Table 1. Electroantennogram responses to a stimulation of male and female queen antennae with puffs of dust impregnated with different chemicals. Talcum powder, or the extracted cuticular dust from hairpencils, was used as dust. See text for details.

Test stimulus	Response
Dust alone	0
Dust + ketone	+
Dust + diol	0
Dust + mineral oil	0
Dust + ketone + diol	+
Dust + ketone + mineral oil	+

pencils in eliciting EAG's from queen antennae, while hairpencils of the monarch butterfly (*Danaus plexippus*) were totally ineffective. This is in accord with the known chemistry of these butterflies —*Lycorea* hairpencils contain the ketone (*6*), whereas monarch hairpencils lack it (*7*). More surprising was the finding that monarch antennae are sensitive to the ketone, even though this species does not produce the compound; full-size EAG's were elicited in male and female monarch antennae in response to pure ketone, as well as queen and *Lycorea* hairpencils, but never in response to hairpencils of laboratory-reared specimens of the monarch itself. In this context it is noteworthy that the monarch is thought by some to belong to a different genus than the queen (*1*).

The fact that both *Lycorea* and the queen have the same ketonic pheromone, and that the monarch, which lacks the ketone, nevertheless responds to it with EAG's, is not in agreement with the original claim that pheromones (and consequently also the pheromone receptors) are species-specific (*8*). This lack of specificity was also found in experiments with diverse moths (*9*).

Electroantennogram tests with the diol suggested that this compound is at most a weak olfactory stimulant for both the queen and the monarch. Responses reached an EAG amplitude only slightly above that of the control, even when high concentrations of the diol were used. The diol response curve was essentially similar to that obtained with farnesol, another terpene alcohol. The relative insensitivity to these terpenoids is not indicative of a general insensitivity to compounds other than the ketone. Significant EAG's were obtained with all three danaid species tested, in response to fruity and flowery odors and fatty acids.

In nature the male of the queen administers its pheromonal secretion to the female on a powdery dust that acts as a carrier. In an effort to imitate this procedure, we stimulated queen antennae with puffs of dust, blown at close range from a metal tube (1 mm in diameter) that had been charged with dust. The dust consisted either of talcum powder, or of the actual cuticular dust from queen hairpencils [freed of intrinsic secretion by chemical extraction (*2*)].

The dust was variously coated chemically, using the same selection of coatings as was used in parallel behavioral experiments (*2*). Only the ketone-containing samples elicited EAG's (Table 1) (*10*). The agreement with the behavioral tests (*2*) is complete, with one exception. Whereas in our case the dust sample bearing ketone alone was fully effective, in the behavioral tests this sample failed to have an effect. Failure was attributed, not to a lack of an effect on the female, but to an inadequate stickiness of the sample, which made it impossible to endow the courting males with a proper load of dust (*2*). Because we administered the dust to the antennae directly, rather than by way of the male, the sample was bound to take effect.

In conclusion, we agree with Pliske *et al.* (*2*) that the ketone is the only sexual releaser-pheromone of the male queen butterfly. Our evidence is that (i) ketone-EAG's are sufficiently large to be attributable to the activity of many odor receptor cells; (ii) diol-EAG's are too small to be based upon the activity of many such cells; and (iii) the unidentified odorous component of the hairpencils is apparently not an odor for the butterflies. Mineral oil, which has the capacity to serve in place of the diol by acting as a substitute glue (*2*), is not an odor for the butterflies (Table 1).

The fact that sensitivity to the queen pheromone occurs in both sexes, rather than just in the sex opposite from the one producing the substance, is not without precedent, since a similar situation is known to prevail in the queen honeybee (*11*). It remains to be seen, however, whether in the queen butterfly the male makes actual use of its potential ability to monitor its pheromonal output.

D. SCHNEIDER, UTA SEIBT
Max-Planck-Institut für Verhaltensphysiologie, 8131 Seewiesen, Germany

References and Notes

1. L. P. Brower, J. van Zandt Brower, F. P. Cranston, *Zoologica* **50**, 1 (1965).
2. T. E. Pliske and T. Eisner, *Science*, this issue.
3. J. Meinwald, Y. C. Meinwald, P. H. Mazzocchi, *ibid.*, this issue.
4. J. H. Myers, thesis, Tufts University (1968).
5. D. Schneider, *Z. Vergl. Physiol.* **40**, 8 (1957); J. Boeckh, K. E. Kaissling, D. Schneider, *Cold Spring Harb. Symp. Quant. Biol.* **30**, 263 (1965); *Symp. Soc. Exp. Biol.* **20**, 273 (1965); B. C. Block, J. Boeckh, E. Priesner, *ibid.* **54**, 192 (1967); D. Schneider, *Science* **163**, 1031 1031 (1969).
6. J. Meinwald, Y. C. Meinwald, J. W. Wheeler, T. Eisner, L. P. Brower, *Science* **151**, 583 (1966); J. Meinwald and Y. C. Meinwald, *J. Amer. Chem. Soc.* **88**, 1305 (1966).
7. J. Meinwald, A. M. Chalmers, T. E. Pliske, T. Eisner, *Tetrahedron Lett.*, in press.
8. P. Karlson and A. Butenandt, *Annu. Rev. Entomol.* **4**, 39 (1959); P. Karlson and M. Lüscher, *Nature* **183**, 55 (1959).
9. D. Schneider, *J. Insect Physiol.* **8**, 15 (1962); *Symp. Soc. Exp. Biol.* **20**, 273 (1965); E. Priesner, *Z. Vergl. Physiol.* **61**, 263 (1968).
10. Because of the difficulty of controlling quantitatively the amount of dust administered to the antennae, the results are only of qualitative significance.
11. K.-E. Kaissling and M. Renner, *Z. Vergl. Physiol.* **59**, 357 (1968).
12. We thank Mrs. U. Heinecke for technical assistance. T. Eisner (Cornell University) suggested this electrophysiological approach. He and his colleagues, J. Meinwald and T. E. Pliske, supplied live monarch and queen butterflies, hairpencil extracts, hairpencil dust, and synthetic hairpencil compounds. We thank L. P. Brower (Amherst College) for *Lycorea* butterflies to test the EAG effect of their hairpencils on the queen and monarch antennae.
13. *Note added in proof*: We have extended our EAG studies to the African danaid *Danaus chrysippos*. Antennae of males and females of this species responded equally to hairpencils of this species, to hairpencils of queen and *Lycorea* butterflies, and to the ketone. This suggests that this Old World species of family Danaidae also possesses the ketone as the male releaser-pheromone. Final proof, however, can only come from chemical analysis. We thank the Natural History Society (Mrs. E. Carswell, Master in Charge) of the Michaelhouse School at Balgowan, Natal, South Africa, for collecting butterflies for us at their locality.

24 January 1969; revised 3 March 1969

Sex Pheromone of the Queen Butterfly: Chemistry

Abstract. *Two major components in the "hairpencil" secretion of the male of the queen butterfly* (Danaus gilippus berenice) *have been identified. One, a crystalline ketone (2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one), is known from another danaid butterfly. The other, a viscous terpenoid alcohol* (trans,trans-*3,7-dimethyldeca-2,6-dien-1,10-diol), is new; its structure is confirmed by an unambiguous synthesis.*

The secretion associated with the extrusible brushlike "hairpencils" found in abdomens of males of the queen butterfly (*Danaus gilippus berenice*) plays an important role in the courtship of these insects (*1*).

To isolate the pheromonal components, hairpencils were removed from live males caught in Florida (*1*) and were extracted with methylene chloride. The infrared spectrum of the extract showed prominent carbonyl absorption at 5.90 μ, and resembled that of 2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one

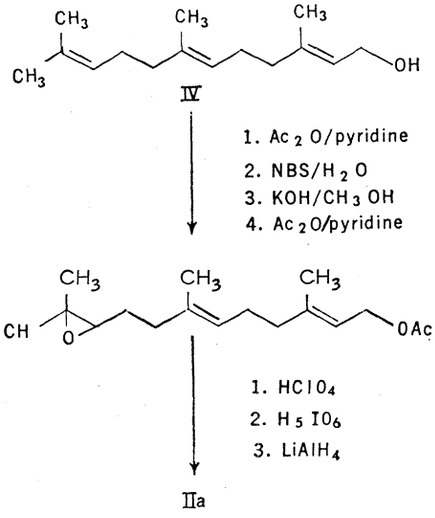
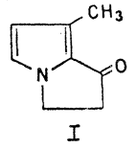


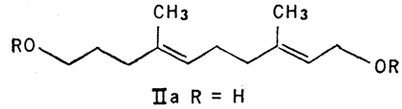
Fig. 1. Synthesis of *trans,trans*-3,7-dimethyldeca-2,6-dien-1,10-diol (IIa).

(I) previously characterized as a major component in the hairpencil secretion of the neotropical danaid *Lycorea ceres ceres* (*2*).



Additional infrared absorption in the 2.7- to 3.0-μ region indicated the presence of hydroxylic components as well. Thin-layer chromatography (TLC) on silica gel (developed with 5 percent methanol in methylene chloride) showed two major spots, along with two minor ones. The least polar component was indistinguishable from an authentic sample of I on the basis of comparisons of thin-layer gas chromatograms. This component could be isolated in crystalline form either by direct sublimation (60° to 65°C at 1 mm-Hg) from the crude extract or by preparative TLC; a mixture melting point with a synthetic sample of I was undepressed.

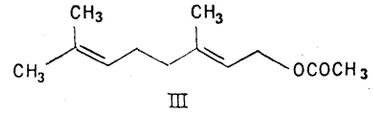
The major polar component of the queen hairpencil extract was most conveniently isolated by preparative TLC after most of the heterocyclic ketone was removed by vacuum sublimation. It was characterized as *trans,trans*-3,7-dimethyldeca-2,6-dien-1,10-diol (IIa) on the basis of the evidence summarized below.



IIa R = H
IIb R = CH_3CO
IIc R = C_6H_5CO
IId R = $Si(CH_3)_3$

The diol (IIa) was eluted from silica gel plates as a colorless, viscous oil with little characteristic infrared absorption beyond hydroxyl and carbon-hydrogen bands. It was converted into the corresponding diacetate (IIb), dibenzoate (IIc), and bis-trimethylsilyl ether (IId) by standard procedures. While neither the diol nor its diacetate could be purified by gas chromatography without decomposition, the mass spectrum (*3*) of the diacetate showed its highest mass/charge (*m/e*) peak at 222, corresponding to expectations for loss of acetic acid from a parent $C_{16}H_{26}O_4$ molecule (molecular weight, 282). The mass spectrum of IId purified by gas chromatography showed a parent peak at *m/e* 342 and an M-15 peak at *m/e* 327, thus confirming the $C_{12}H_{22}O_2$ formula for the diol itself.

The nuclear magnetic resonance (NMR) spectrum (*4*) of IIb showed a striking resemblance to that of geranyl acetate (III), with an additional low-field triplet (τ 6.0, J = 7 hz, 2H) corresponding to a second oxygen-bearing methylene group split by an adjacent methylene. The NMR spectrum of the oily dibenzoate showed all the features to be expected for structure IIc, and the formulation of the diol as an oxidatively degraded sesquiterpenoid closely related to farnesol (IV), appeared to be a most attractive hypothesis on this basis (*5*).



A synthesis of diol IIa from *trans,trans*-farnesol (IV) was accomplished as shown in Fig. 1 (*6*). The final product was identical to the natural diol in all respects. Treatment of the natural product with α-naphthyl isocyanate yielded a bis-α-naphthyl urethane which showed a melting range of 127° to 129°C after recrystallization from an ether-hexane mixture. The synthetic diol gave a bis-α-naphthyl urethane with the same melting range and a mixture melting point was undepressed.

The amounts of I obtainable from the male butterflies varied. Thus, in different batches of insects caught in the wild, recoveries of crude I averaged from 0.1 to 0.02 mg per male. The amount of diol II was very difficult to estimate, since no procedure for efficient isolation and purification of this component was developed. The secretion did not appear to be subject to qualitative variation. Extracted hairpencils from males of another subspecies (*Danaus gilippus strigosus*, from Rodeo, New Mexico) also yielded compounds I and IIa.

Whether these compounds are elaborated *de novo* by the butterflies or are obtained by transformations carried out on closely related precursors which might be available in their food remains to be investigated.

J. MEINWALD
Y. C. MEINWALD
P. H. MAZZOCCHI
Department of Chemistry, Cornell University, Ithaca, New York 14850

References and Notes

1. T. E. Pliske and T. Eisner, *Science*, this issue.
2. J. Meinwald and Y. C. Meinwald, *J. Amer. Chem. Soc.* **88**, 1305 (1966); ———, J. W. Wheeler, T. Eisner, L. P. Brower, *Science* **151**, 583 (1966).
3. We are indebted to Dr. G. Dudek, Harvard University, for the mass spectral results presented in this paper. All measurements were made on an AEI (Calvert Electronics) MS-9 double-focusing instrument. A more thorough study of the mass spectral fragmentation pattern of IIa and many related molecules has been carried out by Drs. S. Shrader and A. M. Chalmers with the M-902 C1 instrument at Cornell University.
4. These NMR data were obtained through Dr. R. Pitcher, Varian Associates.
5. Subsequent to the completion of this work, a C_{13} allenic ketone and a C_{12} diacid, both oxidatively degraded terpenoids, have been characterized as components of other insect secretions [J. Meinwald, K. Erickson, M. Hartshorn, Y. C. Meinwald, T. Eisner, *Tetrahedron Lett.* **1968**, 2959 (1968); J. Meinwald, A. M. Chalmers, T. E. Pliske, T. Eisner, *ibid.*, p. 4893].
6. These results were described at the 4th International Union of Pure and Applied Chemistry Symposium on the Chemistry of Natural Products, Stockholm, June 1966 (Abstr., p. 147). A portion of this synthetic sequence had been carried out earlier by Prof. E. E. van Tamelen at Stanford University, whom we thank for providing us with the appropriate experimental procedures.
8. Supported by NIH grant AI-02908, and by a generous gift from Hoffman-La Roche, Inc. Extracts supplied by R. Alsop, T. Eisner, and T. Pliske.

22 October 1968

Polyvinylsulfate: Interaction with Complexes of Morphogenetic Factors and Their Natural Inhibitors

Abstract. *The inactivation of the mesodermalizing inducing factor by its natural inhibitor is completely abolished by low concentrations of polyvinylsulfate.*

The differentiation of the early gastrula ectoderm of *Triturus* can be channeled into different pathways. The "neuralizing" factor initiates the differentiation of neural tissue, especially forebrain; the "mesodermalizing" factor, which is protein in nature, induces the formation of muscle and notochord (*1, 2*). The formation of complex trunk and tail structures (with spinal cord,