regulatory swelling of *Necturus* gallbladder epithelial cells is the consequence of the transient stimulation of parallel Na^+-H^+ and Cl^--HCO_3^- exchangers in the apical membrane. On the other hand, NaCl entry into the cells during fluid absorption is by a different process in which there is coupled, carrier-mediated movement of NaCl across the apical membrane (*9, 11*). This coupled NaCl entry is unaffected by amiloride (*9*) or by the absence of HCO_3^- (*11*). It occurs at about 25 percent of the rate of volume-regulatory flow. Thus, NaCl may enter the *Necturus* gallbladder epithelial cell across the apical membrane by two modes: (i) the coupled NaCl entry associated with fluid absorption and (ii) the rapid, but transient, exchanges triggered by alteration of the osmolality of the bathing solutions.

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4. The perfusion solutions had the compositions (in millimoles per liter) described here. The HCO_3^- Ringer contained: Na^+, 82.5; Cl^-, 80; K^+, 2.5; Ca^{2+}, 1.8; Mg^{2+}, 1.0; HCO_3^-, 10; $H_2PO_4^-$, 0.5; mannitol, 36; *p*H = 7.6 when gassed with 99 percent air and 1 percent CO_2; HCO_3^--free Ringer contained: Na^+, 82; Cl^-, 86; K^+, 2.4; Ca^{2+}, 1.0; Mg^{2+}, 1.0; HPO_4^{2-}, 1.0; $H_2PO_4^-$, 0.3; mannitol, 36; *p*H = 7.6 when gassed with 100 percent air. The control solution was 188 to 189 mOsm; the hypertonic solutions, 222 to 224 mOsm.
5. The chamber was constructed of plexiglass to create a narrow trough of fluid on both surfaces of the gallbladder. Fluid exchange in either bath was complete in 3 seconds. Further details of the design will be provided on request.
6. The PD and a_{Cl} values were measured by the use of conventional techniques as described in K. R. Spring and G. Kimura [*J. Membr. Biol.* **38**, 233 (1978)]. Since epithelial cells are electrically coupled and similar time-dependent changes of voltage in different cells were observed, PD and a_{Cl} measurements could be made in different cells.
7. Control values in the presence of HCO_3^- were as follows: cell volume, 10,416 ± 463 (14) μm^3; PD, −66.7 ± 1.3 (35) mV; a_{Cl}, 24.9 ± 2.0 (23) m*M*; total quantity of intracellular Cl^-, Q_{Cl}, 25.9 ± 2.6 (14) × 10^{-14} mole. Control values in the absence of HCO_3^- were as follows: cell volume, 11,289 ± 948 (13) μm^3; PD, −66.4 ± 1.3 (31) mV; a_{Cl}, 19.1 ± 2.2 (11) m*M*; Q_{Cl}, 21.6 ± 2.9 (11) × 10^{-14} mole.
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Close-Range Attraction of Female Oriental Fruit Moths to Herbal Scent of Male Hairpencils

Abstract. *A blend of ethyl* trans*-cinnamate, methyl 2-epijasmonate, methyl jasmonate, and* (R)*-(−)-mellein, identified from the hairpencils of male Oriental fruit moths, attracts sex pheromone–releasing females several centimeters away. The chemicals thereby duplicate the behavioral effect elicited by hairpencil-displaying males during courtship; the chemicals also produce the herbal scent emanating from the hairpencils.*

Males of the order Lepidoptera often have accessory scent-producing organs, which usually consist of groups of elongated hairlike scales (hairpencils) that are bundled into special pouches and then everted and splayed in the vicinity of a female during courtship (*1*). Volatile chemicals identified from such structures (*2–4*) or from other specialized scales (*5*) have been described, without reference to the behavior elicited. Studies aimed at defining the behavioral roles played by scent scales, including ablation techniques (*6*), electroantennogram (EAG) assays (*7*), trapping experiments (*8*), or observational inferences (*9*), have revealed that, in most species, courtship pheromones exert a minimal observable effect on female behavior. The lack of an overt female response has hindered the identification of chemicals producing behavioral responses. In rare instances, behavior and chemistry have both been elucidated, but in those instances the compounds described evoked female "acceptance" through inferred quiescence (*10*) or abdominal extension (*11*), rather than attraction.

Courtship in the Oriental fruit moth, *Grapholitha molesta* (Busck), is unusual among the Lepidoptera in that males attract females after they themselves have been attracted to the vicinity of a female by a sex pheromone (*12*). A few centimeters from the female, the male turns away and repeatedly extrudes and retracts its abdominal hairpencils, propelling volatile chemicals over the female with wind generated from wing vibration (Fig. 1). The female immediately walks toward the source of the odor and with her head touches the tip of the male's abdomen, evoking from him a copulatory attempt (*12*). The overt movement of females toward displaying males in this species provided the opportunity to define a lepidopterous courtship pheromone that attracts females. With the use of behavioral and EAG assays, we were able to identify a blend of compounds that duplicates the activity of the natural pheromone. The compounds are ethyl *trans*-cinnamate (**1**), (*R*)-(−)-mellein (**2**), methyl jasmonate (**3**), and methyl 2-epijasmonate (**4**) (Fig. 2).

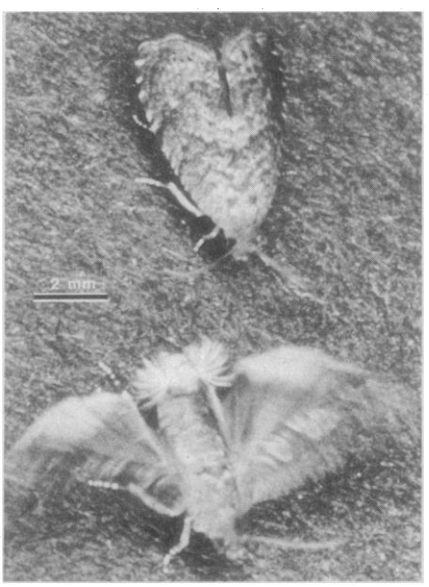

Fig. 1. Male Oriental fruit moth everting his hairpencil organs at the end of his abdomen and attracting a female that is walking toward him.

Approximately 5000 male equivalents (ME) of the hairpencil extract (*13*) were used for the isolation and identification (*14*). The crude extract on filter paper had a pleasant herbal odor, similar to that of the forcibly extruded hairpencils of living *G. molesta* males. Each 1000 ME was concentrated under nitrogen and fractionated on a gas-liquid chromatography (GLC) column [3 percent OV-101 (*15*)] into 12 fractions (Fig. 2). The only fraction to produce an EAG response from female antennae [mean ± standard deviation (S.D.) = 0.60 ± 0.20 mV; *N* =9] above background (0.07 ± 0.05 mV; *N* = 9) was fraction 3 (the crude extract produced 1.25 ± 0.31 mV; *N* = 9) (*14, 16*). The compound from this fraction was collected (approximate yield 0.5 ng per ME) and identified as ethyl *trans*-cinnamate (**1**) by GLC retention times, microhydrogenation, and diagnostic ultraviolet and mass spectra (*14, 17*).

The fractions were tested for their attractiveness to calling females in an arena in moving air (*18*). The test samples on filter paper were placed 4 cm

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Table 1. Behavioral responses of *G. molesta* females to compounds isolated from male hairpencils (Fig. 2); $N = 20$ for each treatment.

Authentic compound* (1 ng of each substance)	Percent walking upwind			Percent walking to 0.5 cm from source			Percent touching source		
	Authentic	Crude†	Blank	Authentic	Crude	Blank	Authentic	Crude	Blank
1	55‡	55‡	20	35	50‡	15	15	40	15
2	15	55‡	20	5	45	15	5	40	10
3	20	55‡	10	5	50‡	0	5	50‡	0
4	20	70‡	15	20	50‡	15	5	45	15
1 + 2	30	65‡	15	20	60‡	5	10	60‡	5
1 + 3	25	40	10	15	30‡	0	5	25	0
1 + 4	80‡	65‡	10	60‡	55‡	10	50†§	55‡	10
1 + 2 + 3	60‡	55‡	0	35‡	30‡	0	30‡	15	0

*Compound **1** was obtained from the Aldrich Chemical Co.; **2**, from Imperial Chemistry Industries. Compound **3** is a synthetic racemic mixture obtained from International Flavors and Fragrances and compound **4** was isolated from lemon peels (*14*). †1 ME. ‡Response under same behavior significantly different from the blank according to a chi-square 2×2 test of independence with Yates' correction ($P < .05$). §Response under same behavior significantly greater than that to **1** alone ($P < .05$).

upwind of individual 4- to 5-day-old females, and the females were scored for their rapid walking toward, and touching of, the paper. Fraction 3 produced a significant amount of upwind walking by females compared to that produced by the solvent blank (*19*), and synthetic **1** was the only compound to elicit significant female attraction (Table 1). A blend of **1** plus fractions 6 and 7 resulted in greater attraction than was elicited by **1** alone (*20*).

Pure compound **2** was obtained directly from fraction 4 (~ 20 ng per ME) and was identified as (*R*)-(−)-mellein by ultraviolet, nuclear magnetic resonance, and mass spectra, as well as by determination of the optical rotation (*14, 21*). Despite its predominance in the extracts, mellein (**2**) was inactive by itself in bioassays (*19*) (Table 1), and fraction 4 did not elicit an EAG response above background (0.16 ± 0.08 mV; $N = 9$). However, a blend of **2** plus **1** and **3** was as attractive to calling females as crude extract (Table 1).

Fractions 6 and 7 produced most of the extract's herbal odor and when mixed with **1** increased the attraction of *G. molesta* females. Compound **3** was identified as methyl jasmonate from fraction 6 (~ 0.3 ng per ME). It was purified by preparative GLC on 3 percent XF-1150 (*15*) and characterized (*14, 22*) by microozonolysis, microhydrogenation, and mass spectral comparisons with an authentic sample (International Flavors and Fragrances). Compound **3** was behaviorally inactive by itself and in combination with **1**, but a blend of **3** plus **1** and **2** attracted a significant number of females toward the source (Table 1).

Compound **4** was obtained from fraction 7 (~ 0.01 ng per ME), purified on XF-1150, and identified as methyl 2-epijasmonate (*14, 23*) by microhydrogenation, GLC retention times, mass spectrometry, and epimerization to **3** in the presence of *p*-toluenesulfonic acid. It was behaviorally inert by itself, but significantly increased attraction of female *G. molesta* when combined with **1** (Table 1).

In this report, we have characterized the male moth hairpencil compounds that are active in attracting female moths. These compounds have not previously been found in the Lepidoptera. Ethyl *trans*-cinnamate is similar to 2-phenylethanol, which has been found in the hairpencils of a number of lepidopteran species (*3*). Mellein is a fungal metabolite (*24*) and has been found in a number of ant species (*25*). Methyl jasmonate, a constituent of jasmine oil, is known in the perfume industry as the queen of aroma. It closely resembles *cis*-jasmone, which was identified by Petty *et al.* (*4*) from hairpencils of the butterfly *Amauris ochlea*.

It is not known whether *G. molesta* are dependent on their various fruit and nut host species for immediate precursors to the hairpencil herbal-scented compounds. Initial studies indicate that *G. molesta* hairpencils from males reared on an artificial diet do not possess the characteristic herbal odor or the GLC peaks **1**, **3**, and **4** of hairpencils from males reared on their usual diet of small green apples.

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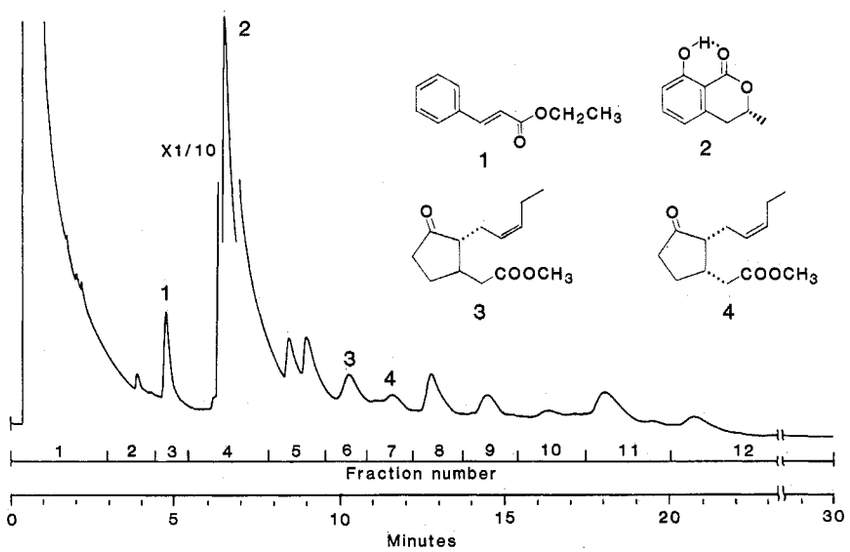

Fig. 2. A GLC tracing (OV-101) of Skellysolve B–extracted, excised hairpencils showing fractions collected for bioassays and further isolation and identification.

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13. Each male's paired hairpencils plus claspers were snipped and immersed in a small vial of Skellysolve B for several minutes. The extract was then separated from the residue and maintained at −20°C until use.
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15. OV-101 is methyl silicone on 100- to 120-mesh Gas-Chrom Q; 2-m glass column (inside diameter, 4mm); 150°C. XF-1150 is cyanoethyl methyl silicone on 100- to 120-mesh Chromosorb W-AW-DMCS; 2-m glass column (inside diameter, 2 mm); 185°C.
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17. Retention times on GLC (OV-101 and XF-1150) coincided with those of authentic ethyl *trans*-cinnamate (Aldrich Chemical Company), whereas authentic ethyl *cis*-cinnamate (*14*) gave different retention times. The ultraviolet spectrum (extinction coefficient 15,000 at wavelength maximum of 270 nm in Skellysolve B) and mass spectrum of **1** were identical to those of ethyl *trans*-cinnamate.
18. The conditions in the sheet metal observation arena (25 by 25 cm) were 20°C; relative humidity 60 to 80 percent; light intensity, 700 lux; and laminar wind flow, 71 cm/sec. A sample (1 μl) of solution containing either 1 ng of synthetic or 1 ME of natural extract followed by 5 μl of Skellysolve B were placed onto a filter paper (5 by 7 mm; Whatman No. 1) skewered to a metal thumbtack. After the solvent evaporated in front of the exhaust tube at the downwind end, the thumbtack was placed with forceps so that the paper hung just above the surface 4 cm upwind of a female. Females were scored on four points: whether they (i) walked upwind and touched the paper; (ii) walked upwind but did not touch the paper; (iii) began walking upwind; or (iv) did not move at all during a 10-second exposure to the treatment.
19. Eleven percent of females walked upwind in response to 3 ME of fraction 4, compared to 3 percent for Skellysolve B blank (not significantly different at $P < .05$ according to a chi-square 2×2 test of independence with Yates' correction); 29 percent walked upwind in response to fraction 3 (significantly different at $P < .05$); $N = 35$ for all treatments.
20. Eighty percent of females walked upwind in response to 10 ng of **1** plus 3 ME of fractions 6 plus 7, compared to 45 percent in response to 10 ng of **1** alone (significantly different at $P < .05$ according to a chi-square 2×2 test of independence with Yates' correction); the response to Skellysolve B blank was 13 percent; $N = 40$ for all treatments.
21. The optical rotation of **2** gave a negative sign $[\alpha]_D^{21} = -133°$, at a concentration of 0.01 g/ml in chloroform. Since H. Arakawa, N. Torimoto, and Y. Masui [*Liebigs Ann. Chem.* **728**, 152 (1969)] determined the absolute configuration of (−)-mellein to be *R*, compound **2** also has the *R* configuration.
22. Compound **3** had GLC retention times on OV-101 identical to the authentic *Z* isomer and different from the authentic *E* isomer.
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Sensory and Motor Functions of Spinal Cord Substance P

Abstract. *Low doses of D-Pro²-D-Phe⁷-D-Trp⁹-substance P, a specific substance P antagonist, depressed the scratching and biting behaviors elicited by intrathecal injections of substance P, and cutaneous application of algesic substances. Higher antagonist doses caused hindlimb paralysis. This suggests that substance P is a neurotransmitter for primary nociceptor afferents and may also have an important function in motor control.*

The principal neurotransmitters for efferent neurons passing through the ventral roots have been known for more than 30 years. However, no sensory neurotransmitter entering the spinal cord via the dorsal roots has been convincingly identified. In 1953, Lembeck (*1*) first suggested that substance P (SP) might be a sensory neurotransmitter. Since the elucidation of the SP structure as Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 (*2, 3*) dorsal root SP has been shown to be present in small diameter primary afferent neurons making synaptic contact onto dendrites of neurons in the spinal cord dorsal horn (*4*). Electrical stimulation of these small diameter afferents leads to a release of SP into spinal cord perfusates (*5*). Furthermore, SP has been shown to excite dorsal horn neurons that can also be excited by intense cutaneous heat (*6*) or by more carefully defined noxious stimuli (*7*).

More recently it has been shown that SP, when injected into intrathecal spaces surrounding the spinal cords of mice, evokes an intense biting and scratching behavior (*8*). This type of behavior, similar to behaviors associated with chronic pain in rodents (*9*), is undoubtedly sensory in nature; the animals precisely direct their mouths and paws to their cutaneous surfaces in an apparently purposeful fashion. In order to further delineate the association of this behavior with pain sensation, we have now mimicked this behavior by coating the skin of the mice with algesic substances. In addition, we have antagonized the responses of these mice to algesic agents by the use of a specific SP receptor antagonist. Combined with the previously obtained data (*1, 4, 8*), this information provides evidence that SP is a neurotransmitter for primary nociceptor afferents. However, data are also presented suggesting that: (i) SP may not be the sole neurotransmitter for nociceptor afferents, and (ii) that spinal cord SP may also play an important role in motor control.

Capsaicin, the active ingredient of Hungarian red peppers, is known to produce intense burning inflammatory pain when applied to human skin (*10*), possi-

Table 1. Capsaicin-induced scratching and biting. The irritant was swabbed on with a Q-Tip. The observation time was the 5 minutes that immediately followed the irritant application. Results are expressed as the mean ± S.E.M. ($N = 8$).

Site	Chemical	Biting and scratching episodes	
		Number	Time spent (seconds)
Ear	Capsaicin	14.1 ± 2.7 (scratching)*	
	Ethanol	1.3 ± 0.5	
Foot	Capsaicin	42.5 ± 9.0 (biting)*	51.9 ± 10.2*
	Ethanol	0	0
Back	Capsaicin	23.4 ± 6.7 (scratching)*	12.6 ± 4.1*
	Ethanol	4.5 ± 1.3	2.1 ± 0.8

*Significantly different from control ($P < .05$, *t*-test).

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