

brane-bound sacs. The latter cells may represent ghosts of spherocytes of previously pitted red cells that, because of their rigid cell membranes or because of damage to their cell membranes during the pitting process (12), are unable to traverse the splenic vasculature and that therefore undergo hemolysis.

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References

1. P. F. Russel, L. S. West, R. D. Manwell, G. MacDonald, *Practical Malariology* (Oxford Univ. Press, London, 1963), p. 429.
2. A. Zuckerman, *J. Infect. Dis.* **106**, 123 (1960).
3. —, in *Immunity to Protozoa*, P. C. C. Garnham, A. E. Pierce, I. Roitt, Eds. (Blackwell Scientific, Oxford, England, 1963), pp. 78–88.
4. H. W. Cox, *Milit. Med.* **131** (Suppl.), 1195 (1966).
5. J. N. George, E. F. Stokes, D. J. Wicker, M. E. Conrad, *ibid.*, p. 1217.
6. A. Zuckerman, S. Abzug, R. Burg, *ibid.* **134** (Suppl.), 1084 (1969).
7. T. Sodeman, J. R. Jumper, P. G. Contacos, C. S. Smith, *J. Trop. Med. Hyg.* **73**, 33 (1970).
8. E. S. Reynolds, *J. Cell. Biol.* **17**, 208 (1963).
9. L. Weiss, *Seminars Hematol.* **2**, 205 (1965).
10. R. A. Rifkind, *Blood* **26**, 433 (1965); N. S. Lawson, B. Schnitzer, E. B. Smith, *Arch. Pathol.* **87**, 501 (1969).
11. W. H. Crosby, *Blood* **12**, 165 (1967).
12. M. E. Conrad, and L. H. Dennis, *Amer. J. Trop. Med. Hyg.* **17**, 170 (1968).
13. J. N. George, D. J. Wicker, B. J. Fogel, C. E. Shields, M. E. Conrad, *Proc. Soc. Exp. Biol. Med.* **124**, 1086 (1967).

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Sex Pheromone of the Codling Moth: Structure and Synthesis

Abstract. *The structure of a sex pheromone of the codling moth, Laspeyresia pomonella (L.), has been determined by spectrometric and chemical degradative techniques and by synthesis to be (2Z, 6E)-7-methyl-3-propyl-2,6-decadien-1-ol. In field cage tests the synthesized sex pheromone was as attractive as the natural, but neither was as attractive as ten virgin females per trap.*

The codling moth is a serious, worldwide pest of most pome and stone fruits. We have studied this insect with the purpose of synthesizing a sex pheromone which can be used to control it. We have reported a laboratory bioassay and a method of isolation of a sex pheromone from females, and chemical tests and gas chromatographic data indicated the pheromone was an unsaturated alcohol (1). Further, the results of the laboratory bioassays were correlated with tests for attractancy in apple orchards when extracts of females at various stages of purification were tested (2). Pure sex pheromone was also attractive in the orchard test, but less so than ten females per trap.

Prior to structural studies, the purity of isolated pheromone was established by demonstrating that a single peak was obtained and the biologically potent fraction was coincident with the peak on gas-chromatographic columns of Apiezon L, SE-30, Zonyl E7, Carbowax 20 M, and diethylene glycol succinate.

Mass spectra (3) of the pheromone and its acetate were nearly identical. Both showed strong peaks at 192, 149, and 121 *m/e* units. Long-chain alcohols and acetates give weak or undetectable molecular ions, because they readily lose water or acetic acid in the mass spectrometer. This fact and the similarity of the two spectra indicated that the mol-

ecular weight of the pheromone was 210. A high-resolution mass spectrum indicated a formula of $C_{14}H_{24}$ for the 192 peak (calculated, 192.1878; found, 192.1886) and therefore the molecular formula is $C_{14}H_{26}O$. The ultraviolet spectrum showed no absorbance between 200 and 320 nm (4).

The infrared spectrum was remarkably similar to that of geraniol (5). The following diagnostic peaks were

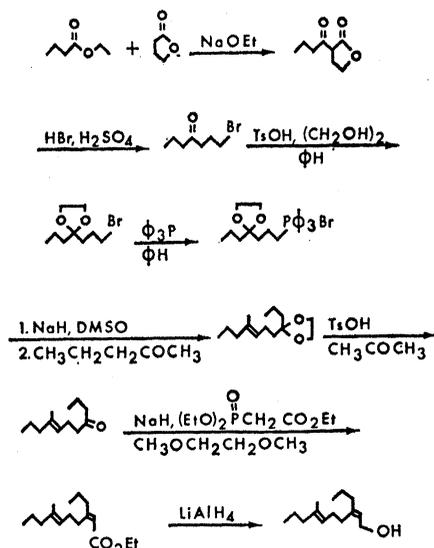
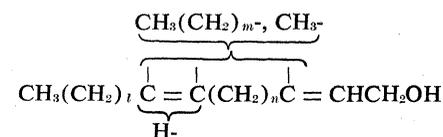


Fig. 1. Synthesis of mixed Z, E isomers of 7-methyl-3-propyl-2,6-decadien-1-ol. Abbreviations: Et, ethyl; Ts, tosyl; ϕ , phenyl; DMSO, dimethyl sulfoxide.

found (KBr pellet; μ): 3.00 (OH), 3.32 (olefin C–H), 3.40 to 3.52 (saturated C–H), 6.05 (C = C), 6.88 ($CH_2 + CH_3$), 7.29 (CH_3), 10.0 (C–O), 12.1 (trisubstituted olefin). The nuclear magnetic resonance spectrum (δ) was (CCl₄; δ): 5.37 (1.00 proton; triplet; $J \sim 7$ hz; C = $CHCH_2$), 5.05 (0.84 proton; broad, unresolved multiplet; C = CH), 4.00 (2.04 protons; doublet; J , 6.7 hz; C = $CHCH_2OH$ coupled to the proton at 5.37 according to decoupling experiment), 2.02 (6.81 protons; distorted quartet; C = CCH_2), 1.66 [3.12 protons; singlet; $C(CH_3)C = C$], 1.27 (4.8 protons; singlet; CH_2 connected to saturated carbon), 0.91 (6.4 protons; triplet; CH_3 connected to saturated carbon). The number of protons assigned to each value of δ was the whole number nearest to the integrated value except for the absorptions at 2.02 and 1.27 ppm where 8 and 4 protons, respectively, were assigned. These were the only possible values consistent with the structural data.

The structural deductions possible from the spectrometric data could be summarized as follows:



where $l \geq 1$; $m \geq 1$; $n \geq 2$; and $l + m + n = 6$. Apart from Z, E isomers, 24 structural formulas satisfied the given conditions.

Further information was obtained by hydrogenolysis (7). Upon hydrogenolysis, primary alcohols lose the carbon atom attached to the OH (8). Consequently, we expected a branched tridecane from the pheromone. The product obtained had a gas-chromatographic retention index (9) of 1223 on SE-30, which suggested a doubly branched tridecane. A mass spectrum also indicated a tridecane ($M^+ 184$) and showed prominent peaks corresponding to loss of propyl and methyl groups and exceptionally weak peaks corresponding to loss of ethyl or butyl groups. By excluding structures containing ethyl or butyl groups, we reduced the possibilities from 24 to 4.

Upon ozonolysis of the pheromone, only one product was detected by gas chromatography. Presumably other products were contained in the solvent front. Its retention index was 1605 on Carbowax 20 M and 1017 on SE-30. This suggested an ozonolysis product with seven carbon atoms and two carbonyl groups. The mass spectrum

showed a molecular weight of 128, which agreed with this assignment. In addition, a prominent peak at 100 *m/e*, corresponding to loss of ethylene from the molecular ion, indicated a MacLafferty rearrangement of a propyl ketone. Another prominent peak was at 110 *m/e*, corresponding to loss of water. The other prominent peaks were the ones expected by α cleavage at the carbonyl groups of 4-oxoheptanal: 85, 71, 57, 43, and 29. Thus, the sex pheromone is one of the *Z*, *E* isomers of 7-methyl-3-propyl-2,6-decadien-1-ol.

A mixture of *Z*, *E* isomers of the pheromone was synthesized by the sequence in Fig. 1. When the synthetic sex pheromone was subjected to gas chromatography on Carbowax 20 M, the first and second isomers, in order of elution, were entirely resolved while the third and fourth were only partially resolved. The second isomer had the same retention time as the pheromone on columns of Apiezon L, SE-30, OV-210, and cyclohexanedimethanol succinate as well as on Carbowax 20 M. The infrared and mass spectra of the second isomer were also identical to those of the pheromone. Since *E* isomers elute after *Z* isomers (10), the first isomer should be *Z*, *Z* and the fourth should be *E*, *E*. Also, the nuclear magnetic resonance chemical shift of the methyl group has been established as 1.65 for the *E* configuration and 1.70 for the *Z* configuration (10). Therefore, the structure of the sex pheromone is (2*Z*, 6*E*)-7-methyl-3-propyl-2,6-decadien-1-ol.

Attractancy tests in a field cage (7.9 m wide by 3.6 m high by 22 m long) indicated that the synthetic and natural pheromones were equally attractive. Each attracted about 15 percent of the males attracted by ten females per trap. Quantities ranging from 1 μ g to 200 μ g per trap were tested and there was no increase in attractancy above 10 μ g per trap. Therefore some other material is also necessary for full attractancy.

The possibility that the sex pheromone was an artifact was considered. To test this possibility, condensed vapors from air passed over virgin females were analyzed by gas chromatography. Although enough pheromone to give a peak was not obtained, the bioassay indicated an active fraction with the same retention time as the pheromone on Carbowax 20 M. The active fraction was collected and reinjected into an Apiezon L column. Again the bioassay indicated an active fraction with the same retention time as the pheromone. Therefore, (2*Z*, 6*E*)-7-methyl-3-propyl-

2,6-decadien-1-ol is a true sex pheromone of the codling moth.

E,E-8,10-Dodecadien-1-ol has been reported to be a sex attractant of the codling moth (11). Also, although structural studies of the sex pheromone were not carried out, it was proposed as the sex pheromone, based primarily on electroantennograms of model compounds (11). In work to be reported elsewhere, we found that the codling moth possesses multiple sex pheromones. As of this writing, however, we have not found the presence of *E,E*-8,10-dodecadien-1-ol in codling moths.

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References and Notes

1. L. M. McDonough, D. A. George, B. A. Butt, M. Jacobson, G. R. Johnson, *J. Econ. Entomol.* **62**, 62 (1969).
 2. L. M. McDonough, D. A. George, B. A. Butt, L. N. Gamey, M. C. Stegmeier, *ibid.* **65**, 108 (1972).
 3. Mass spectra were obtained with a Bendix model 12 and a CEC model 21110B.
 4. The ultraviolet spectrum was determined from 15 μ g of pheromone in 3.4 ml of purified pentane with a Beckman model DB. As a control experiment, the ultraviolet spectrum of 10 μ g of 8,10-dodecadien-1-ol was also determined: maximum wavelength, 224 (molar extinction ϵ is 13,000).
 5. The infrared spectrum was obtained from 80 μ g of pheromone in a 1.5-mm KBr disc with a Perkin-Elmer model 337 equipped with a microbeam condenser.
 6. The nuclear magnetic resonance spectrum was obtained from 900 μ g of pheromone in a 60- μ l microcell with a Varian model HA-100 equipped with a computer of average transients (40 runs).
 7. Hydrogenolysis of 20 μ g of the pheromone was accomplished with National Instruments Laboratories, Inc., Beroza Carbon Skeleton Determinator.
 8. M. Beroza, *Anal. Chem.* **34**, 1801 (1962).
 9. A. Wehrli and E. Kovats, *Helv. Chim. Acta* **42**, 2709 (1959).
 10. G. W. K. Cavill and P. J. Williams, *Aust. J. Chem.* **22**, 1737 (1969).
 11. W. Roelofs, A. Comeau, A. Hill, G. Milicevic, *Science* **174**, 297 (1971).
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Genetics of the Antibody Response to Dextran in Mice

Abstract. *The immune response to dextran having the α -1,3 linkage may be under the control of antibody structural genes. Mice that respond well to this antigen produce antibody restricted with respect to light chain class (λ) and to an antigenic determinant resulting from a particular heavy and light chain interaction. The response to dextran is controlled by a locus linked to the heavy chain locus.*

The antibody light chains, kappa and lambda, have been divided into subgroups on the basis of their variable region sequence similarity to various prototype sequences (1). A comparison of the sequences of mouse λ chains produced by plasmacytomas in BALB/c mice showed that they belong to one λ variable region (V_λ) subgroup. Since the variants within this subgroup were confined to hypervariable regions and could be accounted for by single base changes in DNA, we postulated that the V_λ subgroup is coded for by a single V_λ gene which evolves somatically by mutation and antigenic selection. The sequence coded for by germ-line gene, $V_{\lambda(0)}$, would be the one which occurred most frequently. This prototype sequence, $V_{\lambda(0)}$, was found to be a subunit of those myeloma proteins which bind to α -1,3-dextran (2). These proteins also show a similar antigenic determinant dependent upon a particular light chain and heavy chain combination. This determinant (that is, idio-type) is related to specific antigen binding since nigerose (a disaccharide with the α -1,3 linkage) will inhibit the re-

action between idio-type and antiserum to the idio-type (3). Variable regions of these myeloma proteins could be coded for by two germ-line genes, one of which is $V_{\lambda(0)}$ and the other $V_{H(0)}$, one of the heavy chain variable (V_H) genes. If this were the case, BALB/c mice immunized with α -1,3-dextran should respond with antibody having the characteristics of the myeloma proteins which are antibodies to dextran. Furthermore, germ-line polymorphism in either gene $V_{\lambda(0)}$ or $V_{H(0)}$ might render mice unresponsive to α -1,3-dextran. Responsiveness would show dominant inheritance.

We have tested these possibilities by analyzing the response of inbred mice to dextran containing the α -1,3-glucosyl linkage. Mice were immunized subcutaneously with 100 μ g of dextran B-1355S (57 percent α -1,6; 35 percent α -1,3; and 8 percent α -1,4 linkages) (4) in complete Freund's adjuvant. Antibody response to dextran was tested after 5 days by the Mishell-Dutton modification (5) of the Jerne plaque assay, with the use of sheep red blood cells (SRBC) sensitized with either dextran