the enzyme, is the strongest inhibitor for fertilization. The next most effective inhibitor was acetyl-Ala-Ala-Tyr-MCA, the order being the same as in the case of substrate susceptibility (table). Furthermore, our preliminary experiments indicate that the extent of inhibition with the specific substrate is considerably diminished in the fertilization of naked eggs (egg-investment-free eggs), as previously reported[7] in the inhibition with chymostatin. Thus, chymotrypsin-like enzyme, as well as acrosin-like enzyme[9], plays an important role in fertilization of the ascidian, *H. roretzi,* especially in sperm-penetration of egg investments.

In ascidians, a typical acrosome has not been observed at the apex of the spermatozoon[11], or it is very small[12] in contrast with that of mammals[3] or sea urchins. Therefore, subcellular localization of the chymotrypsin-like enzyme is an important problem in connection with its physiological role in fertilization. We have recently demonstrated the presence of an acrosin-like enzyme at the mitochondrial portion and the apex of the spermatozoon of this ascidian by a histochemical procedure with dansyl-leucylargininal, a specific fluorescent inhibitor of the acrosin-like enzyme (Sawada et al., in preparation). The chymotrypsin-like enzyme may also be localized at this region(s) and may exhibit a cooperative action with the acrosin-like enzyme. Recently, a chymotrypsin-like enzyme has been reported as a lysin, that is a vitelline layer(coat) lytic enzyme, in sea urchins[5,6] and frogs[13]. The existence of such an enzyme has not been proved, however, in spermatozoa of the mammal, although a chymotrypsin-like enzyme, seminin, has been reported in seminal plasma[14]. On the other hand, it has been debated whether acrosin is a real zona-lysin or not, because a purified preparation of acrosin has little or no activity of solubilizing zona pellucida[14] and zona-penetration of sperm is not inhibited with trypsin inhibitors[15]. The involvement of some chymotrypsin-like enzyme in the sperm-penetration through egg investments, therefore, might be a general feature applicable from sea urchins to mammals.

Our investigations on the physiological roles of both the chymotrypsin-like and the acrosin-like enzymes in ascidian sperm, as well as their purification, are now in progress.

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Evaluation of aromatic tetrahydropyranyl ethers as feeding deterrents for the striped cucumber beetle, *Acalymma vittatum* (F.)[1]

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Summary. Laboratory tests with striped cucumber beetle, *Acalymma vittatum,* adults indicated that a number of aromatic tetrahydropyranyl ethers were promising as antifeedants at dosage rates of 0.1 and 0.5%.

In recent years, increasing attention has been given to plant chemicals as sources of insect feeding deterrents. Some of the complexities of this research were reported by Schoonhoven[3]. Plant phenolics were shown to possess such activity when entomologists at the USDA Insects Affecting Man and Animals Research Laboratory in Gainesville, Florida conducted tests early in 1980 with a number of naturally occurring and synthetic phenols and their derivatives. These tests showed that the tetrahydropyranyl (THP) ethers of 2-methoxyphenol, 4-methoxyphenol, 2,3-dimethoxyphenol, and 3,4-methylenedioxyphenol were repellent to adult yellow fever mosquito, *Aedes aegypti* (L.), and the malaria mosquito, *Anopheles quadrimaculatus* Say[4]. The THP ether of 2,3-dimethoxyphenol also protected the arms of human subjects to which the compound had been applied from bites by a saltmarsh mosquito, *Aedes taeniorhynchus* (Wiedemann). The Army Environmental Hygiene Agency subsequently showed that these compounds had low topical hazard potential when tested on rats, rabbits, and guinea-pigs. These results and previous results reported by Reed et

al.[5] using natural products prompted us to evaluate the feeding deterrency of a series of previously unreported aromatic THP ethers toward the striped cucumber beetle, *Acalymma vittatum* (F.), and the results are reported here.

Materials and methods. The THP ethers of all phenolic compounds except those containing an aldehydic group were prepared as follows. A 20% excess of dihydropyran was added dropwise to a stirred solution of 0.5 moles of the phenolic compound and 4 drops of concentrated HCl in 75 ml of reagent grade ether. The resulting solution was then refluxed for 4.5 h, the solvent and excess dihydropyran were removed by evaporation at 20 mm Hg and the residue was dissolved in ether. The solution was washed twice with cold 5% $NaHCO_3$ solution and then with saturated NaCl solution, dried over $NaSO_4$, and freed of solvent at 20 mm. The resulting THP ethers were purified by distillation under reduced pressure to give 95–99% yields of the desired compounds as colorless liquids.

Attempts to prepare the THP ethers of the aldehydophenolic compounds by the above method resulted only in the recovery of unchanged starting material. The substitution of benzene for ether as solvent gave tar-like decomposition products. The THP derivatives were successfully prepared in 97–98% yield by substituting ethyl acetate for the ether as the solvent.

The boiling points and refractive indexes of all THP ethers prepared are given in table 1.

Of the 18 phenolic compounds from which THP ethers were prepared 8 are known to occur naturally in plants, as follows: Phenol and 2-methoxyphenol (guiacol) in a number of essential oils and resins[6]; 4-methoxyphenol in the leaves of *Pirola secunda* L.[7]; 2,6-dimethoxyphenol in the essential oil of *Artemisia herba-alba* var. *densiflora* Boisd.[8]; 3,4,5-trimethoxyphenol in the sap of *Antiaris toxicaria* Lesch.[9]; 3,4 (methylenedioxy) phenol (sesamol) in sesame oil[10]; 2-hydroxybenzaldehyde (salicylaldehyde) in numerous essential oils[11]; and 2-hydroxy-4-methoxybenzaldehyde in many plant roots[12]; These phenols are known to play an important role in the development of plant coloration through their use as intermediates in the biosynthesis of flavonoids[13].

Adult striped cucumber beetle reared on squash plants at 27 ± 1 °C and 60 ± 5% relative humidity, were collected soon after emergence and starved 24 h before testing. Test leaf discs circa 20 mm in diameter were cut from young cantaloupe leaves grown in the greenhouse. These were dipped into the test materials which had been dissolved in acetone and suspended in 0.01% Tween 20[5]. Two of the treated discs and 2 leaf discs treated with a blank of acetone and Tween 20 each were then placed in the bottom of a polyethylene dish (93-mm-diameter × 73-mm-deep). A disposable plastic petri dish bottom (90-mm-diameter × 12-cm-deep) with 4 holes (17-mm-diameter) was placed over the leaf discs to hold them flat and allow a standard area of surface for feeding by 5 beetles which were placed into each container. There were 2 replicates of test containers for each treatment. The plastic containers were covered with a looseweave muslin cover to eliminate fumigant effects and observations were made at 3, 6, and 22 h except for 1 test which was checked at 4 and 24 h. The amount of feeding inhibition was expressed as % inhibition =

$$\frac{\%\text{consumed of treated disc} \times 100}{\%\text{consumed of treated} + \text{untreated disc}}$$

A result of 50 indicates equal consumption of treated and untreated discs while lower numbers indicate antifeedant activity.

Results and discussion. The results are shown in table 2. The most effective compounds at 0.1% were the THP ethers of 3,4-methylenedioxyphenol (20873-c), 3,4-methylenedioxybenzyl alcohol (20702-c), 2,3-dimethoxyphenol (36892), 2-methoxyphenol (36312), 2,6-dimethoxyphenol (36894), and 2-hydroxy-4-methoxybenzaldehyde (36318). The order of effectiveness was 20702-c = 20873-c > 36892 > 36312 > 38618 > 36894. Compounds highly effective at the 0.5% level but much less effective at 0.1% were 30005-b, 36235, 36313, 36893, 38619, 38479, and 38480. Compounds 38695 and 38620 were fairly effective at both concentrations.

Table 1. Boiling point and refractive index of aromatic tetrahydropyranyl (THP) ethers

AI3	THP ether of	Boiling point (°C/mm)	Refractive index (n_D25)
36235	Phenol	68–71/0.5	1.5210
36312	2-Methoxyphenol	105–110/0.3	1.5256
36313	3-Methoxyphenol	110–115/0.3	1.5267
30005-b	4-Methoxyphenol	100–103/0.5	1.5228
36892	2,3-Dimethoxyphenol	110–115/0.5	1.5243
38479	3,4-Dimethoxyphenol	140–142/0.1	1.5331
36894	2,6-Dimethoxyphenol	85–90/0.6	1.5457
36893	3,5-Dimethoxyphenol	124–126/0.3	1.5316
38480	3,4,5-Trimethoxyphenol	150/0.5	1.5295
20873-c	3,4-(Methylenedioxy) phenol	120–125/0.3	1.5368
38620	2,6-Dimethoxy-4-(2-propenyl) phenol	135/0.3	1.5428
38694	3-Ethoxy-4-methoxyphenol	150/0.1	1.5489
38617	2-Hydroxybenzaldehyde	45–48/0.5	1.5600
38619	2-Hydroxy-3-methoxy-benzaldehyde	80–85/0.5	1.5730
38618	2-Hydroxy-4-methoxy-benzaldehyde	75–78/0.4	1.5727
38650	2-Hydroxy-5-methoxy-benzaldehyde	70–75/0.5	1.5565
20702-c	3,4-(Methylenedioxy) benzyl alcohol	131–135/0.4	1.5287
38695	3,4,5-Trimethoxybenzene-methanol	152–155/0.4	1.5223

AI3 represents Beltsville Institute compound numbers.

Table 2. Feeding deterrency of aromatic THP ethers to striped cucumber beetles when presented on leaf discs in a choice experiment

Compound AI3-No.	Percentage of feeding inhibition at indicated times[a] 0.1%			0.5%		
	3 h	6 h	22 h	3 h	6 h	22 h
20702-c	0	0	5	0	0	0
20873-c	0	0	5	0	0	0
30005-b	56	60	33	0	0	0
36235	10	24	49	0	0	0
36312	0	0	11	0	0	0
36313	22	17	39	0	0	0
36892	0	4	5	0	0	0
36893	0	7	18	0	0	0
36894	0	0	32	0	0	0
38617	75	57	48	50	50	68
38618	0	0	23	0	0	0
38619	29	25	7	0	0	0
38620	0	0	16	0	0	13
38479	33[b]		35[c]	0[b]		0[c]
38480	0[b]		22[c]	0[b]		0[c]
38650	18	25	30	0	13	8
38694	0	18	27	20	30	24
38695	0	12	14	7	3	2

[a]Data are expressed as mean values from 4 treated and 4 untreated discs with the amount consumed given as a percentage of the total amount (test disc + control disc) consumed. A value of 0 indicates total inhibition. [b]4 h. [c]24 h.

Compounds 38617, 38650, and 38694 were ineffective even at the 0.5% level. A phytotoxic response was noted at the 0.5% level in only 2 of the materials, 20702-c and 36313.

Although little can be said concerning structure-activity relationships among these compounds, the results suggest that the presence of a methoxy group ortho to the THP group considerably increases the activity of the THP ether of unsubstituted phenol (36235). Even greater improvement is obtained by the presence of a dioxymethylene group on the phenol molecule. Suprisingly, though the THP ether of 3,4-methylenedioxybenzyl alcohol (20702-c) is not derived from a phenol, it proved to be one of the most effective compounds in this series. It was noted that, though the THP ethers of 2-hydroxybenzaldehyde and of 2-hydroxy-5-methoxybenzaldehyde are not effective at either concentration, the THP ether of 2-hydroxy-4-methoxybenzaldehyde is highly effective even at the 0.1% level.

These data add to the numbers of materials which are promising as feeding deterrents for striped cucumber beetle[5]. As the beetle is a significant problem on cucurbits due to both its feeding and transmission of disease, an effective antifeedant would be an attractive alternative to present control tactics.

1 Mention of a proprietary product in this paper does not constitute an endorsement of this product by the USDA.
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3-Methyl-2-hexanone from the triatomine bug *Dipetalogaster maximus* (Uhler) (Heteroptera; Reduviidae)

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Summary. The occurrence of 3-methyl-2-hexanone as a major component of the secretion and possible alarm substance from the metasternal scent glands in the triatomine bug *Dipetalogaster maximus* is reported.

Dipetalogaster maximus (Uhler) is a very large 33-42 mm long blood sucking (triatomine) bug. It occurs naturally in Mexico at the extreme southern tip of semi-arid California Baja (Sur)[2]. *Trypanosoma cruzi*, which causes Chagas' disease in humans, is transmitted by triatomine bugs. Although *D. maximus* is not, because of its restricted distribution, important as a carrier of *T. cruzi*[2] the early larval stages are proving useful in xenodiagnosis, as a natural means of detecting *T. cruzi* in patients suspected of having Chagas' disease[3].

In the efforts which continue to be made to secure improvements in the techniques used to detect and control the noxious triatomines searches have been made for behavior modifying chemicals which the insects themselves produce[4]. Here we should like to report evidence indicating that 3-methyl-2-hexanone is a major component of the secretion and a possible alarm substance from the metasternal (= metathoracic) scent glands of *D. maximus*.

Materials and methods. Larvae and adults of *D. maximus* were obtained from Cambridge, England[1] (the larvae were reared to adulthood in our laboratory, in Cardiff). The metasternal scent glands were isolated by dissection under 200 mM NaCl. They are present only in the adult insect. The electron impact gas chromatographic-mass spectrometric (GC-MS) analyses[1] were carried out using a 7070H VG mass spectrometer at 70 eV with the ion source temperature 190 °C, separator 180 °C and 200 μA ionizing current. Separations were achieved with a 2 m × 2 mm i.d. glass column packed with 3% OV 225 on 100-120 mesh Gas Grom Q; basic programme, 10 ml helium/min, column 70 °C isothermal for 7 min and then temperature programmed at 10 °C/min to 200 °C. The glandular samples were introduced by a simple solventless open column procedure[5]. Standard samples were injected in the usual way in solution in acetone or ethanol. Chemical reductions were carried out with excess sodium borohydride in ethanol (2 μl ethanol for 1 gland). Acidification of the reaction mixture was effected with 2 N hydrochloric acid prior to

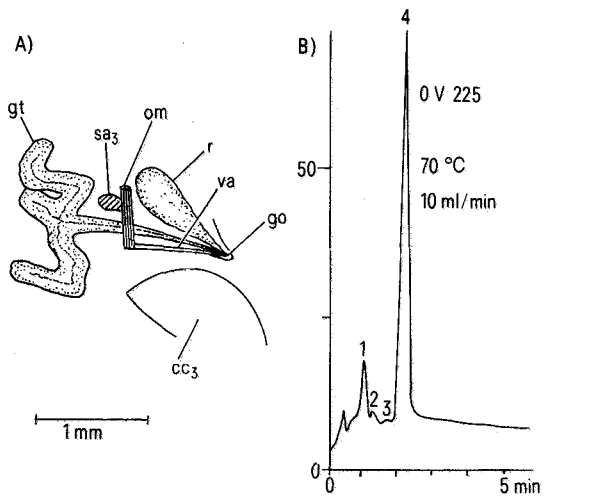

Figure 1. *A* Metasternal scent gland, right-hand side of metathorax; cc_3, 3rd coxal articular cavity; go, position of gland opening; gt, gland secretory tubule; om, opener muscle; r, reservoir; sa_3 metasternal apophysis; va, valve opener arm. *B* Total ion current monitor trace from GC-MS analysis of a single entire gland. 4 peaks were recorded within 3 min after start at 70 °C.