Identification of Odoriferous Compounds from Adults of a Swallowtail Butterfly, *Papilio machaon* (Lepidoptera: Papilionidae)

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Papilio machaon hippocrates, Papilionidae, Butterfly Scent

Adults, particularly males, of a papilionid butterfly, *Papilio machaon hippocrates*, emit a fairly strong scent perceivable by humans. We have identified a variety of volatile compounds (hydrocarbons, alcohols, aldehydes, ketones, esters, and so on) from the wings and bodies of both sexes of the butterfly. Male wings secreted *n*-dodecane, linalool and geranylacetone as major components together with small amounts of camphene, limonene, *p*-cymene, 2-phenylethanol, *n*-hexanal, *n*-decanal, isoamyl acetate, *p*-allylanisole, 2-pyrrolidone and other characteristic volatiles. The overall profile of volatile compounds detected from male body was quite different from that of the wings. Male body was devoid of camphene, 2-phenylethanol, *n*-hexanal but instead contained limonene, acetoin, a sesquiterpene hydrocarbon ($C_{15}H_{24}$), methyl *n*-octanoate, (*E,E*)-hepta-2,4-dienal, and another isomer of heptadienal as principal components, of which the last four compounds were specific to the body. All these substances seem to concurrently characterize the male odor. The chemical patterns of compounds found from female wings and body were essentially the same in quality as those of male wings and body, respectively, although their quantities in females were generally smaller than in males. Females, however, had a larger amount of acetamide than males. The chemical compositions of volatiles from the fore and hind wings of males were not greatly different from each other, and every component was considered to be present on all parts of the wings. This suggests that the scent-producing organs or scent-emitting pores are widely distributed on the whole wings. EAG responses of both sexes to 12 selected compounds identified from the butterfly were not strong at a dose of 1 μg, while both sexes showed relatively stronger responses to *n*-nonanal, methyl *n*-octanoate, D-limonene and linalool at a higher dose (10 μg). Although sexual difference in EAG response was not prominent, females appeared a little more sensitive, and *n*-nonanal and acetoin evoked significantly higher responses from females at 1 μg.

Introduction

Since the finding that males of a danaid butterfly, *Danaus gilippus*, utilize a peculiar dihydropyrrolizine compound (danaidone) as a sex pheromone in their courtship behavior (Pliske and Eisner, 1969), males of some other butterfly species have also been shown to emit species-specific volatile chemicals from the androconial organs or body tissues, although their biological functions are not fully elucidated yet. In some butterflies, however, certain compounds disseminated by male adults have been demonstrated to act as a chemical messenger to elicit a receptive response from conspecific females during the sequence of courtship. Most danaid males secrete dihydropyrrolizine derivatives from the abdominal hair-pencils and/or alar scent organs (Edgar *et al.*, 1971; Komae *et al.*, 1982; Eisner and Meinwald, 1987; Schulz *et al.*, 1988; Honda *et al.*, 1995), and *Idea leuconoe*, one of primitive danaids, makes use of danaidone and viridifloric β-lactone as aphrodisiacs (Nishida *et al.*, 1996). Several male pierids endowed with androconia on their wings also emit characteristic compounds; geranial and neral from *Pieris melete* and *P. napi* (Bergström and Lundgren, 1973; Hayashi *et al.*, 1978; Kuwahara, 1979), 13-methylheptacosane and esters of *n*-hexanol as sex pheromones from *Colias eurythem* and *C. philodice,* respectively (Grula *et al.*, 1980), and a diversity of volatiles from both wings and body of *P. rapae* (Honda and Kawatoko, 1982) and *Delias* species (Ômura *et al.*, 2000). Recently, male odor of *P. melete* was found to elicit leaning behavior

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from courted females (Kan and Hidaka, 1997), and methyl salicylate previously identified from male body of *P. rapae* (Honda and Kawatoko, 1982) was shown to be present also in male body of *P. napi,* to be transferred to the female at copulation, and to act as an anti-aphrodisiac evoking mate-refusal posture from gravid females (Andersson *et al.*, 2000). Males of a lycaenid butterfly, *Lycaeides argyrognomon,* have also been reported to use specific chemicals for intraspecific sexual communication (Lundgren and Bergström, 1975). On the other hand, less publicity has been given to volatile chemicals secreted by papilionid butterflies. The one and only report published to date has dealt with the secretion of *Atrophaneura alcinous*, males of which emit a fragrant odor consisting of a few aromatic aldehydes and terpenoids (Honda, 1980).

We have found that males of some other papilionid butterflies such as *Papilio machaon, P. portenor, P. polytes and P. glaucus* also have fairly strong scent perceivable by humans. In the present study, we report on the chemical composition of volatile compounds from both sexes of adults of *P. machaon hippocrates*, a subspecies inhabiting Japan. Antennal response of the butterfly to the major constituents of the volatiles was also examined electroantennographically.

Materials and Methods

Insects

The offspring of female adults of *P. machaon hippocrates* collected in the field near the campus of Hiroshima University were used in this study. Larvae were reared on *Angelica keiskei* (Umbelliferae) at 25 °C with a 16-h light : 8-h dark regime to produce summer morph adults, while those used for examining the distribution pattern of components on the wings were reared under natural conditions in September (average temperature: 24 °C, photoperiodic regime: 12-h light : 12-h dark). They are diapausing and spring morph-destined larvae and the adults emerged in the following spring. Within 24 hr of eclosion, adults were sexed and kept separately until use in plastic containers (30 × 25 × 20 cm), being fed with 15% aq. sucrose solution once daily.

Collection of volatile components from wings and body

The scent substances were obtained separately from 24 males and 20 females by extraction with dichloromethane which was purified by two distillations. On the fourth day after adult eclosion, wings were amputated from the body. The fore wings, hind wings and body (head, thorax and abdomen were treated together) were extracted separately with 25 ml of the solvent at 0 °C for a month. Each extract was filtered and once concentrated to 500 μl *in vacuo* at 15 °C, and further concentrated to 50 or 100 μl under nitrogen stream at 0 °C.

Distribution of volatile components on the fore and hind wing was also examined using 3-day-old 13 males. The fore wings were divided into two parts (A and B), and similarly, the hind wings, into two parts (C and D) as illustrated in Fig. 3. Respective parts were extracted in the same manner with 10 ml of dichloromethane, and similar workup yielded samples for chemical analyses.

Chemical analyses

Chemical composition of volatile compounds from the wing and body extracts was examined by GC and GC-MS. GC analyses were conducted with a Shimadzu GC-14A gas chromatograph equipped with an FID on an FFAP fused-silica capillary column (0.25 mm I. D. × 50 m, 0.25 μm film thickness). The injection temperature was 250 °C, and the oven temperature was programmed from 50 to 220 °C at a rate of 5 °C/min. The flow rate of carrier gas (N_2) was 1 ml/min. The system was operated by the splitless mode. EI-MS spectra were recorded at 70 eV on a Shimadzu QP2000 mass spectrometer connected to the same GC model, using the same capillary column and under similar operational conditions. Identification of components was based on a comparison of GC retention data and mass spectra with those of authentic samples unless otherwise noted.

Electroantennogram (EAG) recording

The antennal sensitivity of both sexes to selected volatile components from the wings and body was tested electroantennographically by a method similar to that reported previously

(Honda *et al.*, 1998). EAGs were recorded from excised antennae using a DPZ-115 input probe coupled with a DPA-100E amplifier (Dia Medical System), which was interfaced to a personal computer. A test compound dissolved in dichloromethane was deposited on a filter paper strip (5 × 30 mm). After evaporating the solvent at room temperature, the strip was inserted into a glass tube (6 mm I. D. × 50 mm). An odor puff of 1 ml was mixed with a deodorized and humidified air stream blowing continuously over the antenna at a rate of 350 ml/min. Each compound was tested at two doses (1 and 10 μg) on five antennae of both sexes, each of which originated from a different butterfly. EAG responses to a given dose of a compound were measured three times for each preparation. The responses were averaged and expressed as percentages of the response to a standard compound (1 μg of hexan-1-ol). Sexual differences in EAG intensities were analyzed by a *t*-test.

Test chemicals

Pure (*E*)- and (*Z*)-isomers of nerolidol were obtained from an *E/Z* mixture of nerolidol (Tokyo Chemical Industry) by fractional distillation and subsequent chromatographic separation on silica gel. Other authentic chemicals were commercially purchased (Tokyo Chemical Industry, Nacalai Tesque and Aldrich). The purities of the chemicals as assessed by GC were all above 97%.

Results

Identification of compounds detected from the wings and body

Mass spectral data of identified components are summarized in Table I. Compound **10** (Fig. 2) showing a mass spectral fragmentation pattern quite similar to that of (*E,E*)-hepta-2,4-dienal (**12**) was considered to be its geometrical isomer. Compound **17** gave M^+ at *m/z* 204 and represented a fragmentation pattern typical of a sesquiterpene

Table I. Mass spectrometric identification of volatile components from adults of *Papilio machaon*.

Peak No.	Compound	Mass spectral data *m/z* (intensity, %)
1	Camphene	121 (54), 107 (29), 93 (100), 79 (44), 67 (42), 41 (72), 39 (58)
2	*n*-Hexanal	57 (48), 56 (56), 55 (20), 45 (23), 44 (95), 43 (82), 42 (35), 41 (100), 39 (35), 31 (31)
3	Isoamyl acetate	70 (25), 55 (24), 43 (100), 42 (15), 41 (12)
4	Limonene	M^+: 136 (9), 121 (15), 107 (14), 93 (49), 79 (25), 68 (100), 67 (74), 57 (19), 53 (28), 43 (32), 41 (46), 39 (44)
5	*n*-Dodecane	85 (17), 71 (37), 57 (91), 43 (100), 41 (48)
6	*p*-Cymene	M^+: 134 (28), 119 (100), 91 (28), 41 (21), 39 (21)
7	Acetoin	M^+: 88 (5), 45 (100), 43 (61), 42 (7)
8	Methyl *n*-octanoate	127 (6), 115 (7), 87 (34), 74 (100), 59 (19), 55 (20), 43 (42), 41 (28)
9	*n*-Nonanal	98 (16), 82 (17), 70 (24), 69 (21), 57 (78), 56 (52), 55 (48), 45 (20), 44 (74), 43 (62), 42 (31), 41 (100), 39 (30)
10	Heptadienal[a]	110 (16), 81 (100), 68 (15), 67 (13), 53 (33), 51 (13), 41 (41), 39 (54)
11	*n*-Decanal	82 (24), 71 (28), 70 (29), 57 (77), 56 (34), 55 (56), 44 (62), 43 (100), 42 (34), 41 (99), 39 (30)
12	(*E,E*)-Hepta-2,4-dienal	110 (12), 81 (100), 68 (13), 67 (16), 53 (28), 51 (11), 41 (50), 39 (49)
13	Benzaldehyde	M^+: 106 (83), 105 (81), 77 (100), 51 (65), 50 (37)
14	Linalool	136 (4), 121 (12), 107 (4), 93 (50), 71 (95), 55 (65), 43 (100), 41 (98)
15	Phenylacetaldehyde	91 (100), 65 (27), 59 (26), 43 (29)
16	*p*-Allylanisole	M^+: 148 (100), 147 (60), 121 (43), 117 (36), 77 (40), 51 (32), 39 (39)
17	$C_{15}H_{24}$[a]	M^+: 204 (20), 189 (34), 161 (13), 147 (19), 134 (29), 121 (47), 107 (43), 93 (94), 79 (53), 68 (82), 67 (62), 55 (44), 53 (52), 41 (100), 39 (55)
19	Acetamide	M^+: 59 (82), 44 (100), 43 (58), 42 (40)
22	Geranylacetone	151 (4), 136 (4), 121 (1), 107 (4), 93 (3), 69 (21), 43 (100), 41 (29)
25	2-Phenylethanol	92 (55), 91 (100), 65 (24), 43 (34), 41 (29)
26	(*Z*)-Nerolidol	81 (49), 71 (33), 69 (82), 57 (49), 55 (46), 43 (84), 41 (100)
27	(*E*)-Nerolidol	136 (6), 107 (13), 93 (26), 81 (16), 71 (25), 69 (56), 59 (30), 55 (31), 43 (100), 41 (76)
28	2-Pyrrolidone	M^+: 85 (73), 42 (84), 41 (80), 30 (100)

[a] Tentative assignment.

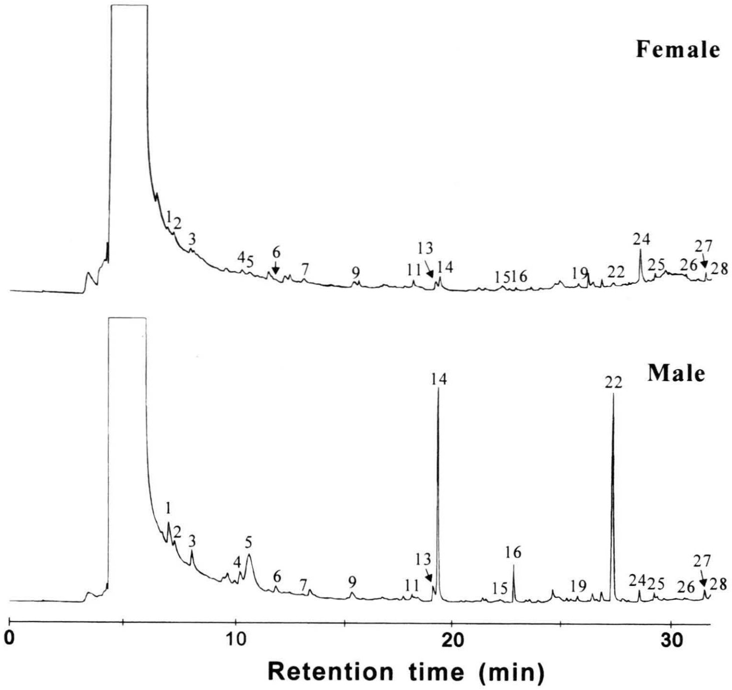

Fig. 1. Gas chromatograms of the extracts from the fore wings of *Papilio machaon*. Chromatograms were obtained with an FFAP fused-silica capillary column (0.25 mm I. D. × 50 m, programmed from 50 to 220 °C at a rate of 5 °C/min).

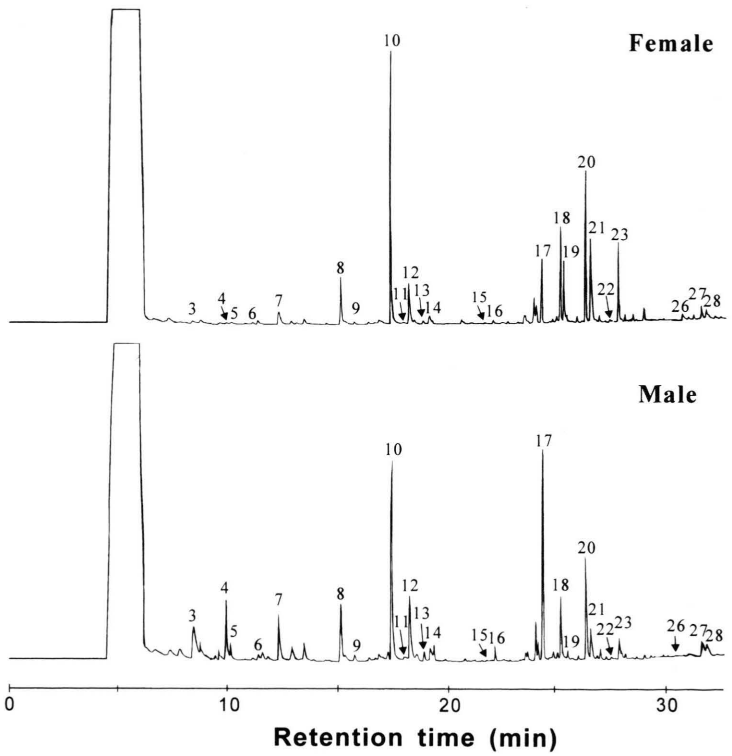

Fig. 2. Gas chromatograms of the extracts from the bodies of *Papilio machaon*. Chromatograms were obtained with an FFAP fused-silica capillary column (0.25 mm I. D. × 50 m, programmed from 50 to 220 °C at a rate of 5 °C/min).

hydrocarbon ($C_{15}H_{24}$). Although its mass spectrum was very similar to that of germacrene A found from the larval osmeterial secretions of some papilionid butterflies (Honda, 1981), identification was unsuccessful because of the inconformity of GC retention data.

Chemical composition of volatile components from the wings

A variety of odoriferous compounds were detected along with ubiquitous higher aliphatic acids from male wings (Fig. 1, Table II). Male wings contained *n*-dodecane, linalool and geranylacetone as major components. Volatiles present in the fore and hind wings were very alike in quality and in the relative proportion of individual components. Female wings also had the same compounds in common, although most of them were present in smaller amounts. Acetoin and *n*-decanal were the major constituents from female wings, and the quantity of acetoin was about three times as large as that of the male wings.

Table II. Chemical composition of volatile compounds from adults of *Papilio machaon*.

		Male							Female						
Peak No.	Compound	Fore wings (%)[a]	Fore wings [ng]	Hind wings (%)	Hind wings [ng]	Body (%)	Body [ng]	Total [ng]	Fore wings (%)	Fore wings [ng]	Hind wings (%)	Hind wings [ng]	Body (%)	Body [ng]	Total [ng]
1	Camphene	3.2	164	3.7	213	–[b]		377	1.3	47	2.7	79	–		126
2	*n*-Hexanal	0.8	75	0.8	83	–		158	1.2	74	0.9	48	–		122
3	Isoamyl acetate	2.4	155	1.9	138	1.9	353	646	0.9	42	1.3	51	0.2	21	114
4	Limonene	7.2	325	0.7	35	6.1	585	945	0.6	19	1.0	28	0.2	5	52
5	*n*-Dodecane	10.2	448	15.7	1141	2.5	233	1822	1.2	38	1.7	43	0.1	5	86
6	*p*-Cymene	1.2	99	1.1	104	1.3	230	433	0.4	25	0.6	26	–[e]		51
7	Acetoin	0.4	45	0.4	55	4.6	716	816	1.8	163	2.2	166	1.1	179	508
8	Methyl *n*-octanoate	–		–		3.0	416	416	–		–		2.8	236	236
9	*n*-Nonanal	1.1	84	1.0	83	0.4	58	225	1.2	62	2.2	95	0.3	13	170
10	Heptadienal[c]	–		–		8.2	1290	1290	–		–		14.4	830	830
11	*n*-Decanal	2.3	169	1.8	150	0.9	145	464	2.4	127	4.7	205	0.1	36	368
12	(*E*,*E*)-Hepta-2,4-dienal	–		–		5.1	805	805	–		–		3.9	322	322
13	Benzaldehyde	1.5	83	1.0	63	1.8	218	364	0.9	33	1.1	35	0.7	29	97
14	Linalool	18.1	1125	11.9	834	2.1	282	2241	1.3	58	2.2	80	0.7	27	165
15	Phenylacetaldehyde	0.2	14	0.2	15	0.2	22	51	0.1	2	0.8	4	0.1	6	12
16	*p*-Allylanisole	3.1	188	2.7	184	1.8	237	609	1.0	43	0.7	27	0.1	7	77
17	$C_{15}H_{24}$[d]	–		–		9.4	1185	1185	–		–		10.6	309	309
18	Unidentified	–	–			3.8			–	–			5.9		
19	Acetamide	0.3	45	0.2	50	0.2	38	133	0.8	100	0.2	19	3.3	228	347
20	Unidentified	–		–		4.8			–				8.7		
21	Unidentified	–		–		2.3			–		–		5.4		
22	Geranylacetone	21.8	1215	13.4	835	0.4	52	2102	0.5	22	0.6	18	0.2	6	46
23	Unidentified	–		–		1.9			–		–		4.1		
24	Unidentified	0.6		0.6		–			0.3		0.6		–		
25	2-Phenylethanol	0.5	48	1.2	117	–		165	0.4	23	1.3	31	–		54
26	(*Z*)-Nerolidol	0.5	30	1.1	72	0.3	31	133	1.8	75	1.2	41	0.2	18	134
27	(*E*)-Nerolidol	0.8	47	1.5	97	0.7	61	205	1.6	67	1.3	45	0.6	65	177
28	2-Pyrrolidone	0.5	53	2.0	247	0.8	118	418	0.6	51	1.6	107	1.4	118	276
	Total	**76.7**		**62.9**		**64.5**			**20.3**		**28.9**		**65.1**		

[a] GC area%.
[b] Not detected (below 0.1%, if any).
[c] Tentative assignment and quantification in terms of (*E*,*E*)-hepta-2,4-dienal.
[d] Quantification based on the GC peak area in terms of β-caryophyllene.
[e] The presence of a trace (< 0.1%) of *p*-cymene was confirmed by MS.

Chemical composition of volatile components from the body

The overall profile of chemical compounds contained in male body was considerably different in both quality and quantity from that of the wing substances, although several minor components were common in the wings and body. A sesquiterpene hydrocarbon (**17**), methyl *n*-octanoate, (*E,E*)-hepta-2,4-dienal and its isomer (**10**) were specifically present in the bodies of both sexes with compounds **10** and **17** being predominant. In addition to these components, male body contained larger amounts of limonene, acetoin and benzaldehyde than the wings. Female body secretion was essentially the same in quality as that of male body, however, the absolute quantity of each component was generally smaller except for acetamide which female body had in a much larger amount than male body.

Distribution of volatile compounds on male wings

To examine the secretion site of volatiles in male wings, several components were determined for parts A to D (Fig. 3). The average of each area was estimated at 3.1, 5.7, 5.3 and 2.3 cm^2, respectively. The compounds in question were distributed in all parts, although the absolute quantities of respective components per unit area varied considerably among the four parts examined (Table III). It seems, therefore, tenable to consider

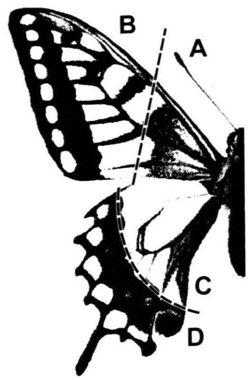

Fig. 3. The upperside of male wings of *Papilio machaon* (spring morph). Wings were amputated along the broken line for quantification of compounds present in each moiety.

Table III. Distribution of volatile components on male wings of *Papilio machaon*.

Compound	Fore wings A[a]		Fore wings B		Hind wings C		Hind wings D	
Camphene	38	(6.1)[b]	33	(2.9)	63	(6.0)	25	(5.4)
n-Hexanal	27	(4.4)	15	(1.3)	20	(1.9)	17	(3.6)
Isoamyl acetate	41	(6.6)	33	(2.9)	69	(6.5)	36	(7.8)
Limonene	14	(2.3)	6	(0.5)	14	(1.3)	12	(2.7)
n-Dodecane	59	(9.5)	32	(2.8)	59	(5.6)	48	(10.5)
p-Cymene	23	(3.7)	16	(1.4)	22	(2.1)	20	(4.3)
Acetoin	12	(2.0)	9	(0.8)	19	(1.8)	10	(2.2)
n-Nonanal	19	(3.1)	29	(2.5)	23	(2.2)	22	(4.8)
n-Decanal	30	(4.8)	43	(3.8)	28	(2.6)	65	(14.2)
Benzaldehyde	10	(1.5)	18	(1.6)	6	(0.6)	14	(3.0)
Linalool	104	(16.7)	169	(14.8)	83	(7.8)	77	(16.7)
Phenylacetaldehyde	5	(0.8)	17	(1.5)	8	(0.7)	10	(2.2)
p-Allylanisole	5	(0.8)	3	(0.3)	2	(0.2)	6	(1.4)
Acetamide	17	(2.7)	39	(3.4)	15	(1.4)	31	(6.7)
Geranylacetone	94	(15.1)	146	(12.8)	119	(11.3)	106	(23.0)
2-Phenylethanol	7	(1.2)	6	(0.5)	10	(0.9)	4	(0.9)
(Z)-Nerolidol	8	(1.2)	11	(1.0)	20	(1.9)	6	(1.3)
(E)-Nerolidol	11	(1.7)	17	(1.5)	20	(1.9)	32	(7.0)
2-Pyrrolidone	39	(6.3)	36	(3.2)	65	(6.1)	51	(11.1)

Amount [ng]

[a] See Fig. 3 for each wing part.
[b] Amounts per unit area (ng/cm^2) are shown in parentheses.

that these compounds are secreted from the whole wings with biased concentrations.

EAG responses to wing and body substances

Excised antennae of both sexes were subjected to EAG experiments using 12 compounds identified from the wings and body. Both males and females did not show strong responses at a dose of 1 μg, however, *n*-nonanal, methyl *n*-octanoate, D-limonene and linalool elicited relatively stronger responses from both sexes at a dose of 10 μg (Fig. 4). Although both sexes exhibited a similar profile of EAG responsiveness, females responded a little more sensitively to most chemicals tested, and showed significantly greater responses than males to *n*-nonanal and acetoin at 1 μg (*t*-test: $p < 0.05$).

Discussion

From the wings and body of adults of *P. machaon,* we have identified more than 20 volatile compounds of various chemical classes, including hydrocarbons, alcohols, aldehydes, ketones, esters, amides, and an ether. Most of them were compounds identified for the first time from papilionid butterflies, though phenylacetaldehyde, benzaldehyde and linalool have already been reported for the male scent of an Aristolochiaceae-feeding papilionid, *A. alcinous* (Honda, 1980). The overall profiles of the chemical composition of the extracts from male wings and body were quite dissimilar: camphene, *n*-hexanal and 2-phenylethanol were constituents specific to the wings, while methyl *n*-octanoate, a sesquiterpene hydrocarbon, (*E,E*)-hepta-2,4-dienal and its isomer were specific to the body. Moreover, *n*-dodecane, linalool and geranylacetone were the major alar components (*ca.* 2 μg each per individual), and the above body-specific components and acetoin were predominant in the body. The chemical patterns of compounds found from female wings and body were essentially the same in quality as those of male wings and body, respectively, although their quantities in females were generally smaller than in males. In particular, males contained much larger amounts of limonene, *n*-dodecane, *p*-cymene, linalool, *p*-allylanisole and geranylacetone. Females, however, had acetamide three times as many as males. The presence of a profusion of volatile chemicals in male wings and perhaps also in the body seems to be responsible for the male characteristic and strong scent.

We have found that in males, alar compounds are distributed on the whole wings, though their concentrations per unit area were different among the four parts (A-D). This finding suggests that the scent-producing organs or scent-emitting pores are scattered on every part of the wings. Miller (1987) reported that adults of certain papilionids possess various types of androconial organs on the wing surface and/or on the distal segment of abdomen. In addition, Honda (1980, 1986) showed that male adults of *A. alcinous* have particular scent-produc-

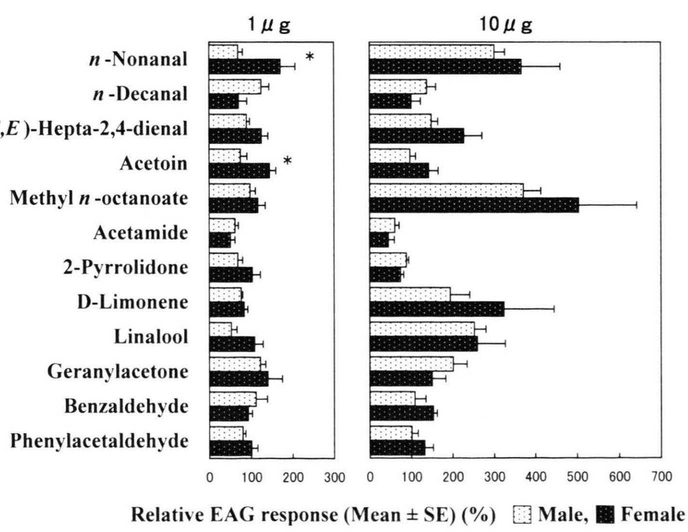

Fig. 4. Relative EAG responses (mean ± SE) of adults of *Papilio machaon* to their exocrine components ($N = 5$). The significance of difference between males and females is represented by an asterisk (*t*-test: * for $p < 0.05$).

ing organs on the inner margin of hind wings. We have carefully scrutinized the integumental structure of fore and hind wings of males by stereoscopy and scanning-electronmicroscopy, however, we failed to discern any particular tissues that seemed to be involved in the emission of odor. The mechanism by which *P. machaon* produces and emits its scent remains to be investigated.

Males and females showed substantially the same EAG responsive pattern at two doses, indicating that the sexual difference in the antennal sensitivity is not remarkable as far as the test chemicals are concerned. Nevertheless, the fact that *n*-nonanal and acetoin elicited weak but significant EAG responses from females at a low dose (1 μg) is suggestive of the implication of these compounds, despite their paucity, in as yet uncharacterized physiological and/or behavioral responses evoked specifically from females.

Although nothing is known about the function of these exocrine substances of *P. machaon* adults, some possible roles may be assumed. First, defensive allomones. Since both males and females possess considerable amounts of odoriferous chemicals, they may serve as defensive substances against predetors. Secondly, a chemical messenger mediating intraspecific male-to-female communication (sex pheromone). In butterflies that utilize some chemical signals such as sex pheromones in mate recognition, it has generally been thought that chemical information provided by males plays a significant role in their courtship (recognition of a male by a female) (*e.g.* Lundgren and Bergström, 1975; Grula *et al.*, 1980; Eisner and Meinwald, 1987; Nishida *et al.*, 1996). In the case of *P. machaon*, however, females also have and may emit characteristic volatiles that are deemed to be received by conspecific males during the sequence of courtship. Therefore, female odor may help a courting male recognize a female as a mate at once. That is, an individual with a strong scent may be regarded as a male, and that with a faint scent, a female. This system would be useful for saving cost to be spent on male pursuit of other males and male-to-male competition. Lastly, an allelochemical mediating interspecific interaction among allied species. In most localities, *P. machaon* lives in sympatry with a Rutaceae-feeding swallowtail, *Papilio xuthus*, that wears a wing color pattern very analogous to *P. machaon*. It has been demonstrated that the approaching behavior of male adults of *P. xuthus* to the female is released by the striped pattern of black and yellow of its wings (Hidaka and Yamashita, 1975). In addition, both sexes of *P. xuthus* appear to have no noticeable scent perceivable by humans. Accordingly, the scent of *P. machaon* may possibly serve to facilitate discrimination between conspecifics and the others by respective species.

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