Zoological Studies **34**(3): 211-214 (1995)

Zoological Studies

Short Note

Chemical Constituents of the Defensive Secretions of the Metasternal Gland of the Stink Bug, *Helopeltis fasciaticollis* Poppius (Hemiptera: Miridae) in Taiwan

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(Accepted March 4, 1995)

Hsiao-Yung Ho, Ru-Shiou Tsai and Yien-Shing Chow (1995) Chemical constituents of the defensive secretions of the metasternal gland of the stink bug, *Helopeltis fasciaticollis* Poppius (Hemiptera: Miridae) in Taiwan. *Zoological Studies* **34**(3): 211-214. A mixture of compounds was found in the metasternal gland of a local stink bug, *Helopeltis fasciaticollis* Poppius (Hemiptera: Miridae). Seventeen components in the mixture were identified by gas chromatography/mass spectrometry (GC/MS) as belonging to different chemical groups: carboxylic acids, aliphatic esters, aliphatic alcohols, and aliphatic aldehydes. The three major compounds which comprised almost 95% of the secretion were acetic acid, 2-octenal, and octyl acetate.

Key words: Acetic acid, 2-Octenal, Octyl acetate.

Information is now available concerning the chemistry of the defensive secretions of over 300 species of carabid beetles (Blum 1981, Dettner 1987). While early characterizations have been restricted to major components of the mixtures, improved instrumentation has made possible the characterization of minor components, even with only a very small amount of sample material. We report here the chemical nature of compounds from the metasternal gland of a local stink bug, which feeds on tea or orange leaves. Large numbers of stink bugs usually stay on tea leaves in tea plantations during the summer. If disturbed, the bug will release a secretion with an unpleasant odor. In this paper, we identify by GC and GC/MS analysis seventeen components from the metasternal gland from samples of just a few microliters of the secretion.

Materials and methods—Adults of *H. fasciaticollis* (Fig. 1) were collected during the summer of 1990 from hills near Academia Sinica, Taipei, Taiwan. The metasternal scent gland was dissected and the contents of the gland were collected with a capillary tube, sealed and stored in a freezer at $-20°C$ for later chemical analysis. Before analysis, the seal was opened and the secretion was withdrawn by microsyringe and 0.5 μl of the secretion was introduced directly into the injector of a GC for analysis.

A Varian 3400 GC equipped with a flame ionization detector was used for the analysis of the components from the gland. Three capillary columns were used , i.e., 30 m × 0.25 mm (I.D.) fused capillary columns each coated with a 0.25 μm film of DB-5, DB-1701, and DB-WAX phase, respectively. GC-MS analysis was carried out on a Varian 3400 gas chromatograph coupled to a Finnigan MAT INCOS 50 mass spectrometer. GC-MS analysis was performed on a DB-5 fused silica capillary column (30 m × 0.25 mm). The oven temperature was held at 40°C for 3 min and programmed at 2°C/min to 120°C, then from 120°C to 280°C at 10°C/min, using helium as the carrier gas. Electron impact (EI) mass spectra were collected at 70 eV with the separator at 250°C and the ion source at 180°C. Chemical ionization (CI) mass spectra were obtained using methane as the reagent gas.

Standards of acetic acid, 2-pentenal, 2-heptenal, and 2-octenal were purchased from Aldrich Chemical Company. Hexanal, 2-hexenal, and 1-octanol were supplied by Sigma Chemical Co. Hexyl acetate, heptyl acetate, octyl acetate, nonyl acetate, 1-hexanol, octanal, 2-nonenal, 2-decenal, and 2-decenol were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 2-Decenyl acetate was synthesized by reacting 2-decenol with acetic anhydride at room temperature overnight, then the product was analyzed by GC-MS.

The relative amounts of each component of the gland secretion were estimated by the built-in computer according to the area on the gas chromatogram.

Results and Discussion—The gas chromatogram of the chemical components of the metasternal gland of the male *H. fasciaticollis* is shown in Fig. 2. Since the molecular ions of some components were not detectable on the EI mass spectra, their molecular weights were determined according to the CI mass spectra. The mass spectral data and the molecular weights of the components are listed in Table 1. A possible structure of each compound was postulated based on its mass

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spectrum through a library search of the NIST data system. Subsequently, seventeen of the eighteen compounds were cross-checked by comparison of the GC retention times and mass spectral data of the natural products with those of the authentic standards under the same GC conditions. This was not done for the component labeled as No. 13. The structure of this thirteenth peak on the gas chromatogram was postulated as 2-octenyl acetate according to the mass spectrum, but no authentic sample was compared for positive identification.

All of the components are listed in Table 2 and classified according to their carbon lengths and functional group. We can see from the table that the mixture is composed of aldehydes, esters, acids, and alcohols. The major components are acetic acid, n-octyl acetate, and 2-octenal. These three components comprise about 95% of the total secretion. All of the eighteen compounds except heptyl acetate were reported as defensive compounds of arthropods by Blum (1981). Heptyl acetate has been found in the defensive secretion of a carabid beetle, *Helluomorphoides clairvillei* (Attygalle et al. 1992). It also has been reported that the volatiles in the secretion of adult *Leptocoris apicalis* (Hemiptera: Coreidae) contain 40% octyl acetate and 40% 2-octenal, accompanied by 2-decenal as a minor concomitant (Baggini et al. 1966), which is quite similar to the present case. Both bugs belong to the order Hemiptera. The compound 2-octenal has been found in the metasternal scent gland of the adult meadow plant bug, *Leptopterna dolabrata* (Hemiptera: Miridae) (Collins and Drake 1965). *L. dolabrata* and *H. fasciaticollis* are both Miridae. There are quite a few insects which use 2-octenal for defense, including insects in the orders Dictyoptera and Hemiptera (Wallbank and Waterhouse 1970, and references in Blum 1981). Octyl acetate has also been detected in the defensive secretions of some Hemiptera (Blum 1981). Thus, the major components of the secretion described in this paper are quite common in Hemiptera.

Fig. 1. The adult stink bug, *Helopeltis fasciaticollis* Poppius.

The minor components in the metasternal gland secretion include several homologs of 2-octenal and octyl acetate. In these homologs, we can see that compounds with chain lengths of even carbon numbers (C-6, C-8, C-10) are more numerous than compounds with chain lengths of odd carbon numbers (C-5, C-7, C-9). In the case of alcohol and saturated aldehyde, only C-6, C-8, and C-10 homologs were detected. It is also interesting that carbon chains of eight are most abundant in all four categories of chemicals, i.e. aldehyde, en-aldehyde, alcohol, and acetate, compounds. This may be due to factors during the biosynthesis of these components in the gland tissue and should be investigated in the future.

Recently, (E)-4-oxo-2-decenal was found to function as an attractant and an arrestant at physiological doses on second

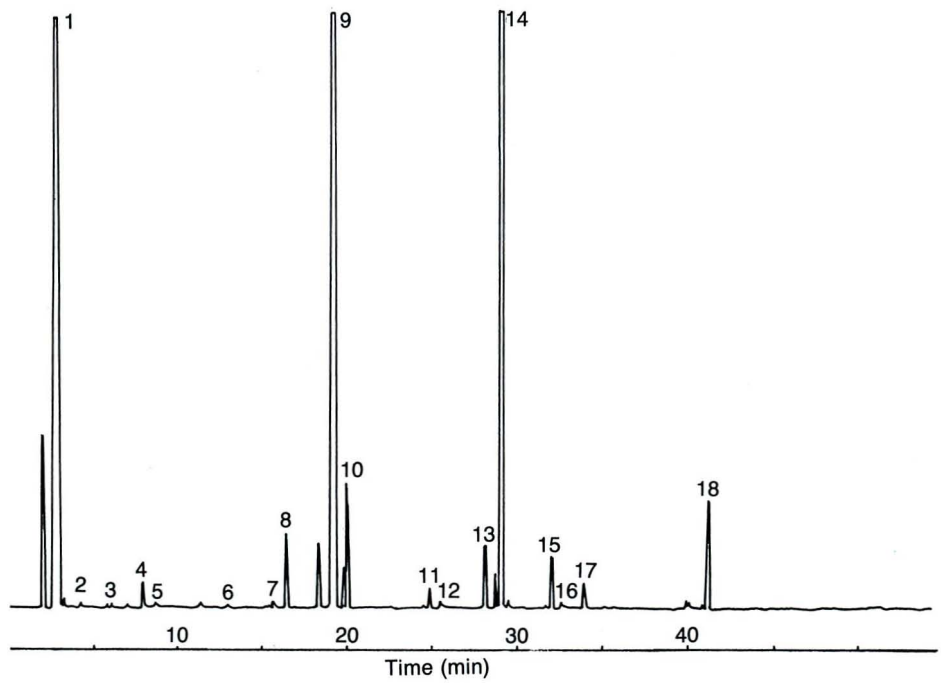

Fig. 2. Gas chromatogram of the chemical constituents of the metasternal gland of the stink bug, *Helopeltis fasciaticollis* Poppius (Hemiptera: Miridae) in Taiwan. Gas chromatography was done on a DB-5 fused silica capillary column (30 m × 0.25 mm). The oven temperature was held at 40°C for 3 min and programmed at 2°C/min to 120°C, then from 120°C to 280°C at 10°C/min.

Table 1. Chemical components of the metasternal gland of *Helopeltis fasciaticollis* and spectral evidence for structure assignments

No.	compound	content (%)	M.W.	mass spectrum (m/z, %)
1	acetic acid	15.3	60	60(M^+,43),45(87),43(100)
2	2-pentenal	0.005	84	84(M^+,100),83(45),69(23),55(68),41(55)
3	hexanal	0.008	100	100(M^+,1),56(75),41(100)
4	2-hexenal	0.11	98	98(M^+,7),83(47),69(30),55(95),41(100)
5	hexanol	0.019	102	84(M^+-18,7),69(58),56(100),43(60)
6	2-heptenal	0.013	112	112(M^+,2),97(4),83(55),68(38),55(74),41(100)
7	octanal	0.031	128	128(M^+,3),110(3),84(30),69(42),55(90),41(100)
8	hexyl acetate	0.49	144	83(M^+-61,3),69(12),61(13),56(29),43(100)
9	2-octenal	59.4	126	111(M^+-15,2),96(12),83(41),70(51),55(75),41(100)
10	octanol	0.98	130	112(M^+-18),97(3),69(40),56(70);41(100)
11	heptyl acetate	0.15	158	158(M^+,1),98(4),70(17),61(12),43(100)
12	2-nonenal	0.05	140	112(M^+-28,1),98(9),70(58),55(90),41(100)
13	2-octenyl acetate	0.47	170	110(M^+-60,20),95(45),81(28),67(28),54(45),43(100)
14	octyl acetate	19.7	172	112(M^+-60,5),84(17),70(22),61(18),43(100)
15	2-decenal	0.4	154	136(M^+-18,0.6),110(3),98(6),83(27),57(70),41(100)
16	2-decenol	0.06	156	156(M^+,0.1),138(15),109(7),95(22),82(38),57(100)
17	nonyl acetate	0.17	186	98(M^+-88,7),83(9),70(17),61(17),43(100)
18	2-decenyl acetate	0.87	198	156(M^+-42,4),138(M^+-60,5),127(4),110(27),96(38),81(38),67(40),43(100)

Table 2. Chemical components of the metasternal gland of *Helopeltis fasciaticollis* classified according to carbon chain length and functional group

class	C-3	C-5	C-6	C-7	C-8	C-9	C-10
acid	acetic acid (15.3)	—	—	—	—	—	—
aldehyde	—	—	hexanal (0.008)	—	octanal (0.031)	—	—
en-aldehyde	—	2-pentenal (0.005)	2-hexenal (0.11)	2-heptenal (0.013)	2-octenal (59.4)	2-nonenal (0.05)	2-decenal (0.4)
alcohol	—	—	1-hexanol (0.019)	—	1-octanol (0.98)	—	2-decenol (0.06)
acetate	—	—	hexyl acetate (0.49)	heptyl acetate (0.15)	octyl acetate (19.7) 2-octenyl acetate (0.47)	nonyl acetate (0.17)	2-decenyl acetate (0.87)

Number in the parenthesis is the relative amount of each component.

instar of the nymphs of the southern green stink bug, *Nezara viridula* (L.) (Pavis et al. 1994). (E)-2-decenal was also found in the nymph of that bug. The physiological importance of the chemicals in the gland during the nymph stage of *H. fasciaticollis* should also be investigated.

In an earlier report on the chemical identification of defensive secretions of a local stick insect (Ho and Chow 1993), the major component, actinidine, was also found in a plant of the stick insect's diet, but at a lower concentration. It was concluded that the stick insect concentrated actinidine from the plant for defensive purposes. In the present case, it would be interesting to know the relationship between plants in the stink bug's diet and the chemical constituents of the metasternal gland.

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臺灣茶角盲椿象 *Helopeltis fasciaticollis* Poppius (Hemiptera: Miridae) 臭腺化學成分之研究

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臺灣茶角盲椿象臭腺中含有複雜的化學成分。本文應用氣相層析質譜法分析並鑑定出十七種化合物。其中包括酸類，酯類，醇類以及醛類。主要成分是乙酸，2-辛烯醛和乙酸辛酯。三者加起來佔總含量的百分之九十五。

關鍵字：乙酸, 2-辛烯醛, 乙酸辛酯。

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