from the circulation, in contrast to its quick uptake and disposition by visceral organs. For relatively equivalent doses, however, the duration of behavioral depression (especially in the early states of morphinization) was much greater after intraventricular injection, and this would indicate a far more prolonged contact of the drug with its receptors in brain. Hug (*4*) has shown that morphine is removed fairly ineffectively from the cerebrospinal fluid into the bloodstream, thus making this long contact possible. It is well known that the establishment of dependence and the development of tolerance are related to the presence of sustained high levels of the narcotic in blood (which will mean also relatively high levels in brain tissue) following repeated subcutaneous or intravenous injection (*1*). For the reasons given above, this may be less necessary when the intraventricular route is used. More important methodologically than the dosage differences is the fact that the cannulas rarely became obstructed and were then easily cleaned, they could not be removed by the animals, and it was not necessary to keep them flushed continuously or to maintain the animals under anticoagulant treatment. These are substantial practical advantages over chronic intravenous catheters (*5*). We have not seen any bacterial infections deriving from the cannulas, even after several months. We used only simple aseptic technique for implantation and injection, and protected the cannulas by a metal cap on the fitting around them. The only serious problem encountered was anorexia, concurrent with prolonged behavioral depression, which developed after each of the first few doses of morphine, and which we treated by gastric tube feeding with a high-calorie liquid diet when necessary.

The findings reported here lend support to the suggestion that some of those neural structures adjacent to the ventricular system (*6*) are major sites of action of the narcotic.

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References and Notes

1. G. A. Deneau and M. H. Seevers, *Advan. Pharmacol.* **3**, 267 (1964).
2. J. Cochin, *Fed. Proc.* **29** (1), 19 (1970).
3. W. R. Martin, *Pharmacol. Rev.* **19**, 463 (1967).
4. C. C. Hug, Jr., in *Narcotic Drugs, Biochemical Pharmacology*, D. H. Clouet, Ed. (Plenum, New York, 1971), pp. 122–136.
5. J. C. Woods and C. R. Schuster, *Int. J. Addict.* **3**, 231 (1968).
6. V. J. Lotti, P. Lomax, R. George, *J. Pharmacol. Exp. Ther.* **150**, 135 (1965); *ibid.*, p. 420; E. Eidelberg, M. L. Bond, C. A. Barstow, reported to the NAS–NRC Committee on Problems of Drug Dependence, Toronto, 1971; A. Herz, K. Albus, J. Metyš, P. Schubert, H. J. Teschemacer, *Neuropharmacology* **9**, 539 (1970).
7. Supported by grant MH 14588-03 of the National Institute of Mental Health and by the Barrow Neurological Foundation. We gratefully acknowledge the advice of Dr. A. S. Schwartz.

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Sex Attractant Pheromone of the House Fly: Isolation, Identification and Synthesis

Abstract. *A sex pheromone isolated from the cuticle and feces of the female house fly attracts the male fly; it has been identified as (Z)-9-tricosene. Chemical and biological comparisons of the natural and synthesized compounds show that they are identical.*

We report the first isolation, identification, and synthesis of a sex attractant pheromone of the common house fly, *Musca domestica* L. The compound, for which the name muscalure is proposed, is (*Z*)-9-tricosene.

The house fly is a danger to the health of man and animals principally because it breeds in manure, garbage, and fermenting crops and carries and spreads typhoid, dysentery, diarrhea, cholera, yaws, trachoma, and many other diseases (*1*). It also serves as an intermediate host of roundworms and tapeworms. The mating behavior of this worldwide pest has been studied extensively (*2*), the studies dating back to Aristotle (*3*). The male is sexually aggressive and the flies are very prolific breeders, so that the prospect that the insect's own sex pheromone could be used to repress its reproductive potential has considerable appeal.

Although olfactorily attractive pheromones of dipterous insects have been sought for many years, none have been isolated or identified. Presence of a house fly sex pheromone has been noted, because live or dead female flies were found to be attractive to males (*4*). Other studies revealed that male flies were also attracted to fly feces and lipid extracts of fly feces (*5*), and cuticular lipids (*6*).

Fractionation of cuticular and fecal lipids disclosed that the attractant, a hydrocarbon, was produced by only sexually mature females (*7*) for the attraction of only sexually mature males (*6*). Accordingly, the attractant was obtained from sexually mature, laboratory-reared female house flies (Orlando Regular strain) by surface washing to remove the cuticular lipids with hexane or ether. The concentrate was chromatographed on a silicic acid column, and the active fraction was eluted with hexane; elution with more polar solvents has yielded no active material (*7*). Thin-layer chromatography (TLC) of the active percolate on silica gel–silver nitrate plates (*8*) with 1 percent ethyl ether in hexane gave four zones. Chromatographic mobility of the zone with most of the activity (R_F 0.70) was consistent with that of a long-chain monoolefin; R_F values of the other zones were consistent with paraffins (R_F 0.95) and polyolefins (R_F 0.25, 0.10). Larger amounts of the monoolefin fraction were obtained by column chromatography (*9*), and gas chromatography (GC) of this fraction (*10*) gave peaks corresponding to 22 percent C_{23}, <1 percent C_{25}, 65 percent C_{27}, 10 percent C_{29}, and 3 percent C_{31}. Materials from the three major peaks were collected by preparative GC (*11*), and the C_{23} material was by far the most attractive. It gave a single peak on poly(diethylene glycol succinate) (DEGS), OV-17, and SE-30 columns (*10*) with retention times [Kovats indices (*12*)] that suggested a straight-chain monoolefin. Its mass spectrum showed a molecular ion at *m/e* 322 corresponding to $C_{23}H_{46}$. Its activity was lost, and its GC peak disappeared on treatment with 5 percent bromine in carbon tetrachloride, verifying the presence of a double bond. Position of the double bond was determined by microozonolysis of a 10-μg sample followed by GC of the products (*13*); retention times of the peaks coincided with those of nonanal and tetradecanal. Configuration of the double bond was established as (*Z*) by TLC on silica gel–silver nitrate plates (*14*). Absence of branching was confirmed by instantaneous hydrogenation (*15*) of 100 μg of the olefin followed by combined GC–mass spectrometry of the saturated product (*16*); the mass spectrum matched that of *n*-tricosane.

The data indicated that the sex at-

tractant was (*Z*)-9-tricosene (*17*). It was prepared by a Wittig synthesis as follows. 1-Bromotetradecane and triphenylphosphine were refluxed overnight in acetonitrile; the resulting salt (82 percent yield) was dissolved in dry dimethyl sulfoxide. *n*-Butyllithium and then nonanal were added. The product of this reaction was chromatographed on a silica gel column with hexane, yielding 9-tricosene (73 percent from the salt; $n_D^{26} = 1.4517$; b.p. 157° to 158°C at 0.1 mm-Hg) which was 93.4 percent pure by GC. Thin-layer chromatography of the product (*18*) showed that 85 percent of the (*Z*) isomer and 15 percent of the (*E*) isomer were present; the isomers were separated on a silver nitrate–silica gel column with hexane (*9*). The (*Z*) isomer had a nuclear magnetic resonance spectrum consistent with 9-tricosene (*19*): ($CDCl_3$,τ) two methyl groups as a triplet at 9.12; 17 CH_2 groups at 8.73; two CH_2 groups adjacent to the double bond as a multiplet at 8.01; CH=CH as a triplet at 4.72. The natural olefin had the same R_F value as the (*Z*) isomer (*14, 18*), and the infrared spectra (*20*) of the two were identical, with no band at 967 cm^{-1} [(*E*) unsaturation]. Further confirmation of identity was obtained by instantaneously hydrogenating both materials in the GC pathway (*15*) to saturated compounds; the products had the same retention time as authentic *n*-tricosane (*21*). A sample of C_{23} olefin isolated from fly feces was indistinguishable from the cuticular C_{23} olefin by infrared spectra, attractant studies, and retention time on two different columns (*22*).

Bioassays were conducted in a laboratory olfactometer as described (*6, 7*). The olfactometer contained 300 virgin male house flies (*23*) in a Plexiglas cage (90 by 45 by 54 cm) to which filtered, humidified outside air was delivered; the air was passed through two ports in the front face of the cage and exited through a screen that formed the rear face of the cage. Each port was a horizontal glass cylinder (8.0 cm, inside diameter, by 40 cm) with a vertical screen at its midpoint and an inverted screen funnel at its inner end. Each test material was applied as a hexane solution to filter paper and positioned in the outer end of one of the cylinders; an internal standard, Edamin (*24*), was similarly positioned in the other cylinder. Incoming air passed over the sample (or standard) and through the cylinder and cage of flies in this order before exiting. Responding flies that penetrated the funnel to reach the attractant were trapped and counted. Sixty milligrams of fecal lipid (*5*) served as an external standard. Each group of 300 newly emerged male flies (less than 3 hours old) was added to the olfactometer under CO_2 narcosis and held 24 hours before testing. Thirty-minute tests with intervening 30-minute recovery periods were then conducted over a 2-day period.

Table 1. House flies attracted to natural and synthetic materials in 30-minute tests, 300 male flies per test.

Material tested	Amount	Tests (No.)	Percentage of flies attracted to		Activity quotient †
			Test material	Internal standard *	
		Natural			
Fecal lipid ‡	60 mg	205	16.9	6.9	
Cuticular lipid	6 mg	5	22.8	5.1	+ 2.2
Cuticular hydrocarbons	6 mg	6	18.4	7.3	+ 1.2
Cuticular paraffins	§	5	4.6	7.9	− 0.5
Cuticular monoolefins	§	10	19.6	12.7	+ 1.5
Cuticular polyolefins	§	6	16.2	13.9	+ 0.2
Cuticular monoolefin C_{23}	100 μg	6	23.0	4.5	+ 1.6
Cuticular monoolefin C_{27}	50 μg	15	6.7	7.0	− 0.1
Cuticular monoolefin C_{29}	150 μg	5	5.6	4.3	+ 0.1
		Synthetic			
(*Z*)-9-Tricosene	10 μg	15	11.9	8.3	+ 0.3
	50 μg	30	18.9	6.2	+ 1.8
	100 μg	15	26.9	18.3	+ 2.1
	250 μg	5	26.1	5.8	+ 1.5
(*E*)-9-Tricosene	10 μg	5	9.4	6.8	+ 0.2
	100 μg	7	9.6	5.2	+ 0.4
	200 μg	5	11.7	6.9	+ 0.4

* One gram of Edamin (*24*). † [(No. of flies attracted to test material — No. attracted to Edamin)/No. of flies in olfactometer] ÷ [(No. of flies attracted to external standard — No. attracted to Edamin)/No. of flies in olfactometer] (*6*). ‡ External standard. § Amount found in 500 mg of cuticular hydrocarbon.

The activity of the natural materials can be ascribed to the C_{23} monoolefin subsequently identified as (*Z*)-9-tricosene (Table 1). A 50-μg sample of the synthetic (*Z*) isomer attracted more flies than 200 μg of the (*E*) isomer or 60 mg of crude fecal lipid. The other cuticular monoolefins (C_{27} and C_{29}) and polyolefins were weakly active. The following bioassays, not included in Table 1, were also performed. Addition of 50 to 100 μg of muscalure to an inactive portion of 100 or 1000 μg of either the cuticular paraffins or *n*-octacosane produced slightly enhanced activity over that of the muscalure alone; at no time was masking (reduction in activity) noted. No masking was observed when (*Z*) and (*E*) isomers were mixed at ratios up to 1 : 3, respectively. In tests of longer duration, 5 μg of muscalure and Edamin were each applied to glass plates; respective captures were 61 and 14 percent in 60 minutes and 76 and 15 percent in 90 minutes.

In addition, male flies apparently became sexually stimulated on exposure to the synthetic or natural pheromone, since the observed number of mating attempts increased markedly near a treated filter paper inserted directly into the olfactometer.

In limited field trials, about twice as many flies landed on a grid treated with 100 μg of muscalure as on an untreated grid. Though not a potent attractant as compared to some sex pheromones, muscalure is expected to be inexpensive to manufacture and it may have good potential for reducing the amount of insecticide needed to control the ubiquitous house fly.

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References and Notes

1. *The House Fly: How to Control It*, USDA Leaflet No. 390 (U.S. Department of Agriculture, Washington, D.C., 1969), p. 2.
2. A. Berlese, *Riv. Patol. Veg.* **9**, 345 (1902); G. C. Hewitt, *Quart. J. Microscop. Sci.* **25**, 495 (1908); R. H. Hutchinson, *Bull. U.S. Dept. Agr.* **345**, 1 (1916); E. B. Basden, *Bull. Entomol. Res.* **37**, 381 (1947); G. W. Barber and E. B. Starnes, *J. N.Y. Entomol. Soc.* **57**, 203 (1949); C. M. Murvosh, G. C. LaBrecque, C. N. Smith, *Ohio J. Sci.* **65**(2), 68 (1965).
3. Aristotle, *History of Animals*, I. Bekker, Ed.

(Royal Prussian Academy of Science, Berlin, 1831), book 5, chap. 8, pp. 542, A6-10.
4. W. M. Rogoff, A. D. Beltz, J. O. Johnsen, F. W. Plapp, *J. Insect. Physiol.* **10**, 239 (1964).
5. M. S. Mayer and C. W. Thaggard, *ibid.* **12**, 891 (1966); M. S. Mayer and J. D. James, *ibid.* **17**, 833 (1971).
6. D. L. Silhacek, M. S. Mayer, D. A. Carlson, J. D. James, *ibid.*, in press.
7. D. L. Silhacek, D. A. Carlson, M. S. Mayer, J. D. James, *ibid.*, in press.
8. Silica gel G containing 25 percent silver nitrate (Uniplates from Analtech, Inc., Newark, Del.).
9. With a column of Adsorbosil-CABN, 100/140 mesh, 25 percent silver nitrate on silica gel (Applied Science Laboratories, State College, Pa.), paraffins, monoolefins, and polyolefins were eluted with 0, 1, and 10 percent ether in hexane, respectively.
10. F & M Model 810 instrument with a flame ionization detector was equipped with stainless steel columns; 5 percent DEGS on 60/80 Chromosorb G-DMCS (1.8 m by 4 mm i.d.); 3 percent OV-17 on 100/120 Gas Chrom Q (2 m by 4 mm i.d.); 5 percent SE-30 on 100/120 Varaport 30 (1.8 m by 4 mm i.d.).
11. Varian Model 90-P instrument, equipped with thermal conductivity detector and the 5 percent SE-30 column (*10*), was temperature programmed from 150° to 280°C.
12. E. Kovats, *Advan. Chromatogr.* **1**, 229 (1966).
13. M. Beroza and B. A. Bierl, *Anal. Chem.* **39**, 1311 (1967).
14. Samples of (*Z*)- and (*E*)-9-octadecene gave spots at R_F 0.70 and 0.90, respectively, on TLC plates (*8*) developed three times with hexane. The natural olefin gave a spot at 0.70.
15. M. Beroza and R. Sarmiento, *Anal. Chem.* **38**, 1042 (1966).
16. Finnigan Model 1015 mass spectrometer interfaced with a Gohlke glass separator to a Varian GC equipped with 3 percent OV-101 on 80/100 Gas Chrom Q (glass column, 2 m by 4 mm i.d.).
17. (*Z*)-9-Tricosene has been reported as one of the major cuticular hydrocarbons of several cockroach species [L. L. Jackson, *Lipids* **5**, 38 (1970)].
18. Spots at R_F 0.70 and 0.90, obtained as described (*14*), for the (*Z*) and (*E*) isomers, respectively, were eluted and quantified by GC.
19. Determined with a Varian XL 100 instrument. We thank K. Scott of the Department of Chemistry, University of Florida, Gainesville, for determining the spectrum.
20. Perkin-Elmer Model 237 instrument, liquid film on NaCl plates.
21. This and other authentic straight-chain hydrocarbons were obtained from Analabs, Inc., North Haven, Conn.
22. DEGS and SE-30 columns (*10*).
23. From a laboratory colony of house flies resistant to phosphate insecticides (Cradson-P).
24. Hydrolyzed milk protein, Sheffield Chemical Co., Union, N.J.

27 August 1971

Natural Occurrence of Fatty Acid Ethyl Esters

Laseter and Weete (*1*) have recently reported the presence of ethyl esters of long-chain fatty acids in extracts of *Rhizopus arrhizus* and their identification by combined gas chromatography–mass spectrometry. This report is interesting in that it extends the known occurrence of such esters to microorganisms but does not constitute their first identification as natural products since they have already been reported from mammalian (*2*) and insect (*3–6*) sources.

As with *Rhizopus*, Skořepa *et al.* (*2*) could attribute no physiological role to the three ethyl esters of long-chain fatty acids which they identified in extracts of ox pancreas. By contrast, specific roles have been suggested for ethyl esters in insects. Thus Bergström *et al.* (*3*) identified ethyl laurate as a component of the volatile territory-marking scent produced by the bumblebee *Bombus terrestris*, and I (*4*) suggested the same function for the series of ethyl esters, of which ethyl myristoleate was the major component, obtained from *B. lucorum*. Kullenberg *et al.* (*5*) have since distinguished a second race of *B. lucorum*, which employs a different series of ethyl esters and have identified these esters in the scents of two other *Bombus* species. As indicated in the original papers, these findings have additional significance as possibly the first examples of chemical characters supplementing and extending classifications based on classical insect taxonomy. Ikan *et al.* (*6*) have identified ethyl esters of long-chain fatty acids as the greater part of the "assembling scent" of the beetle *Trogoderma granarium*, which scent also acts as a repellent for *Tribolium castaneum*.

It may be conjectured that ethyl esters of this type, possessing advantages of volatility and of specificity as natural products, may be used quite widely in the insect world as compounds modifying behavior, that is, "pheromones" in the broadest sense. It is also likely that rather specific, possibly unusual, biosynthetic mechanisms are operating.

In all the above reports, extensive use has been made of modern analytical techniques, notably combined gas chromatography–mass spectrometry which has yielded unequivocal evidence of structure. Their use in reexamination of data obtained by older, less-refined methods and in other contexts should prove illuminating. It is, for instance, possible to confuse a fatty acid ethyl ester with the methyl ester of the corresponding acid containing one more carbon atom if some gas chromatographic methods are used alone. This may explain why ethyl esters have remained undetected in natural sources until recently.

The presence of ethyl esters of short-chain and other acids in flavor and odor volatiles is, of course, well documented [see for example (*7*)].

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References

1. J. L. Laseter and J. D. Weete, *Science* **172**, 864 (1971).
2. J. Skorepa, P. Hrabák, P. Mareš, A. Linnarson, *Biochem. J.* **107**, 318 (1968).
3. G. Bergström *et al.*, *Ark. Kemi* **28**, 453 (1968).
4. D. H. Calam, *Nature* **221**, 856 (1969).
5. B. Kullenberg, G. Bergström, S. Ställberg-Stenhagen, *Acta Chem. Scand.* **24**, 1481 (1970).
6. R. Ikan, E. D. Bergmann, U. Yinon, A. Shulov, *Nature* **223**, 317 (1969).
7. H. E. Nursten and A. A. Williams, *Chem. Ind.* **1967**, 486 (1967).

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Our omission in referencing works by Calam and others stems from the fact (with two exceptions) that no notations of esters of any type were made in their titles, and, therefore, one surveying the literature might easily overlook such information. Two such examples concerning ethyl esters are worth noting at this time. Investigations by Light *et al.* (*1*) demonstrated that cell-free preparations of yeast extract were capable of synthesizing ethyl esters of long-chain fatty acids, while ethanol exogenously added to goat's milk resulted in the synthesis of esters of the same type [Patton (*2*)]. Calam has also related some interesting speculations concerning the functions of ethyl esters of long-chain fatty acids. However, the fungal growth-promoting properties found for the mono- and dienoic C_{18} fatty acid ethyl esters reported by Wardle and Schisler (*3*) and again by Holtz and Schisler (*4*) should not be overlooked. The limited and varied distribution known at this time and the attributed functions of long-chain fatty acid ethyl esters stimulates some interesting questions.

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References

1. R. J. Light, W. J. Lennarz, K. Block, *J. Biol. Chem.* **237**(6), 1793 (1965).
2. S. Patton and R. D. McCarthy, *Nature* **209**(5023) 616 (1966).
3. K. S. Wardle and L. C. Schisler, *Mycologia* **61**, 305 (1969).
4. R. B. Holtz and L. C. Schisler, *Lipids* **6**(3), 176 (1971).

4 August 1971