# Isolation of N,N-Diethyl-m-toluamide (Deet) from Female Pink Bollworm Moths

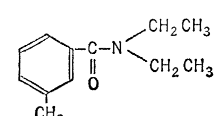
Abstract. *A chemical search for a natural activator of propylure, the sex pheromone of the pink bollworm moth, revealed that the female moths produce* N,N-*diethyl*-m-*toluamide, an outstanding insect repellent synthesized commercially and never before reported from natural sources. The compound occurs in fairly large amount in female adults and in much lesser amount in female pupae, but it is completely absent in female larvae and in all stages of the male insect. Its role in the life of the insect has not yet been definitely determined.*

In 1966, we identified the sex pheromone of the pink bollworm moth, *Pectinophora gossypiella* (Saunders), as 10-propyl-*trans*-5,9-tridecadienyl acetate (*1*). Although the pure compound was extremely active in eliciting sexual excitement and copulatory attempts of male moths in laboratory bioassays, it failed to attract males when baited traps were exposed in field cage tests (*2*). Since a crude methylene chloride extract of the female moths does attract males in the field (*3*), whereas such an extract that has been freed of propylure does not, it was apparent that an activator or activators are necessary to confer attractive powers on propylure under field conditions; this was confirmed when males were lured to propylure admixed with a fraction of inactive female extract (*2*). An attempt was made to isolate and identify the activators.

That portion of the female extract soluble in acetone at −20°C was freed of propylure by being shaken repeatedly at room temperature with portions of methanol (1). The oily fraction (1 g) that is insoluble in methanol was chromatographed on a column of 50 g of silica gel impregnated with silver nitrate (*4*) and eluted successively with 500 ml each of hexane; 5, 10, 25, and 50 percent ethyl ether in hexane; and then ethyl ether (*5*). The fractions eluted with 25 and 50 percent ether in hexane were identical as judged by gas chromatography, each showing a single peak with a retention time of 10 minutes on SE-30 and 12 minutes on DEGS (*6*). The fractions were combined to give 608 mg of colorless, viscous oil.

The infrared spectrum of the undiluted oil showed a very strong band at 1630 $cm^{-1}$ (carbonyl stretching vibration), indicative of a tertiary amide, and aromaticity suggesting meta substitution was shown by C-H out-of-plane bending vibrations in the region of 650 to 900 $cm^{-1}$. The mass spectrum gave the molecular weight as 191, and peak matching on a double-focusing high-resolution instrument established the elemental formula as $C_{12}H_{17}NO$.

The nuclear magnetic resonance spectrum (carbon tetrachloride) gave evidence (chemical shift in values of $\delta$) for four aromatic protons ($\delta$, 7.10), two equivalent ethyl groups (triplet at $\delta$, 1.11; quadruplet at $\delta$, 3.31), and a methyl group on an aromatic ring (three protons, singlet at $\delta$, 2.33).



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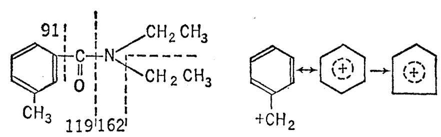

II

m/e 91 m/e 91 m/e 65

The aforementioned evidence enabled us to deduce the structure of the compound as *N,N*-diethyl-*m*-toluamide (Insert I). The mass spectrum was consistent with this structure, showing a base peak at a mass to charge ratio (m/e) of 119 and large peaks at 65, 91, and 162 (Insert II).

Structure I was confirmed by (*9*) comparison of its infrared, nuclear magnetic resonance, and mass spectra with those of synthetic *N,N*-diethyl-*m*-toluamide. Its presence in the moth extract is almost incredible, since it has heretofore been regarded solely as a synthetic product and an outstanding insect repellent known commercially as deet (*7*). The natural product and authentic deet showed identical repellency to *Aedes aegypti* mosquitoes. However, deet has been reported to show some attraction for pink bollworm moths in laboratory tests (*8*).

To preclude the presence of deet as a contaminant in the female moth extract from a single geographic location, we have analyzed batches of both sexes of the insect at various stages of development from three different geographic locations. Deet was identified (by gas chromatography) in large amount from all locations in virgin 3- to 5-day-old female adults, and in much smaller amount in female pupae, but not in female larvae or in males at any stage of development (Table 1). Deet was not found in the synthetic medium on which insects were reared in the laboratory, in the methylene chloride used for shipping the specimens, nor in the screw caps and polyethylene cap liners of the glass shipping containers.

Although preliminary results indicate that deet activates propylure in the field (*2*), its function in the moth is not yet understood.

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Table 1. Content of deet (determined by gas chromatography) in male and virgin female pink bollworms at various stages of development. All adults used were 3 to 5 days old, unless otherwise specified. LA, laboratory adults; WA, wild adults; LP, laboratory pupae; LL laboratory larvae.

| Insects analyzed | | Insects (No.) | Deet content (μg/insect) |
|---|---|---|---|
| Sex | Stage | | |
| *From Brownsville, Texas* | | | |
| Female | LA | 300 | 109.0 |
| Female | LA | 200 | 267.5 |
| Female | WA | 25 | 75.2 |
| Female | LP | 25 | 30.1 |
| Female | LL* | 25 | 0 |
| Male | LA | 200 | 0 |
| Male | LA | 25 | 0 |
| Male | WA | 25 | 0 |
| Male | LP | 25 | 0 |
| Male | LL* | 25 | 0 |
| *From Phoenix, Arizona* | | | |
| Female | WA | 200 | 556.1§ |
| Female | WA† | 142 | 58.1 |
| Male | WA† | 560 | 0 |
| *From Honolulu, Hawaii* | | | |
| Female | WA‡ | 50 | 557.2§ |
| Female | WA | 56 | 604.6§ |
| Male | WA | 127 | 0 |

* Fourth instar. † Extract 1 year old. ‡ Newly emerged. § We have no explanation for the high deet content in these samples as compared with the content of other samples.

## References and Notes

1. W. A. Jones, M. Jacobson, D. F. Martin, *Science* **152**, 1516 (1966).
2. Results of the attractancy tests will be described elsewhere.
3. M. T. Ouye and B. A. Butt, *J. Econ. Entomol.* **55**, 419 (1962).
4. Adsorbosil-CABN (60 to 100 mesh, containing 25 percent silver nitrate) obtained from Applied Science Laboratories, State College, Pennsylvania. The mention of trade names, products, or companies does not constitute

endorsement by the U.S. Department of Agriculture over those not named.
5. The hexane used in these investigations was purified to the equivalent of spectral grade by percolation of reagent-grade hexane through silica gel and subsequent distillation. The ethyl ether was distilled and stored over sodium. All other solvents used were reagent grade.
6. The chromatography was carried out on an F & M Model 500 instrument equipped with a Model 1609 flame ionization attachment with stainless steel columns packed with 5 percent SE-30 on Chromosorb W (3.05 m by 0.31 cm) at 150°C and with 10 percent diethylene glycol succinate (DEGS) on Chromosorb W (3.7 m by 0.31 cm) at 185°C. Nitrogen flow rate on the SE-30 column was 25 ml/min; that on the DEGS column was 14 ml/min.
7. E. T. McCabe, W. F. Barthel, S. I. Gertler, S. A. Hall, *J. Org. Chem.* **19**, 493 (1954).
8. M. Beroza and N. Green, *U.S. Dept. Agr. Handbook No. 239*, 85 (1963).
9. We thank Dr. J. Ruth and Mr. E. L. Gooden, USDA, Beltsville, Maryland, for determining the mass spectra and nuclear magnetic resonance spectra, respectively. We thank Dr. M. T. Ouye (Brownsville, Texas), Mr. L. F. Steiner (Honolulu, Hawaii), and Mr. J. C. Keller (Phoenix, Arizona), all of the U.S. Dept. of Agriculture, for supplying the insects used in this investigation and for carrying out the bioassays, and Dr. C. N. Smith, USDA, Gainesville, Florida, for supplying the results of repellency test.

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## Cryptic Moths: Effects on Background Selections of Painting the Circumocular Scales

Abstract. *A dark moth* (Catocala antinympha) *and a light moth* (Campaea perlata) *rested on appropriate backgrounds in an experimental apparatus allowing a choice between black and white backgrounds. The selections remained unchanged when the circumocular scales of the moths were painted either black or white. These results suggest that selections of background by cryptic moths, with respect to background reflectance, are genetically fixed. Background selections by melanic forms in various species are interpreted in the light of this conclusion.*

Several geometrid and noctuid moths select appropriate backgrounds when presented with a choice of backgrounds differing in reflectance (*1, 2*). These selections, if one assumes that they are visually based, could result from either of two mechanisms: (i) genetically fixed responses to particular background reflectances, and (ii) matching responses, in which the moths compare certain parts of their bodies with their backgrounds. Kettlewell (*1*) and Ford (*3*) have suggested a matching mechanism, involving the circumocular scales, to explain the different background preferences of typical and melanic *Biston betularia*. In an attempt to distinguish between the two alternatives, I altered the reflectances of two moths—a dark noctuid, *Catocala antinympha*, and a pale geometrid, *Campaea perlata*—by painting their circumocular scales. The results provided evidence of genetically fixed selections of background in these species.

The moths were collected near 150-watt bulbs in Leverett, Massachusetts, during the summer of 1967. Individuals to be painted were placed in a cyanide killing jar until their flutterings ceased; this treatment rendered the moths inactive for 3 to 5 minutes. They were then painted with either black or white Flo-Paque paint; I used a very fine red sable brush and viewed them through a × 2 binocular loop. Paint was applied to all scales of the head, of the collar on the thorax, and of the bases of the forewings (Fig. 1). These scales, because of their reflectance (which appeared similar to that of the forewings) and their position around the eyes, were assumed to include any that the moths might use in a reflectance-matching process.

The experimental apparatus consisted of two black and two white pieces of painted blotting paper, each 27.9 by 48.3 cm, formed into a cylinder of alternating black and white sections. The cylinder was set in a plywood box (35.6-cm square by 48.3-cm high) which was covered with a pane of window glass and a double layer of cheesecloth. The entire apparatus was placed in a wooded area where a thick canopy excluded direct sunlight.

Each morning, between 0600 and 0800 hours E.S.T., the background selections of the moths that had been collected and painted the previous evening were noted, and the moths were taken for later determinations of reflectance.

The percentage-reflectance values for the backgrounds and the moths' forewings were determined (*4*). For each species of moth, the forewings of 12 individuals were glued as montages onto black construction paper, and percentage reflectance was measured over a circle, 2.85 cm in diameter, within each montage. The altered reflectances were obtained from montages of painted forewings. The reflectance of the construction paper was 7.33 percent.

The reflectances and background selections of control and experimental groups of both species are shown in Fig. 2. Every group within each species differed significantly from a random distribution on the black and white backgrounds (chi-square tests), but no groups within either species differed significantly from one another (Fisher exact probability tests). These results suggest that selections of background by these moths, with respect to background reflectance, are genetically fixed.

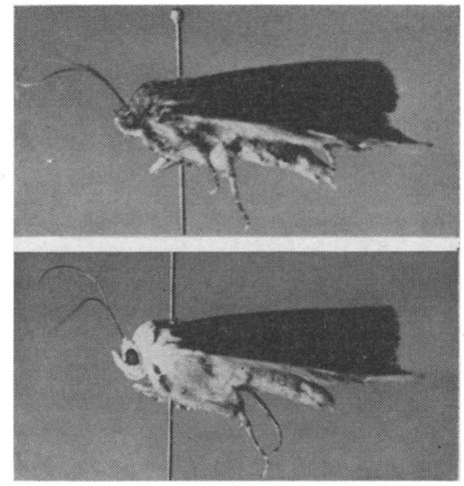

Fig. 1. Extent of the painted area on experimental moths, as shown by comparison of an unpainted (top) and a white-painted (bottom) *Catocala antinympha*. Moths are slightly enlarged.

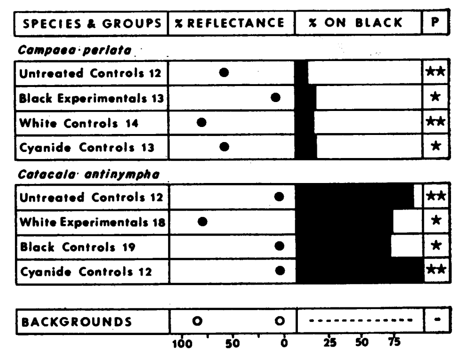


Fig. 2. Reflectances and background selections of experimental and control moths of two species, with reflectances of the experimental backgrounds. The number of individuals tested of each group is given. Significant deviations from chance selections of black and white backgrounds are indicated by stars: one star, $P < .05$; two stars, $P < .01$.

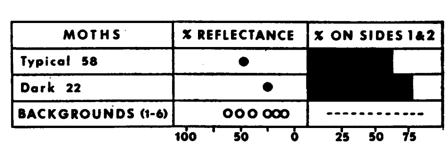


Fig. 3. Reflectances and background selections of two forms of *Cosymbia pendulinaria*, with reflectances of the experimental backgrounds. The number of moths on the lightest two backgrounds was significantly greater than by chance in both forms ($P < .001$), but the two distributions did not differ from one another ($P > .20$, chi-square tests).