Exocrine Substances of the White Cabbage Butterfly, *Pieris rapae crucivora* (Lepidoptera : Pieridae)

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Volatile compounds in the exocrine secretion of the butterfly, *Pieris rapae crucivora*, comprised large amounts of methyl esters of some aliphatic acids and various minor components.

The wings and bodies of both sexes contained methyl palmitate, methyl *n*-heptadecanoate, methyl linolenate, methyl linolate, methyl stearate, 3-methylbutanal and 2-phenylpropenal, while phenylacetaldehyde was present only in the wings. These compounds represented no substantial sexual difference, though quantitative variance was somewhat noticeable between the sexes.

In addition to the above compounds, the male wing secreted indole, $C_{12}H_{18}O_2$, $C_{12}H_{18}O$ and $C_{13}H_{20}O_2$ in small amounts, and the latter three compounds, which were peculiar only to the male wing, were regarded as the constituents of androconial exudate.

In contrast, methyl salicylate and a much larger quantity of indole were also detected from the male body, thereby suggesting the presence of unknown secretory organ(s) in the male body.

INTRODUCTION

During the past two decades, chemical and biological approaches to characteristic substances secreted by certain male butterflies have added to the knowledge of chemical communication between conspecific males and females during the sequence of courtship. Danaid butterflies disseminate from their abdominal hair pencils some dihydropyrrolizine derivatives that serve as a significant chemical messenger to elicit the receptive response of the females (MEINWALD and MEINWALD, 1966; MEINWALD et al., 1966, 1969a, 1971, 1974; EDGAR et al., 1971). A variety of compounds other than dihydropyrrolizines are also contained in the hair pencil secretion of certain danaid males (MEINWALD et al., 1968, 1969a,b, 1971; BELLAS et al., 1974; EDGAR and CULVENOR, 1974; EDGAR et al., 1976; PETTY et al., 1977). Lycaenid and pierid males are generally known to have specialized scales, androconia, that are supposed to be a sort of secretory organ (KAWAZOÉ and WAKABAYASHI, 1976). As wing substances, *n*-nonanal, *n*-hexadecyl acetate and δ -cadinol have been identified from *Lycaeides argyrognomon* (LUNDGREN and BERGSTRÖM, 1975), esters of *n*-hexanol, from *Colias phil-*

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odice, and 13-methylheptacosane, from *Colias eurytheme* (GRULA et al., 1980). In the above Danaidae, Lycaenidae and Pieridae, some of the male exocrine substances are regarded as sex pheromones and substantiated to act as arrestants or aphrodisiacs, inducing the females to copulate (MYERS and BROWER, 1969; PLISKE and EISNER, 1969; LUNDGREN and BERGSTRÖM, 1975; RUTOWSKI, 1977, 1980; GRULA et al., 1980). A recent publication has dealt with a similar topic in the moth, *Grapholitha molesta* whose male hair pencil secretion attracts the females (BAKER et al., 1981), whereas in the armyworm moth, *Pseudaletia unipuncta*, it has been demonstrated that the odoriferous compound released by the male is a deterrent which decreases the tendency of other conspecific males to approach sexually receptive females or to exhibit copulatory behavior (HIRAI et al., 1978).

Other instances of secretion of odorous substances by male butterflies have been described for a papilionid and other pierid species, though no information on their ecological function is yet available. Males of *Atrophaneura alcinous* secrete from the wings (mainly hind wings) phenylacetaldehyde, linalool and other aromatic aldehydes as major constituents (HONDA, 1980). Some authors (BERGSTRÖM and LUNDGREN, 1973; HAYASHI et al., 1978) have reported the identification of geranial, neral and several minor components from *Pieris napi* and *P. melete*, and tricosane is the one identified compound from male wing secretions of *P. rapae* and *P. brassicae*.

The subjects of most of the preceding reports on chemicals mediated by butterflies in mating have been focused principally on male secretion, however, a few research works on lycaenid species (LUNDGREN and BERGSTRÖM, 1975; WAGO, 1978) have documented the involvement of female chemical signals as well as the male's in copulatory behavior. Thus, we now wish to report on volatile compounds in the exocrine secretions of both sexes of *Pieris rapae crucivora*, along with an indication of the components of the male androconial secretion.

MATERIALS AND METHODS

Butterflies. Adults of *Pieris rapae crucivora* derived from pupae collected in a cabbage field in Tokyo University of Agriculture and Technology were used as materials.

Collection of secretion. Within 24 hr of adult eclosion, the wings of the butterflies were excised, and the wings and body (head, thorax and abdomen) were extracted separately for 6 days at room temperature with dichloromethane (Tokyo Kasei Co. Ltd., Spectro Grade). The volume of the solvent used for extraction was 0.4 ml/individual for the wing, and 0.2 ml/individual for the body. Five hundred individuals of each sex were treated in total. The extract was concentrated to 4 μ l/individual for both wings and bodies under a nitrogen stream below 10°C, and the concentrate was subsequently directly subjected to chemical analyses.

Analysis of components. Components of the secretion were examined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). GC analyses were carried out on a Hitachi 163 gas chromatograph equipped with a flame ionization detector, using a glass column (3 mm in diam. $\times$ 2 m) packed with OV-101 (3% on Gas Chrom Q). The flow rate of carrier gas (nitrogen) was 30 ml/min. Injection temperature was 220°C and the oven temperature was programmed from 50 to 220°C at 5°C/min. Mass spectra were obtained by means of EI mode (I.V. : 70 eV) on a JEOL JMS-D 300 mass spectrometer with a JEOL JMA-2000 Data Sys-

tem, interfaced to a JEOL JGC-20 KP gas chromatograph and employing a glass column (2 mm in diam. $\times$ 2 m) packed with the same OV-101, unless otherwise noted. GC parameters in mass spectral measurements were analogous to those described above. CI spectra (reactant gas: isobutane; I.V. : 200 eV) were also recorded for further confirmation when necessary. Identification of the components was tentatively based on mass spectral data and corroborated by comparison of GC retention data and fragmentation patterns of mass spectra with authentic compounds. The percentage composition of each constituent was calculated by estimating its peak area/total peak area on gas chromatograms.

RESULTS

GC analyses of the extracts revealed that the overall pattern of the gas chromatogram of each extract resembles the others both in quality and relative quantity, as shown in Fig. 1 (A-D), though several minor components which are characteristic of the male secretion are obviously discerned.

Male wing

Figure 1-A indicated the presence of more than 23 components. Peaks **b**, **c** and **e** on GC/MS showed the following molecular and fragment ions: Peak **b**: M^+ , m/z 120 (20%), and m/z 92 (21%), 91 (100), 65 (20), 51 (4). Peak **c**: M^+ , m/z 132 (47%), and m/z 104 (43%), 103 (100), 77 (41), 51 (22). Peak **e**: M^+ , m/z 117 (100%), and m/z 90 (52%), 89 (31), 63 (12), 58 (17). These were identified as phenylacetaldehyde, 2-phenylpropenal and indole, respectively.

Peak **a** lacked M^+ but gave significant fragment ions at m/z 71 (13%), 58 (46), 44 (100), 43 (49) and 41 (69), and thus was identified as 3-methylbutanal upon comparison with the authentic standard.

Peaks **i**, **k** and **n** exhibited the base peak equally at m/z 74 and the second most abundant ion at m/z 87, suggesting methyl esters of saturated aliphatic acids. They were readily determined to be methyl palmitate, methyl *n*-heptadecanoate and methyl stearate, respectively, from their molecular weight (**i**: 270, **k**: 284, **n**: 298) and GC retention data.

Peak **j** was deemed to be an isomer of methyl heptadecanoate, presumably a methyl ester of a branched C_{17} acid, since the compound represented a mass spectrum similar to that of peak **k**.

Peaks **l** and **m**, which were not separated from each other under this GC condition, were assumed, in contrast, to be methyl esters of unsaturated fatty acids from the mass spectral fragmentation pattern of the mixture peak. Two ions, m/z 292 and 294, regarded as molecular ions of components **l** and **m**, were suggestive of methyl linolenate and methyl linolate. Another GC/MS measurement on a DEGS glass column (15% on chromosorb AWD MCS, 2 mm in diam. $\times$ 1 m) clearly indicated the presence of the above two esters in approximately equal amounts.

Peaks **f**, **g** and **h** were peculiar to the male wing secretion. They gave the following molecular and base ions: peak **f** (194, 68), peak **g** (180, 95) and peak **h** (208, 81). Their molecular weight was confirmed by measuring the mass spectra with CI mode. By exact mass measurement of the molecular weight, the molecular formula was reasonably assigned to $C_{12}H_{18}O_2$, for **f**, $C_{12}H_{20}O$, for **g** and $C_{13}H_{20}O_2$, for **h**. Fur-

Fig. 1. Gas chromatograms of the extracts from *P. rapae* on a OV-101 glass column, programmed from 50°C to 220°C at 5°C/min. A : male wing, B : male body, C : female wing, D : female body.

ther investigation on their chemical structures is now being undertaken.

Male body

The most prominent feature of the male body secretion is marked by the presence of peak **d** which, showing M^+ at m/z 152 (41%) and fragment ions at m/z 120 (100%), 92 (40), and 65 (18), was identified as methyl salicylate. It is also interesting that phenylacetaldehyde and peaks **f**, **g** and **h** were not detected from the male body.

Female wing

As compared with the male wing secretion, the female's is characterized by the lack of indole and peaks **f**, **g** and **h**, and, again, absolute amounts of other constituents were relatively smaller.

Female body

The gas chromatogram of the extract from female bodies (Fig. 1-D) had a qualitative resemblance to that of the female wings, regardless of the absence of phenylacetaldehyde in the bodies. Each component also displayed close similarity in relative and absolute quantity to the corresponding component in the male bodies, as shown in Table 1.

The average quantity of methyl palmitate per individual in male wings, female

Table 1. Volatile compounds secreted by *P. rapae*

Peak code in Fig. 1	Component	Composition, % ^a			
		Male		Female	
		Wing	Body	Wing	Body
a	3-Methylbutanal	0.7	0.6 (0.38)	1.4 (0.50)	0.2 (0.14)
b	Phenylacetaldehyde	0.2		0.4 (0.50)	
c	2-Phenylpropenal	0.1	0.4 (1.8)	0.3 (0.76)	0.4 (2.0)
d	Methyl salicylate		1.0		
e	Indole	0.1	1.7 (7.5)		
f	C ₁₂ H ₁₈ O ₂	0.8			
g	C ₁₂ H ₂₀ O	1.8			
h	C ₁₃ H ₂₀ O ₂	0.8			
i	Methyl palmitate ^b	6.4	8.4 (0.58)	4.9 (0.19)	8.3 (0.65)
j	Methyl ester (M ⁺ , 284)	0.5	0.9 (0.79)	0.6 (0.30)	1.0 (1.0)
k	Methyl <i>n</i> -heptadecanoate	0.4	0.3 (0.33)	0.3 (0.19)	0.4 (0.50)
l	Methyl linolenate	69.3 ^c	71.3 (0.45)	71.0 (0.26)	68.9 (0.50)
m	Methyl linolate				
n	Methyl stearate	14.1	10.7 (0.33)	13.7 (0.24)	10.0 (0.36)

^a Quantitative relation to the male wing is shown in parentheses, where the quantity of each component in the male wing secretion is expressed as 1.0.

^b Absolute amount of methyl palmitate in respective parts is given in the text.

^c Combined percentage of components **l** and **m**.

wings, male bodies and female bodies was estimated at 4.6 µg, 0.9 µg, 2.7 µg and 3.0 µg, respectively.

DISCUSSION

Volatile substances present in the wings and bodies of *Pieris rapae crucivora* were found to consist of large amounts of methyl esters of several aliphatic acid and various minor components. In the bodies of both sexes, the relative proportion of methyl palmitate was greater than in the wings, while that of methyl stearate was smaller. Additional characteristics are that the body contains a larger quantity of 2-phenylpropenal than the wing and that phenylacetaldehyde is detectable only from the wing. It is of interest that phenylacetaldehyde and 2-phenylpropenal have been reported to be significant odoriferous substances secreted by males of a papilionid butterfly, *Atrophaneura alcinous* (HONDA, 1980).

Although the amounts of compounds **f**, **g** and **h** were quite small, they were characteristic of the secretion from male wings. Males of *Pieris rapae*, like other pierid species, are known to possess androconia, which are considered to be scent-producing organs (BERGSTROM and LUNDGREN, 1973; RUTOWSKI, 1980). The scent scales are generally present only on male wings. Therefore, components **f**, **g** and **h**, at least, can be regarded as the constituents of male androconial secretion. However, as the extraction of secretion was conducted from young adults which, except for the females, may be sexually immature, some qualitative and/or quantitative alteration of secretion components with age would be expected, as documented in *Pieris melete*, (KUWAHARA, 1979). Compound **f** was also detected from a related *Pieris* species,

P. canidia, and the quantity of this compound appeared larger in this species (unpublished data).

It is to be noted that from male bodies methyl salicylate was found which did not occur in any other parts. This substance has been reported to be present also in the hair pencil secretion of *Amauris ochlea* (PETTY et al., 1977). In addition, male bodies contained indole in a far larger amount than male wings. This knowledge coupled with the absence of androconia on male bodies suggests that the male body is also provided with a distinct secretory organ(s).

As is well known, the males of *Pieris napi* disseminate a strong geranial and neral odor (BERGSTRÖM and LUNDGREN, 1973), whereas the males of *P. rapae* are almost scentless to humans, which seems due to the meager quantities of odoriferous compounds secreted. It is noteworthy that conspicuous qualitative disparity was observed between the secretion components in these two related species.

In *Pieris*, visual cues appear to be very important in the incipient step of the courtship sequence (recognition of a female by a male), as demonstrated in *P. rapae crucivora* (OBARA and HIDAKA, 1968; OBARA, 1970); however, it is probable that the exocrine substances of males play a mediating role during courtship and that they also serve as a species-specific chemical signal assuring reproductive isolation among related species.

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