai* detect them and use them as a cue to locate the host.

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REFERENCES

- IWAMOTO, M., Y. TAKAGI, K. KOGAMI and K. HAYASHI (1983) Synthesis of characteristic flavor constituents of watermelon (*Citrullus vulgaris*) essential oil. *Agric. Biol. Chem.* **47**: 115–116.
- TAKABAYASHI, J., T. NODA and S. TAKAHASHI (1985) Effect of kairomones in the host searching behavior of *Apanteles kariyai* WATANABE (Hymenoptera: Braconidae), a parasitoid of the common armyworm, *Pseudaletia separata* WALKER (Lepidoptera: Noctuidae). I. Presence of arresting stimulants produced by the host larvae. *Appl. Ent. Zool.* **20**: 484–489.
- The Aldrich Library of NMR Spectra (1974) **1**: 201A.