Neryl Formate: Alarm Pheromone of the Cheese Mite, *Tyrophagus putrescentiae* (Schrank) (Acarina, Acaridae)

The cheese mite, *Tyrophagus putrescentiae* (Schrank), is a cosmopolitan species capable of infesting many types of foods and stored products, and is commonly found in house dust[1]. While maintaining a culture of the mite, it was found that the mites including both sexes and nymph tended to aggregate into a colony in the feeding medium. Their aggregation was also observed even on a glass surface. If one or several mites at the centre of their aggregation were disturbed by patting with a soft drawing brush or squashed, aggregating mites around them escaped and eventually their aggregation came loose within several minutes, as shown in Figure 1. The same escaping behavior of the mites was observed when a small piece of filter paper impregnated with the pentane extract of mite bodies was placed at the centre of the aggregation on a glass plate (Figure 2). These findings suggested that the cheese mite contains an alarm pheromone in the body and excretes it on being disturbed[2]. We now report here the isolation and the chatacterization of neryl formate, as the alarm pheromone of the cheese mite.

Material and methods. The mites were fed on a mixture of rice bran and dry yeast (1:1 by volume) at 25 °C, 70–80% relative humidity in a rearing vial (13 cm in diameter, 7 cm in height) covered with a round glass plate having a 3 cm diameter hole in the centre which was sealed with a piece of thin paper for ventilation. The rearing vial was kept in a glass container (a desiccator was conveniently employed) to prevent escape and also to maintain the humidity.

Bioassay for the alarm pheromone activity was conducted on a round glass plate (13 cm in diameter) on which an appropriate number of the mites (more or less than 100,000) had been placed and kept for 1 h. Small filter papers (5 mm²) were impregnated with the candidate material by dipping for a *monent* into its successive 10-fold dilution series in pentane. After evaporation of the solvent, each filter paper was placed on the tested glass plate. Every 2 min the number of mites crawling over the tested paper was counted for 10 min. The alarm pheromone activity was evaluated by the lowest concentration of the tested solution at which repellency against mites was observed (see Table III).

Results and discussion. For the isolation of the pheromone, mites which escaped out of the culture medium and aggregated on the inside of the cover glass were brushed off everyday into a conical flask containing pentane and were stored. Mites thus collected (about 9 ml in sedimented volume) were filtered, and the residue was re-extracted twice with 30 ml of pentane. The combined filtrate was dried and concentrated through a short

[1] M. Sasa, S. Oshima, K. Matsumoto and R. N. Sinha, Jap. J. sanit. Zool. *18*, 216 (1967).
[2] C. G. Butler, Biol. Rev. *42*, 42 (1967).

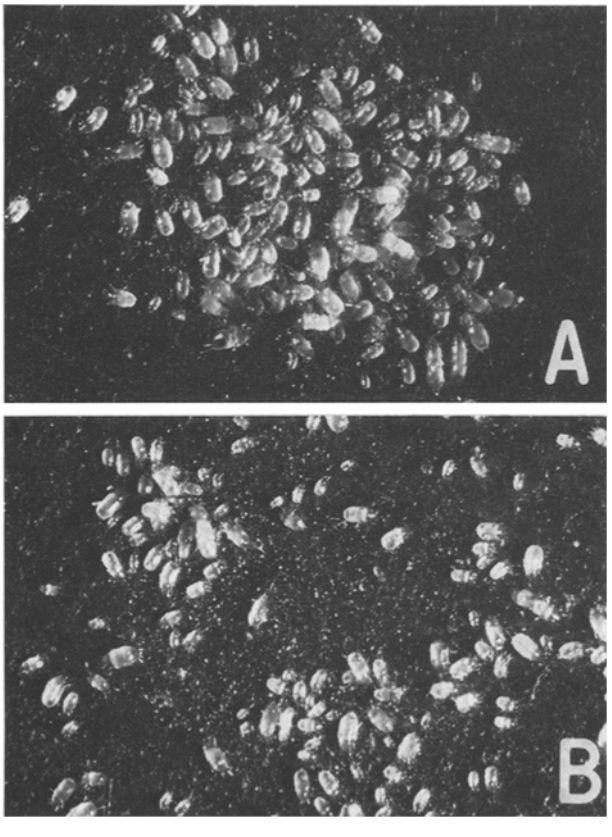

Fig. 1. A) indicates a colony just after several mites are disturbed at the centre of the aggregation, and B) shows 1 min later. Aggregation is about to come loose.

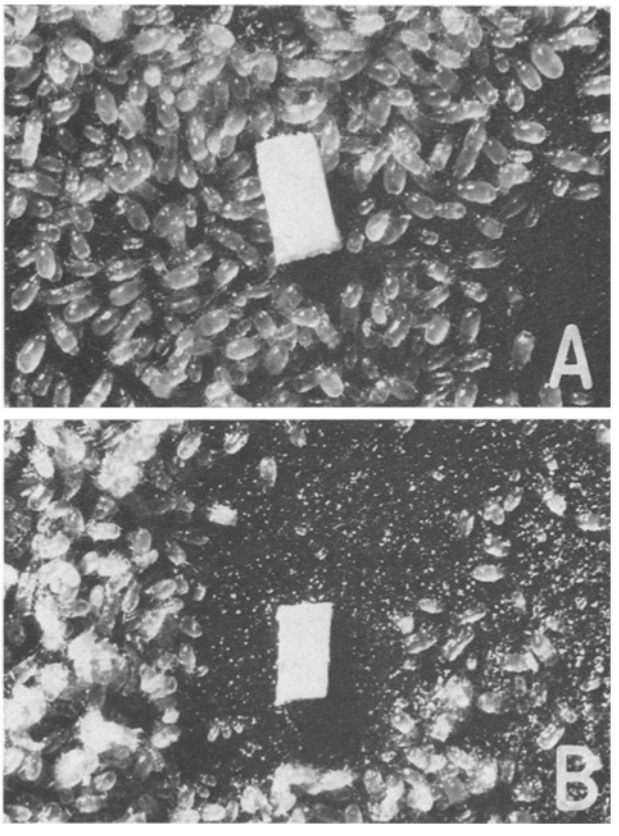

Fig. 2. A) indicates just after a small filter paper impregnated with the pentane extract of mite bodies is placed at the centre of the aggregation. B) shows 1 min later. Around the filter paper, all mites are evacuated.

Table I. Gas liquid chromatographic analysis of some monoterpenoids

Compound	Column temperature[a]	
	120 °C	100 °C
n-Decyl acetate	6.7 min	16.4 min
Lynalyl acetate	3.7	8.2
Geranyl acetate	9.7	24.3
Geranyl formate	7.6	18.0
Neryl acetate	8.4	20.5
Neryl formate	6.4	15.0
The isolated pheromone	6.4	15.0

[a] 75 cm in length × 3 mm in diameter, packed with 15% PEG-20 M, flow rate; 30 ml helium/min.

Table II. Identification of reaction products from the pheromone and neryl formate by gas liquid chromatography

Reaction product	Analytical condition	
	10% BDS (90 °C)	15% PEG-20M (100 °C)
Hydrogenation product		
from the pheromone	4.6 min	5.1 min
from neryl formate	4.5	5.1
Hydrolysis product		
from the pheromone	16.7	22.3
from neryl formate	16.4	22.3
Hydrogenation and hydrolysis product		
from the pheromone	7.8	11.0
from neryl formate	7.7	11.0
Ozonolysis product[a]		
from the pheromone	8.9	6.3
from neryl formate	8.9	6.2

[a] Identified as 4-pentanon-1-al by mass spectrometric analysis coupled with gas liquid chromatograph.

Table III. Alarm pheromone activity of the isolated pheromone and authentic neryl formate

Time after applying the tested paper	Number of mites crawling over the tested paper						
	Control	Neryl formate (ppm)			Isolated pheromone (ppm)		
(min)		0.1	1	10	0.1	1	10
2	9	12	8	0	7	10	0
4	18	16	17	2	>20	11	1
6	>20	>20	>20	3	>20	>20	0
8	>20	>20	>20	0	>20	>20	0
10	>20	>20	>20	2	>20	>20	0

Widmer column. The concentrate was chromatographed on a silicic acid column (10 g silicic acid; Mallinckrodt Ltd., 1.4 cm in diameter, 16 cm in height) by eluting successively with each 50 ml of ether: pentane mixture of the following ratio; 0:10, 0.5:10, 1:10, 2:10, 10:10, and finally with 50 ml of methanol. The bioassay results showed that the activity was found only in the fraction of ether:pentane 0.5:10 mixture. Further purification of this fraction was made by preparative thin layer chromatography on a Kieselgel-HF_{254} plate (0.25 mm thick, 5 × 20 cm) using ether pentane: 0.5:10 mixture as developing solvent. The pheromone activity was found in the fractional zone of Rf-value between 0.4–0.5, which was scraped off and extracted with ether to give, on evaporation, pure pheromone (121 µg), showing Rf; 0.44 (Rf of myristyl acetate; 0.38), and the relative retention time to *n*-decyl acetate on GLC; 0.91 (on PEG-20M at 100 °C with 30 ml helium per min flow rate) was obtained.

The mass spectrum of the isolated pheromone measured by a mass-spectrometer (Hitachi RMS-4) coupled with gas liquid chromatograph gave fragment ions at *m/e* 41 (C_3H_5), 69 (C_5H_9), 93 (C_7H_9), 121 (C_9H_{13}), and 136 ($C_{10}H_{16}$), of which 41 and 69 were base peak, and the highest mass ion 136 was not supposed to be the molecular ion but a radical ion resulted by elimination of a neutral fragment such as acid, water etc. This mass spectrum pattern is possibly suggestive of a doubly unsaturated acyclic monoterpene derivative for the structure of the pheromone[3].

The result of trial comparison of the isolated pheromone with several available monoterpenoids on GLC is shown in Table I, which indicates that the pheromone seems to be neryl formate. The Rf-value of the pheromone on TLC was also identical with that of authentic neryl formate. Further evidence was provided by comparison on GLC between each product of the following chemical reactions of the pheromone and authentic neryl formate; hydrogenation, hydrolysis with dilute alkali, hydrogenation followed by hydrolysis, and ozonolysis. As shown in Table II, it is concluded that the isolated pheromone is identical with neryl formate in all respects. The biological activity of neryl formate was also identical with that of the isolated pheromone as shown in Table III.

In the present study, neryl formate is demonstrated to be an alarm pheromone of the cheese mite. The natural occurrence of neryl formate in the animal kingdom is new.

Zusammenfassung. Aus Käsemilben, *Tyrophagus putrescentiae* (Schrank), wurde Nerylformiat isoliert und als Alarmpheromon erkannt.

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[3] G. R. WALLER, *Biochemical Application of Mass Spectrometry* (Wiley-Interscience, New York 1972), p. 354.

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