The trail and alarm pheromones of the ant, Pristomyrmex pungens Mayr[1]

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Summary. The trail and alarm pheromones of P. pungens were investigated. The nine fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:4}$, $C_{20:5}$) were the components of the trail pheromone, while the alarm pheromone was found to be the mixture of 4 monoterpene hydrocarbons (α-pinene, camphene, β-pinene, limonene).

In the recent years, the nature of pheromone and other considerable chemical release used by insects has aroused considerable interest. A wide variety of ant species are known to utilize trail and alarm pheromones[2] as a means of orientation and communication. The chemical investigation of the groups of natural products is still in its initial stage. The first isolation and identification of the trail pheromone was achieved by Tumlinson et al.[3], who reported the major volatile component of the trail pheromone produced by Atta taxana to be methyl-4-methyl pyrrole-2-carboxylate. Recently Huwyler et al.[4] demonstrated the trail pheromone of the ant, Lasius fuliginosus to be the mixture of 6 fatty acids (hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, decanoic acid, dodecanoic acid). A number of alarm pheromone of ant species have been reported[2] such as dimethylsulfide, aliphatic ketones, alkanes, terpene aldehydes and formic acid. Recently Wheeler et al.[5] reported the presence of 7 pyrazine derivatives in the alarm pheromone of Ponerine ants: 2,6-dimethyl-3-n-pentyl pyryzine, 2,6-dimethyl-3-n-butyl pyrazine, 2,6-dimethyl-3-n-propyl pyrazine, 2,6-dimethyl-3-ethyl pyrazine, 2,5-dimethyl-3-isopentyl pyrazine, 2,5-dimethyl-3-ethyl pyrazine. In this preliminary communication the analysis of the trail and alarm pheromones is reported.

Material and method. The whole bodies of worker ants (63 g), Pristomyrmex pungens Mayr, obtained from Hiroshima, were macerated in n-pentane. After evaporating the solvent, an oily matter (446 mg) was obtained. The trail and alarm responses of the minor workers from the laboratory colony was used to monitor this and all subsequent isolation step. The oily matter was fractionated by preparative high speed liquid chromatography (HSLC) into 3 fractions as shown in the table. The

Isolation and identification of trail and alarm pheromones

1. Preparative HSLC of total extracts of the ants

Fraction	Retention volume (ml)	Composition[10]	Compound	Activity
1	26.3	20.1	Pigment	–
2	28.8	23.1	Fatty acid	Trail
3	35.4	56.8	Hydrocarbon	Alarm

2. Composition of trail pheromone (fraction 2 in HSLC)

Fatty acid	Composition[10]	Identification[12]			
		GC-MS			GC
		EI (M^+)		CI (QM^+)	
$C_{14:0}$	0.1		242	243	GC
$C_{16:0}$	0.2	$C_{17}H_{34}O_2$	270.2564	271	GC
$C_{16:1}$	0.2		268	269	GC
$C_{18:0}$	2.1	$C_{19}H_{38}O_2$	298.2860	299	GC
$C_{18:1}$	13.8	$C_{19}H_{36}O_2$	296.2663	297	GC
$C_{18:2}$	3.3	$C_{19}H_{34}O_2$	294.2529	295	GC
$C_{18:3}$	0.1		292	293	GC
$C_{20:4}$	0.6	$C_{21}H_{34}O_2$	318.2510	319	GC
$C_{20:5}$	0.6		316	317	–

3. Composition of alarm pheromone (fraction 3 in HSLC)

Peak No.	Alarm pheromone	Composition[10]	Identification
1	α-Pinene	9.1	IR, GC
2	Camphene	9.4	IR, GC
3	β-Pinene	33.0	IR, GC
4	Limonene	5.3	IR, GC

HSLC was performed by means of a Waters Ass. Model ALC 202 liquid chromatograph with M6000 pumping system and detected with a Waters Ass. Model R401 refractive index detector. 2 Shodex GPC columns (A801 × 1, A802 × 3, porous polymer of styrene divinylbenzene) was used. For analysis of trail pheromone, the methylation of the fatty acids (fraction 2 in HSLC) was carried out using diazomethane and then analyzed by gas chromatography-mass spectrometer (GC-MS)[6]. The instrument used consists of JGC-20KP gas chromatography (JEOL Co. Ltd, Japan) equipped with the column which was coupled to a JMS-D100 mass spectrometer (JEOL Co. Ltd, Japan) having electron impact (EI) and chemical ionization (CI) combination sources and measured the folowing conditions: column, 10%-DEGS Gaschrom Q (100–200 mesh) glass column (3 m × 3 mm); column temperature programmed 150° to 200 °C (5 °C/min); injection temperature 300 °C; carrier gas He 2.1 kg/cm^2; ionization voltage 23eV. When the CI source was used, iso-butane was used for the reactant gas. The individual components showing alarm response of fraction 3 in HSLC were isolated by preparative gas chromatography (GC), and then identified by IR-spectra on comparison with those of authentic specimens. The S-assay described by Hangartner[7] and O-assay by Tumlinson[3] were employed to check the biological activity of the trail pheromone. While the alarm activity was tested by the Blum's method[8].

Results and discussion. The trail pheronome[9]. The biological activity of the trail pheromone was detected in fraction 2 which was obtained from preparative HSLC. The threshold of detection of the trail pheromone was 10^{-4} to 10^{-7} g/ml. The IR-spectrum of the fraction showed the absorption band at 1700 cm^{-1} due to carbonyl group (showing the presence of the acid). The acid fraction was reacted with diazomethane and submitted to GC-MS analysis. Some of the methyl ester of the fatty acids showed no molecular ion (e.g. $C_{20:5}$) in MS using EI source, so the CI source was used instead of EI in case of $C_{20:5}$ acid. The 9 fatty acids ($C_{14:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:4}$, $C_{20:5}$) were identified by GC-MS as the components of the trail pheromene. The individual fatty acids were further identified by GC. The isolation and identification of trail and alarm pheromones are shown in the table.

Alarm pheromone. The ants were irritated and then they secreted the alarm pheromone in test tube. After extracting the ants with n-pentane, an odorous oil was obtained. The gas chromatogram of the oil showed 4 peaks which were very similar to those of the fraction 3 in HSLC. The individual components were further isolated by preparative GC and identified by IR-spectra on the comparison with those of authentic specimens. Further identification was performed by GC. The main constituents of alarm pheromone were β-pinene together with limone, α-pinene and camphene. It is of interest that the monoterpene hydrocarbons were detected as the alarm pheromone in P. pungens as well as Nasutitermes exitiosus (Hill)[11].

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The effect of ultraviolet light (UVL) on the lysosomes of hairless mouse epidermis

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Summary. An increased release of acid phosphatase from the lysosomes of UVL irradiated hairless mouse epidermis is demonstrated. The results indicate that lysosomal membrane stability is decreased when the hairless mouse is exposed to either acute or chronic UVL.

The lysosomes are single membrane, subcellular organelles which contain many acid hydrolases[2,3]. Ultraviolet light (UVL) is capable of lysing isolated rat liver lysosomes[4–6] causing release of their catalytic enzymes. Johnson[7] using mice, and Fand[8] using hairless mice, rats and human foreskin found that the acid phosphatase activity in skin exposed to 10 times the minimal erythema dose (MED) of UVL was lower than the activity from skin irradiated through window glass[7] or when the skin was protected with a sunscreen[8]. Histochemical studies of human skin, by Johnson and Daniels[9], indicated that the lysosomes of human skin exposed to 10 times the MED of UVL began significant lysis after 1 h post irradiation and increased to a peak at 6–8 h post exposure. Little effect of UVL was observed on mitochondrial 'marker' enzymes[7–9], indicating an accelerated leakage of acid phosphatase from the lysosomes. No attempt was made to determine the ultimate fate of the enzyme.

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