A Propylthio Moiety Essential to the Oviposition Attractant and Stimulant of the Onion Fly, *Hylemya antiqua* MEIGEN¹⁾

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Trapping by a Porapak Q column and GC-MS analysis of onion volatiles revealed 9 alkyl di- and trisulfides. Among these, dimethyl, methyl propyl and dipropyl trisulfides were newly identified in the headspace onion odor and the last two were found, for the first time, to have attractant-oviposition stimulating activity. From the bioassay tests of 24 synthetic and commercially obtained compounds, it was concluded that a propylthio moiety is essential for a compound to express the activity. These findings were discussed in relation to the host plant selection of onion fly and synergism among the onion odor components.

INTRODUCTION

By exposing the onion fly, *Hylemya antiqua* MEIGEN to the headspace odor of sprouting onions, MATSUMOTO demonstrated the oviposition stimulating activity of onion volatiles (unpublished data). Based on the chemical analysis of steam-distilled onion oil by NIEGISH and STAHL (1956) and CARSON and WONG (1961), MATSUMOTO and THORSTEINSON (1968a) tested many sulfur compounds and found n-propyl mercaptan and di-n-propyl disulfide as the attractant-oviposition stimulants to onion fly. Recently, PIERCE et al. (1978), with their chemical analysis of onion volatiles, added methyl propyl disulfide and cis- and trans- propenyl propyl disulfides to the active components.

To newly hatched larvae, not only di-n-propyl disulfide and propyl mercaptan which are onion odor components but also many other monosulfides, disulfides and mercaptans which are not likely to occur in onion volatiles were significantly attractive (MATSUMOTO and THORSTEINSON, 1968b). The broader spectrum of the compounds attractive against the newly hatched larvae naturally necessitates the gravid flies to be more strict in oviposition site selection to avoid wrong selection of host plants by the larvae.

In the present work, we first re-investigated the host plant odor which was actually emitted from growing, stored and fresh cut onions. Then, we clarified the structural characteristic of the attractant-oviposition stimulant molecules with synthesis of onion volatile analogues.

MATERIALS AND METHODS

Onion volatiles trapping. To trap the volatiles emitted from growing stored, and fresh

¹ Studies on the oviposition attractants and stimulants of the onion fly, *Hylemya antiqua* (Diptera: Anthomyiidae). Part 1.

cut onions, Porapak Q, a gas chromatograph packing, was employed (BYRNE et al., 1975; VERNON et al., 1977). About 1 kg of sprouting onions with the leaf length of 30–40 cm or 15 kg of stored onions were put in a bell jar (35 cm diam $\times$ 50 cm high) and the headspace volatiles were pumped through a Porapak Q column (3 cm diam $\times$ 40 cm long) at a flow rate of 3 litres/min. Air was introduced through a charcoal column (3 cm diam $\times$ 30 cm long) to eliminate any contamination. The Porapak was pre-conditioned by washing with distilled pentane over 20 hr in a continuous infusion extractor. After 1 week aeration, the Porapak was extracted with pentane for 24 hr. The extract was concentrated to 0.5 ml first by distilling pentane off through a 20-cm fractionating column and then under a gentle nitrogen stream. Five μ l of the concentrate was injected into a gas chromatograph. In the case of fresh cut onion, 500 g was placed in a small glass pot (10 cm diam $\times$ 20 cm high) and aerated for 4 hr.

Chemical analysis by GC and GC-MS. A Shimazu GC-6A gas chromatograph equipped with a flamephotometric detector and a 30 m long, 0.25 mm wide glass capillary column coated with OV-101 was used. The nitrogen flow at 60 ml/min was split at 29:1, the temperature was programmed from 50 to 190°C at 2°C/min. For identification of compounds the mass spectra were obtained with a Hitachi GC-MS, K-53; a Hitachi 063 gas chromatograph equipped with a 3 m long, 3 mm wide stainless steel column packed with OV-101 on 60–80 mesh Celite 545 was interfaced through a Biemann separator with the mass spectrometer which was monitored by a total ion detector at ionization potential of 70 eV.

Test Chemicals. n-Propyl mercaptan; dimethyl and di-n-propyl sulfides; dimethyl, diethyl, di-n-propyl, di-n-butyl and di-n-amyl disulfides were commercial products in reagent grades. The other compounds tested were prepared according to the previous workers as follows; mercaptans were synthesized by alkaline hydrolysis of the corresponding alkylisothioureia hydrobromide or alkylthiomagnesium bromide (STREITWIESER and HEATHCOCK, 1976), monosulfides to TARBELL and LOVETT (1956), di- and trisulfides to CARSON and WONG (1959) and sulfonate to BACKER and JONG (1948). To attain at least 95% purity, some chemicals were purified by the preparative gas chromatography with a Shimazu GC-4B gas chromatograph equipped with a 3 m long, 3 mm wide stainless steel column packed with OV-101 on Celite 545. All the synthetic compounds were characterized with the mass spectrometer.

Bioassay. The onion fly, *Hylemya antiqua*, MEIGEN was reared by the method of MATSUMOTO and THORSTEINSON (1967) for several generations in our lab and chosen for the bioassay at their maximum ovipositional stage, 15–35 days after emergence. Into a bioassay cage (30 $\times$ 30 $\times$ 30 cm) 25 gravid females and 5 males were put with a supply of sugar, dried milk, water and either Brewer's yeast or Difco yeast extract. The cage was illuminated with a fluorescent lamp 10 cm above for 16 hr a day at 22–25°C. Five μ l of a test chemical was dissolved in 0.2-ml ethyl ether and applied onto 2-g granule charcoal. The charcoal was packed into a glass tube (1.2 cm diam $\times$ 1 cm high), which was then set vertically at the center of a petridish (9 cm diam $\times$ 1 cm high). Around the glass tube were placed evenly with 30 g of moistened glass beads (2–4 mm diam), on which onion fly oviposited. For the control charcoal, only 0.2-ml ether was applied. After evaporation of the solvent, these 2 dishes were set at 10 cm apart in a bioassay cage. Usually 8 bioassay cages in 2 groups were maintained and used alternately every other day. For each chemical, the bioassay was replicated 8 times with 8 different cages so that possible biases arising from the batches of onion fly could be

eliminated. The dishes were kept in the cage for 22 hr. For counting the eggs, the dish was flooded with water, gently stirred to float them and decanted onto fine cheese cloth. The activity of test compound is expressed in terms of the total number of eggs laid in both dishes, which indicates the oviposition stimulating activity, and the difference of the egg number between them, which reflects the attractancy and/or arrestancy of test compound over the untreated. For significance tests of the difference of egg numbers, they were transformed to square roots and subjected to t-tests.

RESULTS

Identification of onion volatiles

All major components of onion volatiles from stored, sprouting and fresh cut onions were illustrated in Fig. 1 A, B and C. They are dimethyl disulfide, methyl n-propyl disulfide, di-n-propyl disulfide, methyl propenyl disulfide, n-propyl propenyl disulfides (2 geometrical isomers), dimethyl trisulfide, methyl n-propyl trisulfide and di-n-propyl trisulfide. Of these compounds, di-n-propyl disulfide was included in the largest quantity, followed by n-propyl propenyl disulfides. Three trisulfides were recognized in the headspace onion volatiles as BOELENs et al. (1971) detected previously in the onion steam-distillate. These play an important role in the oviposition of onion fly as tested followingly by us. Although n-propyl mercaptan was known to occur in onion steam-distillate (BOELENs et al., 1971) and to attract onion fly (MATSUMOTO and THORSTEINSON, 1968a), this was not detected in this study. There were little qualitative differences among the volatile components when condensed from the stored, sprouting and fresh cut onions, but the gross yields of the volatiles from the three differed greatly (Fig. 1). This fact suggests that onion bulbs are the major source of onion odor but onion leaves are not. The importance of onion bulbs in host plant selection of onion fly through olfaction has also been tested by VERNON et al. (1977).

Attractant-oviposition stimulating activity of the synthetics of onion volatile components

The activity of several synthetic compounds which were found in onion volatiles is summarized in Table 1. In addition to the previously known methyl n-propyl disulfide(2) and di-n-propyl disulfide (3), methyl n-propyl trisulfide (5) and di-n-propyl trisulfide(6) were newly found as the active components. Dimethyl di- and trisulfides did not elicit significant oviposition. Although 3 propenyl compounds were detected, they were not bioassayed in this work. However, according to PIERCE et al. (1978), n-propyl cis- and trans-propenyl disulfides showed some activity but methyl cis- and trans-propenyl disulfides did not.

Activity of onion volatile analogues

From the above findings we hypothesized that a propylthio moiety is essential in an active compound. To confirm this hypothesis, analogues were synthesized and their activity were examined. The activity of analogues with a propylthio moiety and those without it are summarized in Tables 2 and 3, respectively. All the propylthio compounds tested showed significant activity, whereas none of the non-propylthio compounds did. To clarify the structure-activity relationship further, Tables 2 and 3 are studied in detail according to the following characteristics.

a) Effect of varying the number of sulfur atoms in a molecule. Methyl n-propyl

Figure 1. Gas chromatograms of the headspace volatiles of stored(A), sprouting(B) and freshly cut (C) onions. Me: Methyl, Pr: Propyl, Pe: Propenyl.

sulfide (8) and di-n-propyl sulfide (9) were neither detected in this study nor found in onion steam-distillate (BOELENs et al., 1971). Nevertheless, these monosulfides and such di- and trisulfides as methyl n-propyl disulfide(2), di-n-propyl disulfide(3), methyl n-propyl trisulfide(5) and di-n-propyl trisulfide (6) showed the high activity. Especially, methyl n-propyl disulfide and di-n-propyl disulfide showed the profound activity in terms of the total numbers of eggs laid and the difference of egg numbers between the treated and untreated; they were 340 and 333, and 172 and 169, respectively. n-Propyl mercaptan was also quite active with 298 egg deposition in total and 102 eggs in difference. In contrast, n-propyl alcohol have little activity at an optimal dose

(MATSUMOTO and THORSTEINSON, 1968a). Therefore, at least one sulfur atom in a molecule or two for optimal is necessary to show activity.

b) Effect of elongation of an alkyl chain. In a monosulfide with a propyl group fixed on one side, an alkyl group on the other side was lengthened from methyl(8) to stearyl(12) to examine the effect of alkyl chain length on the activity. Methyl n-propyl sulfide(8), di-n-propyl sulfide(9) and n-propyl n-amyl sulfide (10) showed the prominent activity comparable to the native onion volatiles, namely 200—290 egg deposition in total or 100—150 eggs in difference. The activity decreased gradually as the alkyl chain was elongated further, but the statistically significant activity was recognized even up to 18-carbon length of n-propyl stearyl sulfide(12) which was despite a solid at room temperature. In disulfide, only n-propyl n-amyl disulfide (14) was tested and this also exhibited activity as strong as the onion volatiles.

c) Effect of introduction of an aromatic ring. The effect of introducing aromatic-

Table 1. ATTRACTANT-OVIPOSITION STIMULATING ACTIVITY OF ONION VOLATILES

No.	Chemicals	Formulae	Mean numbers of eggs laid		
			Treated	Control	Difference ^a
1	Dimethyl disulfide	CH ₃ SSCH ₃	33.1	57.3	-24.2 ns
2	Methyl n-propyl disulfide	CH ₃ SSC ₃ H ₇	256.0	83.8	172.2 ***
3	Di-n-propyl disulfide	C ₃ H ₇ SSC ₃ H ₇	251.0	81.9	169.1 ***
4	Dimethyl trisulfide	CH ₃ SSSCH ₃	10.6	17.1	-6.5 ns
5	Methyl n-propyl trisulfide	CH ₃ SSSC ₃ H ₇	182.1	104.6	77.5 *
6	Di-n-propyl trisulfide	C ₃ H ₇ SSSC ₃ H ₇	200.0	60.3	139.7 ***
7	n-Propyl mercaptan	C ₃ H ₇ SH	199.9	98.3	101.6 *

^a ns: Not significant, * ** *** significant at 5%, 1% and 0.1% respectively.

Table 2. ATTRACTANT-OVIPOSITION STIMULATING ACTIVITY OF ONION VOLATILE ANALOGUES WITH A PROPYLTHIO MOIETY

No.	Chemicals	Formulae	Mean numbers of eggs laid		
			Treated	Control	Difference ^a
Monosulfides					
8	Methyl n-propyl sulfide	CH ₃ SC ₃ H ₇	219.4	68.3	151.1 ***
9	Di-n-propyl sulfide	C ₃ H ₇ SC ₃ H ₇	156.0	49.8	106.2 **
10	n-Propyl n-amyl sulfide	C ₃ H ₇ SC ₅ H ₁₁	179.9	62.9	117.0 ***
11	n-Propyl n-octyl sulfide	C ₃ H ₇ SC ₈ H ₁₇	96.6	39.8	56.8 ***
12	n-Propyl stearyl sulfide	C ₃ H ₇ SC ₁₈ H ₃₇	127.3	83.8	43.5 *
13	n-Propyl phenyl sulfide	C ₃ H ₇ S-	97.9	17.1	80.8 ***
Disulfides					
14	n-Propyl n-amyl disulfide	C ₃ H ₇ SSC ₅ H ₁₁	208.9	48.5	160.4 ***
15	n-Propyl phenyl disulfide	C ₃ H ₇ SS-	167.9	59.6	108.3 **
Sulfonate					
16	<i>n</i> -Propyl propane thiosulfonate ^b		253.4	49.0	204.4 ***

^a See the note of Table 1.

^b Found in extract of freshly cut onion (BOELEN, et al., 1971).

Table 3. ATTRACTANT-OVIPOSITION STIMULATING ACTIVITY OF ONION VOLATILE ANALOGUES WITH NO PROPYLTHIO Moiety

No.	Chemicals	Formulae	Mean numbers of eggs laid		
			Treated	Control	Difference ^a
Mercaptans					
17	iso-Propyl mercaptan	(CH ₃) ₂ CHSH	20.1	49.6	−29.5 ns
18	Allyl mercaptan	CH ₂ =CHCH ₂ SH	59.3	46.3	13.0 ns
19	n-Amyl mercaptan	C ₅ H ₁₁ SH	67.5	40.9	26.6 ns
Monosulfides					
20	Dimethylsulfide ^b	CH ₃ SCH ₃	40.8	34.5	6.3 ns
21	Methyl allyl sulfide ^b	CH ₃ SCH ₂ CH=CH ₂	42.6	57.6	−15.0 ns
Disulfides					
22	Diethyl disulfide	C ₂ H ₅ SSC ₂ H ₅	58.0	41.8	16.2 ns
23	Di-n-butyl disulfide	C ₄ H ₉ SSC ₄ H ₉	27.9	14.5	13.4 ns
24	Di-n-amyl disulfide	C ₅ H ₁₁ SSC ₅ H ₁₁	58.4	72.1	−13.7 ns

^a See the note of Table 1.^b Found in steam-distillates of onion and crushed garlic (BOELENs et al., 1971).

city in the analogues was tested with 2 phenyl sulfides, although these were included in neither onion steam-distillates nor headspace odors of growing and fresh cut onions. Aromaticity provided the molecule with an odor quality quite different from the preceding di-alkyl sulfides. Nevertheless, n-propyl phenyl mono- and disulfides(13, 15) showed somewhat weak but consistent activity to onion fly, possibly due to their low volatility.

d) Effect of introduction of a double bond in the propylthio moiety. It is important to determine if a compound loses its activity by introduction of a double bond into the propylthio moiety, because many such unsaturated compounds as allyl, cis- and trans-propenyl sulfur compounds are included in onion (BRODNITZ et al., 1969; PIERCE et al., 1978). Allyl mercaptan (18) and methyl allyl sulfide(21) did not show any activity, in contrast to the high activity of the corresponding compounds such as n-propyl mercaptan(7) and methyl n-propyl sulfide(8). Besides, methyl cis- and trans-propenyl disulfides showed little activity according to PIERCE et al. (1978). Thus, retention of a propylthio moiety was again essential for a compound to express the activity.

e) Effect of oxidation of the sulfur atom. n-Propyl propane thiosulfonate(16), an oxidized form of di-n-propyl disulfide, was found in onion extracts (BOELENs et al., 1971) and has been suspected to play an important role in formation of the raw onion flavor (WHITAKER, 1976). So this was also synthesized to bioassay. As expected from the possession of a propylthio moiety, this compound showed very strong activity comparable with methyl n-propyl disulfide(2) and di-n-propyl disulfide(3), 302 egg deposition and 204 eggs in difference.

DISCUSSION

Di-n-propyl disulfide was used in much larger quantity than actually found in onion volatiles to attract onion fly in the fields (MATSUMOTO, 1970). Besides, this compound *per se* was not so effective as cut onions in the fields (TOMIOKA, 1977). The superi-

ority of onion odor in the fields would be explained in part by synergistic interactions among the active propylthio compounds, and between these and inactive sulfur or even non-sulfur components. In fact, the propylthio compounds, which are thought to be produced from their precursor trans(+)-propenyl-cysteine S-oxide by allinase, characterize onion flavor *in toto* (SCHWIMMER, 1968; WHITAKER, 1976).

Of the 45 flavor constituents identified in onion steam-distillate by BOELENS et al. (1971), 16 compounds have a propylthio moiety and constitute over two-thirds in quantity of the compounds emanated from onions. In the present study, although many small peaks on the chromatographs in Fig. 1 remained unidentified, over half of them were determined to have a propylthio moiety. Our preliminary study with electroantennography also revealed that not only the propylthio compounds but the non-stimulative components give the good EAG responses of onion flies. The EAG-active components, regardless of their oviposition-stimulancy, may be more prone to synergize the propylthio compounds.

From the side of onion fly, their adaptation to an array of propylthio compounds and others, instead of a single compound, in host plants would be so advantageous that they could find the plants without fail.

Addendum—VERNON et al. (Personal communication) reached the similar results independently of our work.

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REFERENCES

- BACKER, H. J. and G. J. DE JONG (1948) [The properties of the sulfonyl group. II. Isomerization of allyl benzyl sulfone to propenyl benzyl sulfone.] *Rec. Trav. Chim.* **67**: 884-893. (*Chem. Abst.* **43**: 6995d).
- BOELENS, M., P. J. DE VALOIS, H. J. WOBLEN and A. VAN DER GEN (1971) Volatile flavor compounds from onion. *J. Agr. Food Chem.* **19**: 984-990.
- BRODNITZ, M. H., C. L. POLLOCK and P. P. VALLON (1969) Flavor components of onion oil. *J. Agr. Food Chem.* **17**: 760-763.
- BYRNE, K. J., W. E. GORE, G. T. PEARCE and R. M. SILVERSTEIN (1971) Porapak-Q collection of airborne organic compounds serving as models for insect pheromones. *J. Chem. Ecol.* **1**: 1-7.
- CARSON, J. F. and F. F. WONG (1959) Separation of aliphatic disulfides and trisulfides by gas-liquid partition chromatography. *J. Org. Chem.* **24**: 175-179.
- CARSON, J. F. and F. F. WONG (1961) The volatile flavor components of onion. *J. Agr. Food Chem.* **9**: 140-143.
- MATSUMOTO, Y. (1970) Volatile organic sulfur compounds as insect attractants with special reference to host selection. In "Control of Insect Behavior by Natural Products." (ed. D. L. WOOD, R. M. SILVERSTEIN and M. NAKAJIMA) pp. 133-160. Academic Press, New York.
- MATSUMOTO, Y. and A. J. THORSTEINSON (1967) A simple method for rearing onion maggot, *Hylemya antiqua* MEIG. (Diptera: Anthomyiidae) in the laboratory. *Appl. Ent. Zool.* **2**: 58-59.
- MATSUMOTO, Y. and A. J. THORSTEINSON (1968a) Effect of organic sulfur compounds on oviposition in onion maggot, *Hylemya antiqua* MEIGEN (Diptera: Anthomyiidae). *Appl. Ent. Zool.* **3**: 5-12.
- MATSUMOTO, Y. and A. J. THORSTEINSON (1968b) Olfactory response of larvae of the onion maggot,

- Hylemya antiqua* MEIGEN (Diptera; Anthomyiidae) to organic sulfur compounds. *Appl. Ent. Zool.* **3**: 107-111.
- NIEGISH, W. D. and W. H. STAHL (1956) The Onion: Gaseous emanation products. *Food Research* **21**: 657-665.
- PIERCE, Jr., H. D., R. S. VERNON, J. H. BORDEN and A. C. OEHLSCHLAGER (1978) Host selection by *Hylemya antiqua* (MEIGEN): Identification of three new attractant-oviposition stimulants. *J. Chem. Ecol.* **4**: 65-72.
- SCHWIMMER, S. (1968) Enzymic conversion of trans-(+)-S-1-propenyl-L-cysteine S-oxide to the bitter and odor-bearing components of onion. *Phytochemistry* **7**: 401-404.
- STREITWIESER, A. Jr. and C. H. HEATHCOCK (1976) *Introduction to Organic Chemistry.*, 1279 pp. Collier-MacMillan, London.
- TARBELL, D. S. and W. E. LOVETT (1956) The isomerization by base of alkyl allyl sulfides to alkyl propenyl sulfides. The Mechanism of the reaction. *J. Amer. Chem. Soc.* **78**: 2259-2264.
- TOMIOKA, T. (1977) [Bionomics of *Hylemya antiqua* MEIGEN and *Hylemya platura* MEIGEN.] (in Japanese) *Shokubutsu-boeki* **31**: 206-209.
- VERNON, R. S., J. H. BORDEN, H. D. PIERCE, Jr. and A. C. OEHLSCHLAGER (1977) Host selection by *Hylemya antiqua*: laboratory bioassay and methods of obtaining host volatiles. *J. Chem. Ecol.* **3**: 359-368.
- WHITAKER, J. R. (1976) Development of flavor, odor, and pungency in onion and garlic. *Adv. Food Res.* **22**: 73-133.