Pheromone Study on Acarid Mites

XVI. Identification of Hexyl Linolate in Acarid Mites and its Distribution among the Genus Tyrophagus

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\[ n\text{-Hexyl linolate, } n\text{-hexyl } (Z,Z)\text{-9, 12-octadecadienoate was identified commonly in all strains of four species of acarid mites belonging to the genus Tyrophagus; however, the compound was not detected in three other species of related acarid mites. Although origin and function of the ester is obscure, it may be one component of the exudate from the opisthodonotal gland and serve as a bio-solvent of the alarm pheromone present in the gland. The other possible function of the wax may be as a component of cuticle to prevent moisture loss from the body. The ester was demonstrated to be produced biosynthetically. This is the first example reported of the occurrence of \( n\text{-hexyl linolate in nature.} \]

INTRODUCTION

Many simple waxes are known in nature such as that in honeybee comb (Tulloch, 1970), in waxy body-coverings of scale insects (Tamaki, 1970; Takahashi and Nomura, 1982) and in a white woolen-yarn-like secretion of the mulberry sucker (Kuwahara 1980). Those esters function either as material for building blocks or as a kind of defensive secretion. Other types of esters are also known whose alcohol moieties are biologically active, such as bombykol linolate from pupal Bombyx mori females (Yamamoto et al., 1985), functioning possibly as a stored form of the pheromone bombykol, and ecdysone esters of several common fatty acids (Kubo et al., 1987) acting as metabolites of the artificially administered 20-hydroxyecdysone. There are other types of simple waxes which are components of the glandular secretion of many insects; their functions are not clear but they are assumed to be a “bio-solvent” for the active ingredient(s) of the gland, along with lower hydrocarbons.

Astigmatid mites possess a pair of opisthodonotal glands (formerly called latero-abdominal gland or oil gland) in their body, and several acarid mites use the gland exudate as their alarm pheromone and/or defense substance (Kuwahara et al., 1980). The active principles of five stored product mites have been identified as neryl formate and/or citral (Kuwahara et al., 1980; My-Yen et al., 1980). Isopiperitene is also known as the alarm pheromone of an agricultural pest mite Tyrophagus similis (Kuwahara et al., 1987). Natural product chemistry of the hexane extract, possibly the gland exudate of genus Tyrophagus, revealed the common presence of an unknown wax and squalene other than the alarm pheromone with \( C_{13}-C_{15} \) hydrocarbons (Howard et al.,...
Hexyl Linolate in Acarid Mites and Its Distribution 339

1988). Squalene is a common component found in dry yeast used for rearing mites, however, the wax is not detected in the yeast. The wax was identified and its distribution among related species was also studied.

MATERIALS AND METHODS

Mite species. Four species of genus *Tyrophagus*: *T. putrescentiae* (3 strains: strain 1, Shiga; strain 2, Goki; strain 3, Tokyo), *T. neiswanderi* (2 strains: strain 1, Hokkaido; strain 2, Katoerya), *T. similis* and *T. perniciosus* were used, along with *Aleuroglyphus ovatus*, *Carpoglyphus lactis*, and *Rhizoglyphus robini*. All mites except *C. lactis* and *R. robini* were reared by feeding dried yeast at 70–80% RH and at room temperature. *C. lactis* was reared on sugar and yeast (1:1) and *R. robini* on dry onion powder. One strain of *T. putrescentiae* was raised on dried onion powder to detect the effect of food on the composition of the gland exudates.

Extraction. Each species of mites was freed from its culture medium by a conventional saturated saline flotation method. The mite bodies, after weighing, were soaked in hexane for 3 min in order to avoid contamination of in-body materials such as triacylglycerol, and the hexane layer was collected by filtration. The residual mite bodies were then rinsed twice with a small amount of hexane. The combined hexane extracts with rinses from each mite were concentrated in vacuo and used separately either for isolation or for quantitative determination of the wax and hydrocarbons. Similar hexane extracts were also prepared as controls from dry yeast and dry onion powder.

Synthesis of hexyl linolate. Methyl linolate (1 g) was mixed with *n*-hexanol (5 g) with a catalytic amount of *p*-TsOH (50 mg); the mixture was then kept overnight at 150°C. After addition of ether and water, the ether layer was separated. The extract, after washing with satd. NaHCO₃ and drying over Na₂SO₄, was concentrated under reduced pressure. The residue was then transferred to an SiO₂ column, eluted with a hexane-ether (100:10) mixture to give hexyl linolate (1.1 g, purity by GLC >98%).

Analytical method. Gas liquid chromatograms were obtained by a Yanaco G-180F or a Hitachi 263-30 gas chromatograph (GLC) with flame ionization detector, using a conventional packed column (5% FFAP, 75 cm x 3 mm id) and chemically-bonded phase silica capillary columns (0.25 mm id x 25 m); FFAP (Quadrex) and CP-sil (Chrompack). All GLC data were processed using a SIC 7000B Intelligent Integrator, and the content of the wax and squalene were calculated. Mass spectra were obtained by a direct or a gas chromatograph introduction method (GC/MS), using a Hitachi 367-0200 gas chromatograph interfaced to a Hitachi M-80B High Resolution Mass Spectrometer operated at low and high resolution mode. An OV-1 bonded phase capillary column (0.25 mm id x 50 m, Gasukuro Kogyo) and 2% OV-1 column (3 mm id x 1 m, on Chromosorb W) were used for the GC/MS system.

Dimethyl disulfide derivative of the methylated natural wax was prepared and the position of double bond of the wax was elucidated by analyzing the mass spectrum, following the method reported (Vincenti et al, 1987).

Proton nuclear magnetic resonance (NMR) spectra were obtained using a JEOL FX-100 and a Bruker 500MHz NMR spectrometer. Samples were dissolved in CDCl₃ and TMS was used as the internal standard.

Infrared (IR) spectra were obtained as a liquid film on NaCl plates using a JASCO IRA-1 infrared spectrometer (Japan Spectroscopic Co.).
RESULTS

GLC analyses of crude hexane extracts of *T. putrescentiae* strain 2

Hexane extract (250 mg) of the mites (34.94 g), analyzed by GLC using a CP-sil capillary column at a temperature programmed condition as shown in Fig. 1, indicated two main peaks at $t_R 24.28$ min and 28.79 min. The former peak was isolated and identified as stated below. The latter peak, which was also detectable in dry yeast, was identified as squalene, based on GC/MS results ($M^+$ ion at $m/z$ 410, the characteristic base ion at $m/z$ 69, and fragmentation pattern) (Fig. 2), and NMR spectrum on a sample separately obtained from *A. ovatus*: $\delta$ 1.56 and 1.65 ($s$ 12H and $s$ 12H, allylic methyl), 2.00 (br. 20H, methylene) and 5.08 (br. 6H, vinyl).

Isolation of the ester from *T. putrescentiae* strain 2

The hexane extract (250 mg) was chromatographed on an SiO$_2$ column (5 g). The column was eluted with 50 ml each of hexane, mixtures of hexane and ether (10: 1, 1:1), ether and MeOH. The 10:1 fraction gave an oily material (70 mg) after evaporation of the solvent, and contained the ester. The material was again purified through a SiO$_2$ column (10 g) with the 10:1 mixture of hexane and ether by collecting 5 g of each of the eluates. The major ester was recovered in fractions No. 15–17 (31.49 mg) and was further purified by an SiO$_2$ column (5 g) using hexane and benzene (3:1). The ester was obtained in fraction No. 27–51, weighing 7.18 mg. In a separate experiment, 10 g of mites gave 38 mg of hexane extractables and 2.10 mg of the ester.

Structure elucidation

Molecular ion was observed at $m/z$ 364.3319 (calcd. for C$_{24}$H$_{43}$O$_{2}$; 364.3339) and following diagnostic ions: base ion at $m/z$ 43, 279 (10%), 263 (13%) and 262 (14%), along with characteristic n-hydrocarbon fragments (Fig. 2). The fragment at $m/z$ 279 is identifiable as $M^+$—C$_9$H$_{18}$, and those at $m/z$ 263 and 262 as elimination products of hexyloxy and hexanol, respectively. Therefore, the compound was suggested to be hexyl ester of octadecadienoic acid. The NMR spectrum (Fig. 3, 100 MHz) gave the following signals: at $\delta$ 5.35 (vinyl, 4H, m), 4.05 (alkoxy methylene, 2H, t, $J=6.5$ Hz),

![Fig. 1. A typical GLC of hexyl linolate and squalene from Tyrophagus putrescentiae strain 2 by CP-sil CB capillary column at temperature programmed condition (150–250/4°C). A: hexyl linolate, B: squalene.](image-url)
Fig. 2. GC/Mass spectra of hexyl linolate and squalene. Hitachi GC/MS M-80B, 70 eV. A: hexyl linolate, B: squalene.

Fig. 3. 100 MHz NMR spectrum of isolated hexyl linolate in CDCl₃. Partial spectra indicated in the figure were obtained at 500 MHz.

Fig. 4. Fragmentation pathway of cyclic reaction products from methyl linolate and dimethyl disulfide by mass spectrometry.
2.76 (homoallyl methylene, 2H, t, J=4.1 Hz), 2.29 (methylene adjacent to carbonyl, 2H, t, J=7.0 Hz), 2.04 (allyl methylene, 4H, br. m), 1.60 (methylene, 4H, br. t), 1.26–1.30 (long chain methylene, br) and 0.88 (terminal-methyl, 3H, t, J=5.8 Hz).

As a result, the compound was suggested to be n-hexyl octadecadienoate with methylene-separated diene in the acid moiety. Ir spectrum also supported this structure, indicating the presence of an ester carbonyl group (1,725 and 1,160 cm⁻¹).

Position of the double bond was assigned as 9 and 12 by mass spectrum, based on the diagnostic ions derived from cyclic reaction products of methyl octadecadienoate with dimethylsulfide. All diagnostic ions as shown in Fig. 4 except m/z 289 were observed in the mass spectrum with M⁺ ion at m/z 420 (42%) and the base ion at m/z 155. The geometry of two double bonds was also assigned as Z and Z, because of there being no absorption band around 970 cm⁻¹ by Ir spectrum and of the small coupling constants (11.5 Hz) for vinylic protons from the 500 MHz NMR spectrum.

The compound was reduced by LiAlH₄ in ether. Two products were detected by GLC, one of which gave the molecular ion at m/z 266 (13.6%) and the base ion at m/z 67, and therefore, was concluded to be octadecadienol. The other product which gave M⁺—H₂O at m/z 84 and base ion at m/z 56 was identified as n-hexanol. Both compounds were completely identical to those corresponding products from n-hexyl linolate by GC/MS and also by capillary GLC. Neutral fraction of alkaline hydrolysis also gave n-hexanol. Acid fraction after methylation gave the molecular ion at m/z 294 and the base ion at m/z 81, suggesting methyl octadecadienoate. The product also had the same retention time as methyl linolate by capillary GLC. The natural compound gave the same NMR spectrum as that of the synthetic n-hexyl linolate. The compound was therefore identified as n-hexyl linolate, n-hexyl (Z,Z)-9,12-octadecadienoate.

Distribution of the wax and squalene among acarid mites.

Mite species used were from two families, three subfamily and four genera. The hexane extract of each mite was directly analyzed by GLC using a 2% OV-1 packed column in isothermal condition (200°C), and the peak areas corresponding to hexyl linolate (tR; 6.29 min) and squalene (tR; 16.83 min) were quantitatively determined as shown in Table 1. Each corresponding peak was also confirmed by a capillary GLC (CP-sil) and by GC/MS. The wax was detected in all species and strains belonging genus Tyrophagus, however, no corresponding compounds were found in any of the other species (A. ovatus, C. lactis or R. rothi). The dry yeast used for rearing medium contained a considerable amount of squalene, but none of the ester corresponding to hexyl linolate. T. putrescentiae strain 2 reared on the dry onion powder in which neither squalene nor hexyl linolate was detected, contained a certain amount of the ester, indicating its origin was biosynthetic. Therefore, the presence of the ester was concluded to be a characteristic of genus Tyrophagus and the wax to be a biosynthetic product. Squalene was detected in varying degree in all mites fed on dry yeast.

DISCUSSION

Neryl myristate, a similar simple wax, is known in A. ovatus, although its function is unclear (Leal et al., 1988), and this is the second example of wax found in mites. Simple waxes of insects are classifiable into three categories as mentioned in the Intro-
Hexyl Linolate in Acarid Mites and Its Distribution

Table 1. Content of hexyl linolate and squalene in hexane extracts of astigmatid mites

<table>
<thead>
<tr>
<th>Mite</th>
<th>Hexyl linolate</th>
<th>Squalene</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mg/10 g</td>
<td>%</td>
</tr>
<tr>
<td>A. ooctus</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>C. lactis</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>R. robini</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>T. putrescentiae</td>
<td>st. 1</td>
<td>4.55</td>
</tr>
<tr>
<td></td>
<td>st. 2</td>
<td>2.10</td>
</tr>
<tr>
<td></td>
<td>st. 3</td>
<td>1.73</td>
</tr>
<tr>
<td>T. neiswanderi</td>
<td>st. 1</td>
<td>3.84</td>
</tr>
<tr>
<td></td>
<td>st. 2</td>
<td>3.13</td>
</tr>
<tr>
<td>T. similis</td>
<td></td>
<td>8.06</td>
</tr>
<tr>
<td>T. pernicius</td>
<td></td>
<td>2.13*</td>
</tr>
<tr>
<td>Dried yeast</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>Onion powder</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>T. putrescentiae</td>
<td>st. 2</td>
<td>5.90</td>
</tr>
</tbody>
</table>

*: Value obtained from the isolated material.
—: Not detected.

duction. The presence of hexyl linolate characteristic in genus Tyrophagus seems to be a third type. The role in the gland and/or on the body surface is at present obscure, because active ingredients of the opisthontotal gland in those mites are neryl formate, citral (Kuwahara et al., 1980 and 1988, and My-Yen et al., 1980), isopiperitenone (Kuwahara et al., 1988) and 2-hydroxy-6-methyl-benzaldehyde (Curtis et al., 1981 and Leal et al., in preparation), and none of these compounds is related to n-hexyl linolate as a precursor or degradation product.

There were no higher cuticular hydrocarbons (C20-C40) found among these Tyrophagus mites, which are common components of insect cuticles functioning to prevent moisture loss from the surface. Therefore, these waxes, neryl myristate and n-hexyl linolate, seem to function to protect the mite body from desiccation, although these mites were not tolerant to dryness.

From a biosynthetic viewpoint, n-hexyl linolate was supposed to be a product between linolic acid and the reduction product of hexanal derived from linolate by two successive enzyme reactions of lipoxygenase and fatty acid hydroperoxide lyase (Sekiya et al., 1986). The ability to reduce hexanal to hexanol is conceivable in these mites, judging from the presence of an aldehyde-reductase-like substance in Tyrophagus putrescentiae (Kuwahara et al., 1983). This is the first example known of the occurrence of n-hexyl linolate in nature.

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REFERENCES


