

# The Presence of Disparlure, the Sex Pheromone of the Gypsy Moth, in the Female Nun Moth

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Disparlure, (*cis*-7,8-epoxy-2-methyloctadecane) the sex attractant of the gypsy moth, *Porthetria* (*Lymantria*) *dispar*, attracts the male nun moth, *Lymantria monacha*, in the field and is a highly effective olfactory stimulus in electroantennogram (EAG) and single-cell recordings. We have now analyzed the extract of 2000 abdominal tips of the female nun moth. Physical and chemical tests, which included gas-chromatographic retention times, elution volumes from silica gel and silica gel-silver nitrate columns, mass spectra, epoxide functionality, EAG-activity of chromatographic fractions with gypsy moth antennae, presence of disparlure precursor, all indicated that disparlure is present in the extract of nun moth sex glands. The optical activity of the natural disparlure of the two species has not yet been determined.

Several authors have reported <sup>1–5</sup> that disparlure, *cis*-7,8-epoxy-2-methyloctadecane <sup>6</sup>, the sex attractant of the gypsy moth, *Porthetria* (*Lymantria*) *dispar* (L.), is a highly effective attractant for the nun moth, *Lymantria monacha* (L.). Further evidence for the presence of disparlure in the nun moth rests on electroantennogram (EAG) and single cell data. Additional physical data were sought to establish the presence of disparlure in this species.

## Material

In July 1973, 2000 pupae of female nun moths were collected during an outbreak of *Lymantria monacha* <sup>5</sup> in a 60-year-old 300-ha forest in the Silkeborg district, Middle-Jütland/Denmark. The forest consisted of *Pinus silvestris* L. with some *Picea excelsa* L. underwood of varying density. The moths emerged in cages in the forest, and 1–2 days after emergence, the abdominal tips were cut off and preserved in benzene. Further treatment was then delayed for several weeks.

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## Methods and Results

In the search for disparlure in the nun moth, the 2000 female sex glands were homogenized and then extracted with benzene and cyclohexane. The concentrated extract was chromatographed on 10 g of

silica gel, and the following hexane-ether fractions (10–15 ml each) were collected: 3 of 100% hexane, 5 of 2% ether in hexane, 7 of 5% ether in hexane, 3 of 15% ether in hexane, and 5 of 100% ether. Electroantennogram (EAG) recordings (gypsy moth antennae) showed activity in the third fraction of 5% ether in hexane, which was the elution volume of disparlure when chromatographed identically. The concentrate from this fraction and adjacent ones (to avoid loss) was further chromatographed on 30 g Hi-Flosil-Ag<sup>®</sup> (silver nitrate on silica gel, Applied Science Lab, State College, Pa., USA) with 100 ml 100% hexane, 150 ml 2% ether in hexane, and then 5% ether in hexane; following 30 ml of the latter solvent found inactive by EAG, active material was eluted in the next 50 ml. Gas chromatography (GC) of the concentrate of this fraction on SE-30 (5% on 70/80 mesh Anakrom ABS, copper column 1.8 m × 4 mm i.d., 190°) gave a peak with a retention time (*t<sub>R</sub>*) (8 min) coinciding with that of disparlure. (GC on DEGS, Carbowax 20M, and Silar 10C failed to separate disparlure from gross interference.)

A mass spectrum of this peak was obtained from the partially purified extract as previously described <sup>7</sup> on a Finnigan 1015 quadrupole mass

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spectrometer equipped with a gas chromatographic inlet and a Systems Industry 150 magnetic tape-computer data system. (The effluent, He at 15 ml/min, from a 1.5 m  $\times$  2 mm i.d. glass column containing 3% OV-1 on Varaport at 170 °C was directed into the spectrometer, operated at 70 eV and 200  $\mu$ A, through a Gohlke glass separator, with scans recorded every 2 sec.) Computer-generated reconstructed gas chromatograms (RGC) were obtained from the total ionization current (total  $m/e$  range) and from individual  $m/e$  values found in the mass spectrum of disparlure. The RGC of the total  $m/e$  range showed an interfering peak emerging before disparlure and other background material (Fig. 1). This interference was suppressed by re-

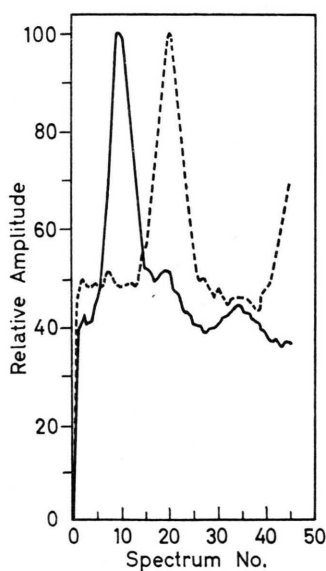


Fig. 1. Computer-generated reconstructed gas chromatograms obtained on injection of partially purified extract of female nun moth sex glands: solid line, total ion current; dashed line,  $m/e$  264 only. Retention time of dashed-line peak (spectrum No. 19–20) coincides with retention time of disparlure.

cording RGC's at  $m/e$  values characteristic of disparlure and as a result, a peak appeared at the  $t_R$  of disparlure, providing evidence for its presence in the extract. At the low concentrations injected, disparlure lost water and gave rise to a characteristic  $m/e$  264 (mol. ion – 18) peak. Presence of this fragment is clear in the comparison of the RGC of the total ion current and the RGC of  $m/e$  264 only shown in Fig. 1. In the RGC from  $m/e$  264, a peak appears at spectra 19–20, precisely the  $t_R$  of disparlure. The mass spectrum at this point, corrected

for background, was consistent with that of pure disparlure. When 0.4  $\mu$ g of pure disparlure was added to another portion of the extract and injected, the peak on the RGC of  $m/e$  264 occurred at the same retention time, and the peak size was markedly increased.

For further confirmation of structure, tests for the epoxide group were made<sup>8</sup>. In one, extract equivalent to about 40 glands was evaporated to near dryness and then diluted to 100  $\mu$ l with dichloromethane. An aliquot was chromatographed on the SE-30 column as described, followed by chromatography of a second aliquot that had been reacted with dry  $H_5IO_6$  for 7 min. The height of the GC peak having the  $t_R$  of disparlure was reduced 50% by the reaction, and no further reduction occurred after an additional 5 min of reaction time. A small peak with a  $t_R$  matching that of undecanal was generated by the reaction, thereby providing evidence that not only was an epoxide present but that the position of the epoxide group in the chain was the same as in disparlure. This experiment was repeated on a SCOT column (15 m, 0.05 mm i.d., OV-101, injected sample at 80 °C, temperature programmed 8 °C/min to 200 °C and held,  $t_R$  disparlure 17.1 min) to secure improved resolution. Again 50% of the peak was eliminated by the reaction; therefore only half of the peak can be attributed to disparlure; the composition of the other half is unknown. EAG tests were consistent with the foregoing findings since the reaction caused diminution of EAG response.

In the second test for epoxide functionality<sup>8</sup>, the extract was spotted at two points on a silica gel TLC plate (Brinkmann) and only one of the spots was allowed to react for 15 min with 10%  $H_3PO_4$ . After the plate was developed, the spots were scraped off and eluted with 15% ether in hexane; each eluate was concentrated and analyzed by GC on the SE-30 column. Again the peak with the  $t_R$  of disparlure was reduced by 50% as a result of treatment, indicating the presence of an epoxide probably of *cis* configuration<sup>8</sup>. Reaction of 7  $\mu$ g of disparlure in the same way was 98% complete.

Finally, the olefin precursor of disparlure, *cis*-2-methyl-7-octadecene, that was found in the extract of gypsy moth glands<sup>6</sup> was sought in the inactive hydrocarbon fraction of the nun moth extract<sup>9</sup>. Epoxidation followed by chromatography on silica

gel produced a material with an above control EAG response at the elution volume of disparlure.

Thus, all physical and chemical tests for disparlure in the extract of the female nun moth were positive: GC  $t_R$ , elution volumes from columns of silica gel and silica gel-silver nitrate, mass spectra derived from gas chromatography-mass spectral analysis, functionality (positive for epoxy group, indication of proper epoxide position by the undecanal generated, and *cis* configuration from TLC), presence of the disparlure precursor, and the better than control EAG response to fractions corresponding to those of disparlure in the various chromatographies. Since the observed peak on SE-30, estimated to be about 10 ng per gland, was decreased by 50% in the epoxide tests, the amount of disparlure found per gland is estimated to be about 5 ng per gland.

### Discussion

The identification of disparlure in the female lure gland of the nun moth is in accordance with all previous behavioral and electrophysiological tests:

a. Males of both species are equally attracted to synthetic disparlure in the field<sup>1-6</sup>. Both species prefer disparlure over several closely related epoxides<sup>2</sup>.

b. Electrophysiologically (EAG and single-cell recordings), disparlure was the most effective of 50 related epoxides in both species. As in a., no species differences have been found in the response pattern to disparlure modifications<sup>2, 10</sup>.

c. In EAG cross tests (see Priesner<sup>14</sup>, below) with female glands and male antennae of both species, the naturally produced pheromones elicited reciprocal responses.

d. The two species show cross attraction in field experiments (see below).

The evidence that two partially sympatric species produce the same compound as a sex attractant raises the question of the means by which they maintain their reproductive isolation. With regard to the role of pheromones, one may ask whether disparlure is indeed the only luring principle? Are there other pheromones, *e.g.* from the males, playing a role? Or are there additional sensory signals used for species recognition?

In the context of this question it is necessary to consider a recent paper by Iwaki *et al.*<sup>11</sup>, who syn-

thesized the two possible optical isomers (enantiomers) of disparlure in approximately 96% pure form. They found in behavioral and EAG tests that the gypsy moth males respond to at least 100-times lower concentrations of the (+) disparlure isomer than the (-) isomer. Could it be that the two species produce different disparlure enantiomers?

While there exists at present no chemical information to answer this question, a comparison of the biological effects of the female lures gives some indicative evidence. The degree of cross-attraction of the two species in the field has already been investigated in two earlier papers<sup>12, 13</sup> and is presently the subject of a detailed study by one of us (H.S.). The results show that females of both species attract males of both species, but in a competitive test, nun moth females were more effective than gypsy moth females in attracting nun moth males. In EAG cross tests with both species<sup>14</sup>, the glands of *P. dispar* and of *L. monacha* females were found to be equally effective olfactory stimuli to male antennae of both species. Whatever the basis for the behavioral discrimination of the species may be, it is not apparent in the EAGs. The reported 1:100 differences in the EAG-effectiveness of the two enantiomers<sup>11</sup> on the one hand, and the full reciprocal EAG effect of the gland products on the other hand are not compatible with the concept that the gypsy moth produces *only* the (+) enantiomer and the nun moth the (-) enantiomer.

The above results do not rule out the possibility that one or both species produce both disparlure enantiomers. If such optical isomerism is relevant for species recognition, this would imply the existence of a minimum of two types of disparlure receptors which respond differently to the enantiomers. Optical isomerism would then play a role similar to the well established one in which pheromones of various closely related moth species contain different proportions of geometrical isomers of a compound<sup>15-19</sup>.

Single olfactory receptor cells discriminating between optical isomers have been documented in the migratory locust and the honeybee<sup>20</sup>. Although the stimulating chemicals were not pheromones in those studies, it was recently reported that the naturally produced (+)-isomer of an ant alarm pheromone is clearly more effective than the (-) form in behavior tests<sup>21</sup>.

- <sup>1</sup> J. Schönherr, *Z. angew. Entomol.* **71**, 260 [1972].
- <sup>2</sup> D. Schneider, R. Lange, F. Schwarz, M. Beroza, and B. A. Bierl, *Oecologia (Berl.)* **14**, 19 [1974].
- <sup>3</sup> V. Skuhravý, M. Čapek, and R. Hochmut, *Anz. Schädlingskd. Pflanzen-Umweltschutz* **47**, 59 [1974].
- <sup>4</sup> M. Boness, W. Schulze, and U. Skatulla, *Anz. Schädlingskd. Pflanzen-Umweltschutz* **47**, 119 [1974].
- <sup>5</sup> J. Schröter and R. Lange, *Z. angew. Entomol.* **77**, 337 [1975].
- <sup>6</sup> B. A. Bierl, M. Beroza, and C. W. Collier, *Science* **170**, 87 [1970].
- <sup>7</sup> M. Beroza, B. A. Bierl, and H. R. Moffitt, *Science* **183**, 89 [1974].
- <sup>8</sup> B. A. Bierl, M. Beroza, and M. H. Aldridge, *Anal. Chem.* **43**, 636 [1971].
- <sup>9</sup> G. Kasang, D. Schneider, and M. Beroza, *Naturwissenschaften* **61**, 130 [1974].
- <sup>10</sup> D. Schneider, W. A. Kafka, M. Beroza, and B. A. Bierl, in preparation.
- <sup>11</sup> S. Iwaki, S. Marumo, T. Saito, M. Yamada, and K. Katagiri, *J. Amer. Chem. Soc.* **96**, 7842 [1974].
- <sup>12</sup> K. Görnitz, *Anz. Schädlingskd.* **22**, 145 [1949].
- <sup>13</sup> I. Schwink, *Z. angew. Entomol.* **37**, 349 [1955].
- <sup>14</sup> E. Priesner, *Fortschr. Zool.* **22**, 49 [1973] and *Z. Naturforsch.* **30 c**, 676 [1975] (see also <sup>2</sup>).
- <sup>15</sup> M. Beroza, G. M. Muschik, and C. R. Gentry, *Nature* **244**, 149 [1973].
- <sup>16</sup> J. A. Klun, O. L. Chapman, K. C. Mattes, P. W. Wojtowski, M. Beroza, and P. E. Sonnet, *Science* **181**, 661 [1973].
- <sup>17</sup> A. Comeau and W. L. Roelofs, *Entomol. Exp. Appl.* **16**, 191 [1973].
- <sup>18</sup> W. L. Roelofs and R. T. Cardé, *Pheromones (M. Birch, ed.)*, p. 96, North-Holland, Amsterdam/London 1974.
- <sup>19</sup> J. H. Tumlinson, C. E. Yonce, R. E. Doolittle, R. R. Heath, C. R. Gentry, and E. R. Mitchell, *Science* **185**, 614 [1974].
- <sup>20</sup> W. A. Kafka, G. Ohloff, D. Schneider, and E. Vareschi, *J. Comp. Physiol.* **87**, 277 [1973].
- <sup>21</sup> R. G. Riley, R. M. Silverstein, and J. C. Moser, *Science* **183**, 760 [1974].