

# Semiochemistry of Cabbage Bugs (Heteroptera: Pentatomidae: *Eurydema* and *Murgantia*)<sup>1, 2, 3</sup>

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J. Entomol. Sci. 31(2): 172-182 (April 1996)

**ABSTRACT** The semiochemistry of the common North American pest of crucifers, *Murgantia histrionica* (Hahn) (the harlequin bug), and two related European species, *Eurydema ventrale* L. and *E. oleraceum* L., was investigated. The metathoracic scent glands of these warningly-colored stink bugs (Pentatomidae) are smaller than the scent glands of most cryptically-colored pentatomids, and the secretions from the scent glands of *Murgantia* and *Eurydema* species include two heretofore unknown natural products: (2*E*,6*E*)-octadienedial and (2*E*,6*E*)-octadiene-1,8-diol diacetate. It also was discovered that when harlequin bug adults are squeezed, they expel a frothy fluid from the margins of the prothorax with a distinctive odor due to the presence of 2-*sec*-butyl-3-methoxypyrazine and 2-isopropyl-3-methoxypyrazine. The presence of alkylmethoxypyrazines in the expelled fluid of harlequin bugs extends the known distribution of these compounds to include the Heteroptera, and strengthens the argument that this class of pyrazines constitute a universal warning order equivalent to the color red as a visual warning signal.

**KEY WORDS** Harlequin bug, allomone, pheromone, pyrazine, aposematic, scent gland, octadienedial, diacetate

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The harlequin bug, *Murgantia histrionica* (Hahn) (Heteroptera: Pentatomidae), is an important pest of cabbage, broccoli, and other cole crops in the United States (McPherson 1982), as are some *Eurydema* species in Europe and Asia (e.g., Hori et al. 1984, Bonnemaïson 1952). In fact, these are closely-related genera whose members specialize on crucifers, and related plants such as cappers (Capparaceae) (English-Loeb and Collier 1987), containing mustard oil glycosides (glucosinolates) (Louda and Mole 1991). Despite the economic importance of these insects, the semiochemistry of only two Japanese *Eurydema* spp. has been examined, and this research was conducted prior to the advent of capillary column gas chromatography (Ishiwatari 1974, 1976).

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<sup>1</sup> This paper is dedicated to Dr. Murray S. Blum, University of Georgia, on the occasion of his retirement. The senior author wishes to thank Murray for letting him pursue his interest in bugs as a graduate student, and for all the encouragement over the years.

<sup>2</sup> Received 25 July 1995; Accepted for publication 22 January 1996.

<sup>3</sup> Mention of commercial products does not necessarily constitute endorsement by the USDA.

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As their name implies, most stink bug adults (Pentatomidae) have highly developed metathoracic scent glands, but *M. histrionica* and *Eurydema* spp. are exceptional in this respect. These conspicuously colored bugs have less "evaporative" cuticle surrounding the scent gland openings than do most pentatomids (Schaefer 1972), and the gland reservoir is much smaller in *Eurydema* and *Murgantia* species than in cryptically-colored stink bugs (Bonnemaïson 1952, Aldrich unpublished observations). Sequestration of mustard oils may render *Eurydema* and *Murgantia* bugs unpalatable to predators (Bowers 1990), relaxing the selection pressure for production of the usual stink bug defensive chemicals (Aldrich 1988).

Recently, one of us (JRA) had the opportunity to collect *Eurydema* spp. in Italy, and to chemically analyze their exocrine gland secretions. Similar research was subsequently conducted with adult harlequin bugs in the U.S. We now report discovery of novel bifunctional natural products from these insects, and the identification of warning odorants (alkylmethoxypyrazines) in a previously overlooked secretion released from the prothorax of adult harlequin bugs.

### Materials and Methods

*Eurydema ventrale* L. and *E. oleraceum* L. adults were found on various wild crucifers in and around Perugia and Colifiorito, Italy, July through August, 1994. Adult and late-instar harlequin bugs were collected from cabbage at an organic farm in Anne Arundel Co. MD, in late August, 1994. Bugs were maintained on fresh host-plant material until dissection.

Metathoracic scent glands for gas chromatography (GC) and GC-mass spectrometry (GC-MS) were excised from CO<sub>2</sub>-anesthetized bugs submerged in tap water, and extracted in approximately 100 µl of hexane or CH<sub>2</sub>Cl<sub>2</sub> (5 to 10 glands per sample). Samples were analyzed on a DB-5™ column (0.25 µm film, 30-m × 0.25-mm ID; J&W Scientific, Folsom, CA) in a Varian 3500 GC with helium as carrier (50 cm/sec linear velocity), a temperature program from 50°C for 2 min to 235°C at 15°/min, with a flame ionization detector. Data were recorded using the Varian GC Star Workstation™ software on a Gateway 2000™ 386/25 computer. Electron impact MS (EI-MS) were obtained using Hewlett Packard 5890 GC-MS instruments at 70 eV, with HP-5™ columns (instrument in USA: 0.11 µm film; 25-m × 0.2-mm ID; instrument in Italy: 0.25 µm film; 30-m × 0.25-mm ID), programmed from 50°C for 2 min to 250°C at 15°/min. Chemical ionization MS (CI-MS) were collected using a Finnigan 4510 GC-MS equipped with an INCOS Data System, a 30-m DB-1 column programmed from 60°C for 2 min to 250°C at 5°/min, and using either NH<sub>3</sub> or ND<sub>3</sub> as reagent gas.

Prothoracic fluid from harlequin bugs squeezed with forceps was collected in micropipettes, and expelled into 50 to 100 µl of HPLC-grade water for analysis of alkylmethoxypyrazines. An Empore™ extraction disk (SDB-XC, 1-cm OD, 3M Company, St. Paul, MN) was placed in a 1-ml glass-fritted funnel, washed with 0.5 ml aliquots of diethylether, methanol, and water (HPLC-grade MeOH and H<sub>2</sub>O). Solvents were filtered through the disk by applying a slight positive pressure above the liquid layer. Prothoracic fluid samples were added to the funnel and filtered through the disk by positive pressure. Organic compounds

adsorbed on the disk were eluted with approximately 300  $\mu$ l of freshly-distilled ether. Extracts were dried over sodium sulphate and concentrated to 1 to 2  $\mu$ l for injection into the GC-MS. One sample was prepared from 101 harlequin bug females, and two samples were processed similarly for groups of 37 and 60 harlequin bug males. These samples were analyzed on a Finnigan INCOS XL GC-MS instrument operated in the EI mode at 70 eV, with a 60-m DB-1™ column, helium as carrier (50 cm/sec), a temperature program from 50°C for 2 min to 230°C at 5°/min.

Identifications of previously known natural products were verified by comparisons to commercial standards: benzyl alcohol, nonanal,  $\alpha$ -pinene, *n*-dodecane, *n*-tridecane, 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine, and 2-isobutyl-3-methoxypyrazine (Aldrich Chemical Company, Milwaukee, WI); (*E*)-2-hexenal, (*E*)-2-octenal, and (*E*)-2-octenyl acetate (Bedoukian Research Inc., Danbury, CT); (*E*)-2-octenoic acid (California Aromatics and Flavors, Belleville, NJ). Identifications of new natural products were verified by comparisons to synthetic standards prepared as follows. Dimethyl 2,6-octadieneoate was prepared (Scheffer and Wostadowski 1972), giving a mixture of *E/Z*-isomers. The identities of the isomers was verified by comparison to standards provided by Dr. L. A. Paquette, Ohio State University, and, as reported, dimethyl (2*E*,6*E*)-octadieneoate was the predominant isomer (ca. 80%). Dimethyl (2*E*,6*E*)-octadieneoate was purified by flash chromatography (> 95% by GC) and reduced by diisobutylaluminum hydride to the corresponding (2*E*,6*E*)-octadienediol (Miller et al. 1959). (2*E*,6*E*)-Octadienyl diacetate was prepared by acetylation of the diol with acetyl chloride and pyridine in ether by standard procedures. (2*E*,6*E*)-Octadienedial was prepared by oxidation of the diol with active MnO<sub>2</sub> in CH<sub>2</sub>Cl<sub>2</sub> at room temperature (Chan et al. 1968).

## Results

The metathoracic scent glands of *E. ventrale*, *E. oleraceum*, and *M. histrionica* are morphologically similar, with no obvious differences between the sexes. The gland reservoir is reduced relative to that of other pentatomids, especially medially, creating a bilobed appearance (Bonnemaison 1952). However, the pair of accessory glands which, in other pentatomids are thought to empty into the reservoir (Aldrich et al. 1978), are at least as large as in other pentatomids, and are attached close to the external opening of the reservoir.

While dissecting *Eurydema* spp. in Italy, one of us (JRA) noticed that the bugs produced an odor reminiscent of that from the blood of ladybird beetles (Coccinellide). It was assumed that this odor was due to constituents of the methathoracic scent gland secretion. However, another of us (JCG) observed that harlequin bugs collected in the field produced a frothy material with an odor unlike that from the metathoracic scent gland secretion of carefully anesthetized and dissected individuals. Further examination showed that when *M. histrionica* adults are gripped dorso-ventrally with forceps and squeezed, a drop of this fluid is exuded from each epiprothorax. The fluid usually includes bubbles, suggesting that its emission may somehow be connected to the tracheal system (Fig. 1). This fluid has the unmistakable odor attributed to lady

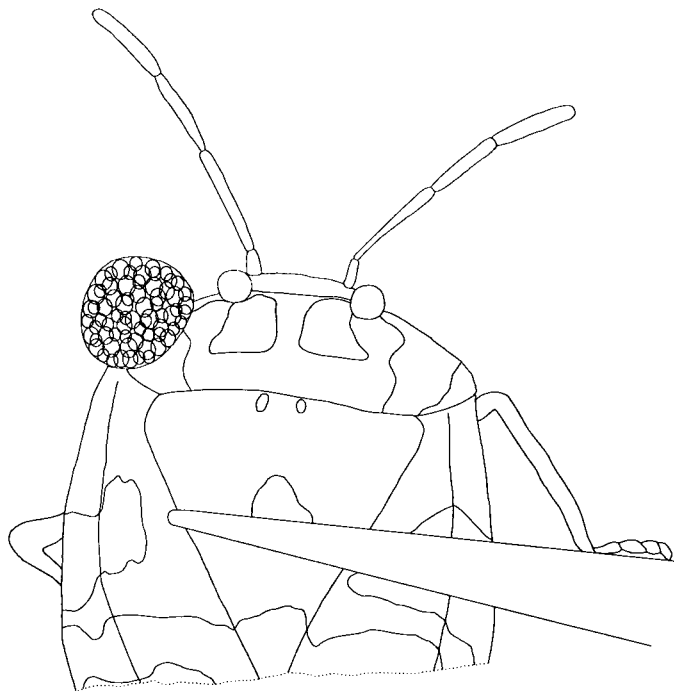


Fig. 1. Dorsal view of a *Murgantia histrionica* adult showing the position of fluid froth emitted from the left prothoracic margin when the bug was squeezed with forceps.

beetles from alkylmethoxy-pyrazines (Moore et al. 1990). Blood from harlequin bug adults (e.g., a cut leg) did not smell like alkylmethoxy-pyrazines. Fourth and fifth-instars also produced fluid from the anterior margin of the pronotum when squeezed, but this fluid lacked air bubbles, nor did it possess the distinctive methoxy-pyrazine odor (J. Aldrich, personal observation).

The scent gland secretion from the two *Eurydema* spp. examined here are considerably different from each other (Fig. 2). *Eurydema oleraceum* produces a blend typical of stink bugs, except for  $\alpha$ -pinene (compound 2, Fig. 2A). The predominant compounds are (*E*)-2-octenal (4), (*E*)-2-octenyl acetate (8), and *n*-dodecane (11) (Fig. 2A). On the other hand, the scent gland secretion of *E. ventrale* is very unusual (Fig. 2B); benzyl alcohol (3) is a prominent component, along with two new natural products (10 and 12). The CI-MS of (10) indicated a molecular weight (MW) of 138 with no exchangeable protons;  $m/z$  (%) 294 ( $[2M + NH_4]^+$ , 5%), 173 ( $[M + (NH_3)_2H]^+$ , 100), and 156 ( $[M + NH_4]^+$ , 85). The CI-MS of (12) indicated a MW = 226 with no exchangeable protons;  $m/z$  (%) 261 ( $[M + (NH_3)_2H]^+$ , 8), and 244 ( $[M + NH_4]^+$ , 100). The EI-MS of (12) matched the spectrum of (2*E*,6*E*)-octadiene-1,8-diol diacetate in the proprietary Hewlett-Packard™ computerized mass spectral library (Baltes et al. 1979), suggesting

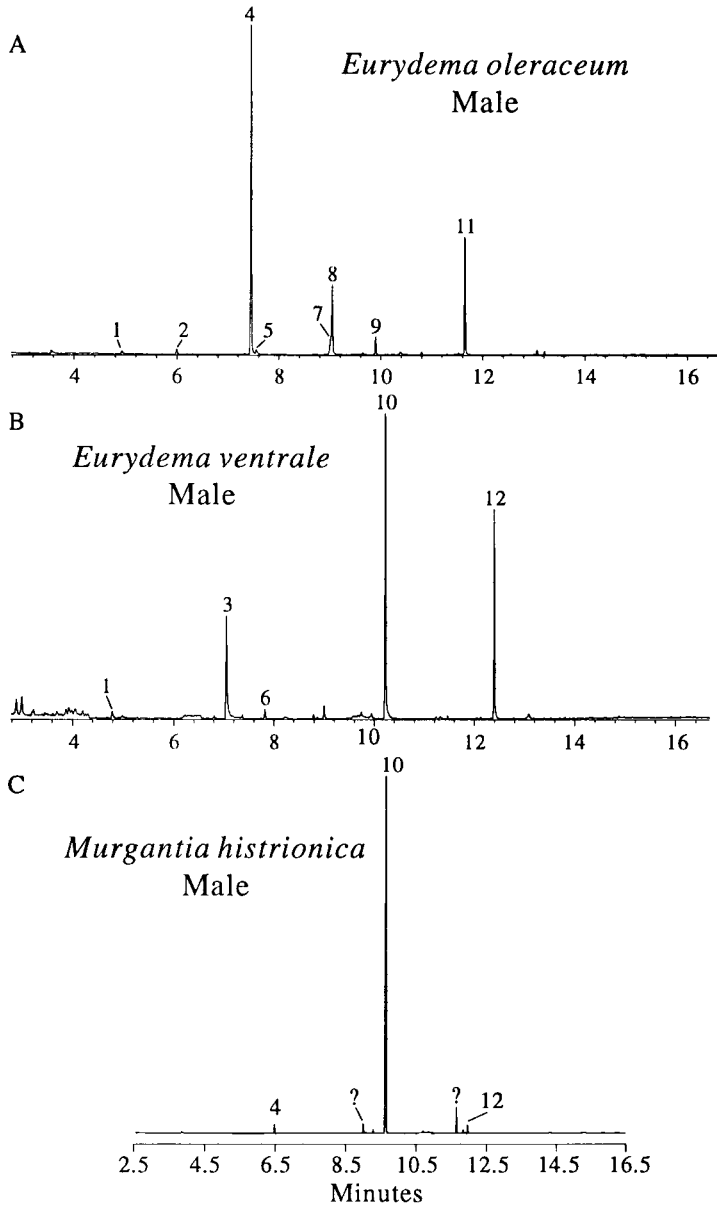


Fig. 2. Gas chromatograms of methathoracic scent gland extracts from (A) seven *Eurydema oleraceum* males, (B) seven *E. ventrale* males and, (C) one *Murgantia histrionica* male (1 = (*E*)-2-hexenal, 2 =  $\alpha$ -pinene, 3 = benzyl alcohol, 4 = (*E*)-2-octenal, 5 = (*E*)-2-octenol, 6 = nonanal, 7 = (*E*)-2-octenoic acid, 8 = (*E*)-2-octenyl acetate, 9 = tridecane, 10 = (2*E*,6*E*)-octadienedial, 11 = pentadecane, and 12 = (2*E*,6*E*)-octadiene-1,8-diol diacetate).

that (10) could be the corresponding dialdehyde. The four isomers of synthetic 2,6-octadiene-1,8-diol diacetate were separated on the HP-5<sup>TM</sup> column of the GC-MS. The major (2*E*,6*E*)-isomer eluted last (11.9 min), the (2*Z*,6*Z*)-isomer eluted first (10.8 min), and the mixed geometrical isomer had an intermediate retention time. Synthetic standards of (2*E*,6*E*)-octadienedial and (2*E*,6*E*)-octadiene-1,8-diol diacetate produced mass spectra and had retention times on the HP-5 and DB-5 column matching those of natural products (10) and (12), respectively (Fig. 3). The scent gland secretion of *M. histrionica* is like that of *E. ventrale*, except that (2*E*,6*E*)-octadienedial (10) is by far the major component (>85% by GC peak area integration). Nevertheless, a trace (ca. 1%) of (2*E*,6*E*)-octadiene-1,8-diol diacetate (12) is present (Fig. 2C).

Under our GC-MS conditions, standards of 2-isopropyl-3-methoxypyrazine, 2-*sec*-butyl-3-methoxypyrazine (Fig. 4A), and 2-isobutyl-3-methoxypyrazine (1 ng/μl hexane) eluted at 17 min, 19 min 33 sec, and 19 min 47 sec, respectively. Compounds from the prothoracic fluid of 60 male harlequin bugs that eluted at 17 min 3 sec and 19 min 34 sec (Fig. 4B) produced EI-MS matching spectra of 2-isopropyl-3-methoxypyrazine and 2-*sec*-butyl-3-methoxypyrazine (Fig. 4A), respectively, with the latter component being about 4 times more concentrated than the former. The efficiency of our extraction procedure for alkylmethoxypyrazines was not determined; however, based on the ion abundances of the EI-MS from the standard and natural product, we estimate that harlequin bug males release on the order of 0.25 ng of 2-*sec*-butyl-3-methoxypyrazine/bug. 2-*sec*-Butyl-3-methoxypyrazine was also detected from the sample of 101 *M. histrionica* females, but 2-isopropyl-3-methoxypyrazine was not detected.

In the sample of 101 harlequin bug females, and in the two samples processed similarly for groups of 37 and 60 harlequin bug males, the three most abundant compounds produced EI-MS suggestive of mustard oils. The mass spectra of these compounds matched published spectra of *sec*-butyl isothiocyanate (Kjaer 1963), methylthiopropyl nitrile, and methylthiobutyl nitrile (Spencer and Daxenbichler 1980), respectively. These volatiles correspond to glucosinolates commonly found in cabbage (Fenwick et al. 1983).

## Discussion

Dialdehydes are known from ants (dolichodial and iridodial), beetles (chrysolimial), and termites (ancistrodial and cavaiidial), but these compounds are all cyclic mono- and sesquiterpenoid molecules quite unlike (2*E*,6*E*)-octadienedial (Wheeler and Duffield 1985). Male olive fruit flies, *Dacus oleae* (Geml.) (Tephritidae), produce the diethyl ester of 5-oxo-1,9-nonadioic acid (Gariboldi et al. 1983), and many birds and mammals secrete high molecular weight diester waxes (Jacob 1978, Nicolaidis et al. 1969), but these compounds are structurally distinct from the scent gland diacetate of *Eurydema* and *Murgantia* bugs. A search of the Chemical Abstracts database for (2*E*,6*E*)-octadienedial and (2*E*,6*E*)-octadiene-1,8-diol diacetate retrieved only 3 references to these compounds, none of which involved natural product chemistry (Baltes et al. 1979). To our knowledge, volatile straight-chain dialdehydes and diol esters are previously unknown in insects. However, we have recently identified 9-hydroxygeranyl diacetate (2,6-dimethyl-2*E*,6*E*-octadiene-1,8-diol diacetate), and

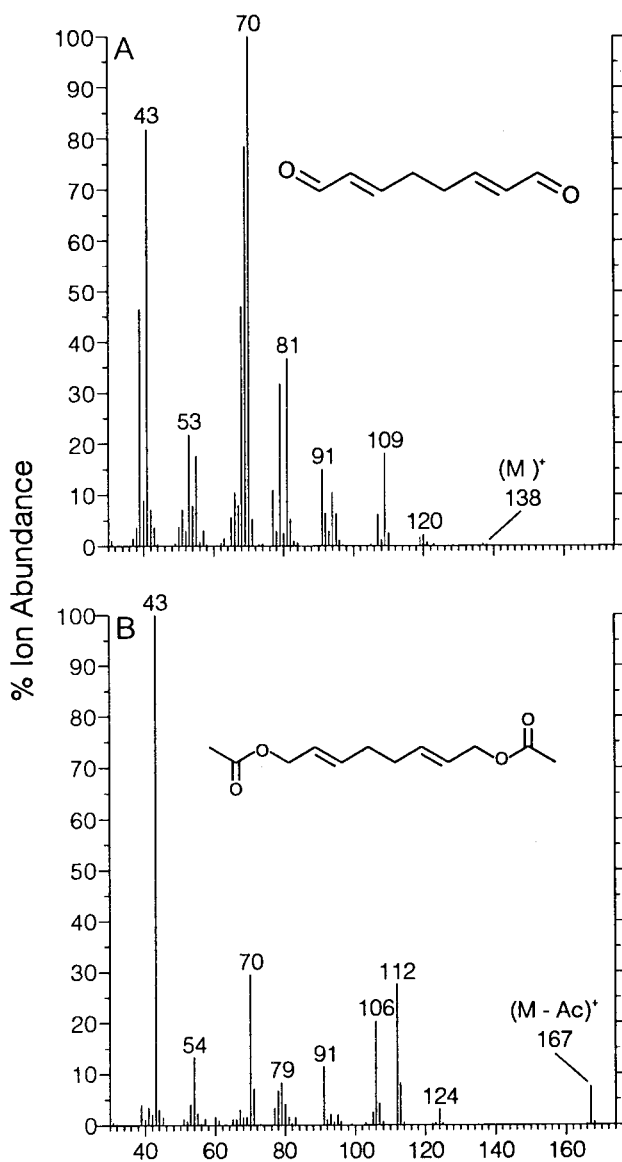


Fig. 3. The electron impact mass spectra of synthetic standards of (2E,6E)-octadienedial (A), and (2E,6E)-octadiene-1,8-diol diacetate (B).

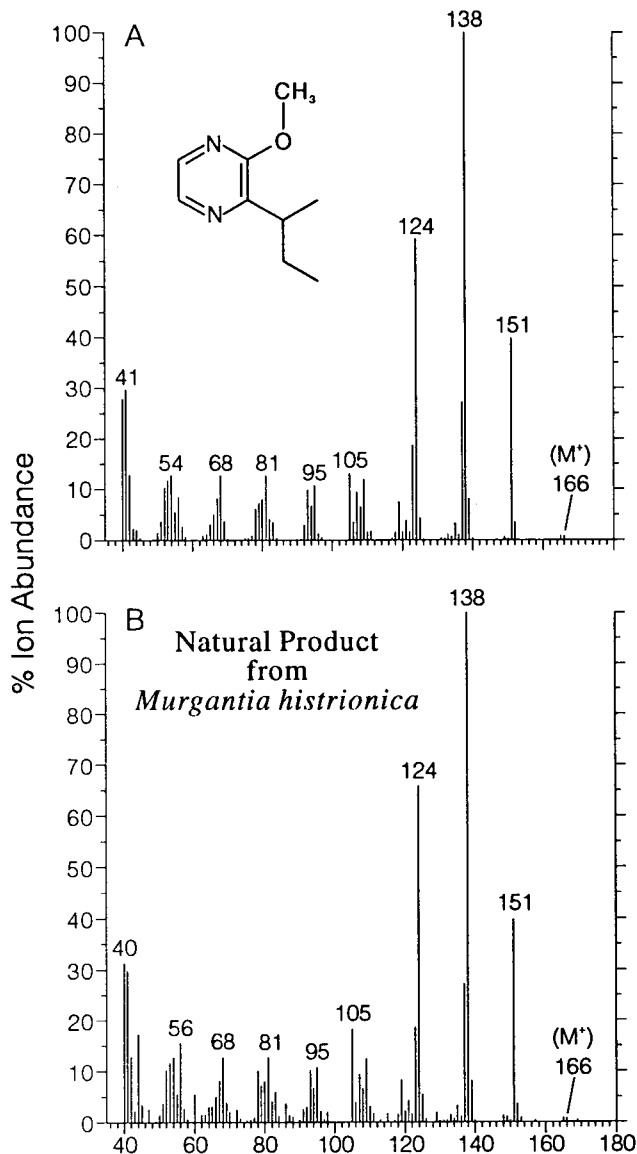


Fig. 4. The electron impact mass spectrum of a commercial standard of 2-sec-butyl-3-methoxypyrazine (A), and the natural product isolated from the prothoracic exudate from 60 *Murgantia histrionica* males.



related dibutyrate and diols from males of *Oechalia schellenbergii* (Guérin-Ménéville) (Pentatomidae), a common predator in Australia and South Pacific islands (Aldrich et al. 1996).

Our observation that squeezing *M. histrionica* adults causes them to release fluid dorso-laterally from the prothorax is apparently a new discovery for this group of insects. A similar phenomenon has been documented for various aposematic lygaeid bugs, most thoroughly for the large milkweed bug, *Oncopeltus fasciatus* (Dallas). These bugs sequester cardiac glycosides from Asclepiadaceae and, when attacked, exude discrete droplets laden with the poisons from intersegmental weak points (Duffey 1980, Scudder et al. 1986). Mass spectral data indicate that the fluid exudate from harlequin bugs is laden with the aglucones of glucosinolates, which probably renders the secretion repulsive to at least some vertebrate predators (Fenwick et al. 1983, Louda and Mole 1991).

We also were interested in identifying the distinctive odor principles of the adult harlequin bug prothoracic secretion and were successful in doing so: 2-sec-butyl-3-methoxy-pyrazine is primarily responsible for the odor, along with 2-isopropyl-3-methoxy-pyrazine. The presence of known alkylmethoxy-pyrazines in the expelled fluid of harlequin bugs extends the distribution of these intensely organoleptic compounds to include the Heteroptera and strengthens the argument that this class of pyrazines constitute a universal warning odor equivalent to the color red as a visual warning signal (Guilford et al. 1987, Moore et al. 1990). We suspect that *E. ventrale* has an exudate system similar to that of *M. histrionica* and that alkylmethoxy-pyrazines occur in many other aposematic Heteroptera.

Why did *Murgantia histrionica* and *Eurydema ventrale* evolve dialdehydes and diol esters? Again, the situation in the large milkweed bug, *O. fasciatus*, may provide a clue. *Oncopeltus fasciatus* is among the few bugs whose metathoracic scent gland secretion is sexually dimorphic, perhaps because sequestration of host-plant toxins superseded the need for *de novo* synthesis of defensive compounds; the secretion of males is enhanced in acetate esters, while the secretion of females contains mainly the corresponding aldehydes (Games and Staddon 1973). Although the precise significance of this chemical dimorphism has yet to be positively established, circumstantial evidence suggests that milkweed bug males attract females with a pheromone (Aldrich 1988). There is some indication that the metathoracic scent gland secretions of *Murgantia* and *Eurydema* bugs are sexually dimorphic (Aldrich unpublished data); therefore, the possibility that these secretions are attractant pheromones is currently under investigation.

### Acknowledgments

We thank L. A. Paquette, Ohio State University, for samples of dimethyl (*E,E*)-, (*E,Z*)-, and (*Z,Z*)-2,6-octadienedioates, D. Reed, National Research Council of Canada, Saskatoon, for help in locating spectral data for glucosinolates, and T. M. Barros for technical assistance. The research was partially supported by the National Research Council of Italy, Special Project RAISA, sub-project No. 2188.

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