solar radiation budget. The exclusion of the albedo effect may even lead to predicted changes in surface temperature of the wrong sign (*9*). The effect of solar absorption by NO_2 should also be included in these models for case studies involving significant changes in the NO_2 column density.

At biologically important wavelengths in the ultraviolet region (UV-B) (280 to 320 nm), solar absorption by NO_2 has only a small effect on the amount of solar radiation reaching the earth's surface. In this spectral region the optical thickness of O_3 is several orders of magnitude greater than the optical thickness of NO_2; thus the flux of UV-B radiation incident at the earth's surface is much more sensitive to changes in the O_3 column density than to changes in the NO_2 column density. Neglecting solar absorption by NO_2 leads to a slight overestimate of the biological effects from stratospheric injection of NO_x. For the 17- and 20-km injection cases considered above, neglecting solar absorption by NO_2 caused the change in UV-B radiation at the earth's surface to be overestimated by a factor of 1.02.

FREDERICK M. LUTHER
Lawrence Livermore Laboratory,
University of California,
Livermore 94550

References and Notes

1. *Climatic Impact Assessment Program Monograph 3* (Document DOT-TST-75-53, U.S. Department of Transportation, Washington, D.C., 1975); *Climatic Impact Assessment Program Monograph 4* (Document DOT-TST-75-54, U.S. Department of Transportation, Washington, D.C., 1975).
2. For example, see S. Manabe and R. Strickler, *J. Atmos. Sci.* **21**, 361 (1964).
3. J. V. Dave and J. Gazdag, *Appl. Opt.* **9**, 1457 (1970).
4. R. Gelinas, *Lawrence Livermore Lab. Rep. UCRL-74944* (1974) (prepared for inclusion in *Climatic Impact Assessment Program Monograph 3*).
5. *U.S. Standard Atmosphere* (Government Printing Office, Washington, D.C., 1962).
6. F. Luther, in *Proceedings of the 4th Climatic Impact Assessment Program Conference*, A. J. Broderick, Ed. (U.S. Department of Transportation, Washington, D.C., in press).
7. *Climatic Impact Assessment Program Report of Findings* (U.S. Department of Transportation, Washington, D.C., 1974).
8. J. Chang and H. Johnston, in *Proceedings of the 3rd Climatic Impact Assessment Program Conference*, A. J. Broderick and G. M. Hard, Eds. (Document DOT-TSC-OST-74-15, U.S. Department of Transportation, Washington, D.C., 1974), pp. 323–329. This paper compares results from several investigators.
9. V. Ramanathan, L. Callis, R. Boughner, in preparation; in *Proceedings of the 4th Climatic Impact Assessment Program Conference*, A. J. Broderick, Ed. (U.S. Department of Transportation, Washington, D.C., in press).
10. I thank Dr. R. Greenstone for first suggesting the importance of NO_2 in the perturbed stratosphere and Dr. J. V. Dave for his guidance and assistance in developing the radiative transfer model. This work was carried out under the auspices of the U.S. Energy Research and Development Administration, under contract W-7505-Eng-48, and was supported in part by the U.S. Department of Transportation High Altitude Pollution Program.

30 September 1975; revised 15 December 1975

Sex Pheromone Specificity in the Pine Sawflies: Interchange of Acid Moieties in an Ester

Abstract. *3,7-Dimethylpentadecan-2-ol was identified as the free alcohol in three species from two genera of pine sawflies (Hymenoptera: Diprionidae). In* Neodiprion lecontei *and* Neodiprion sertifer *the acetate of this alcohol is the major component of their sex attractant; in* Diprion similis *it is the propionate. By examining the responses of the male antennae of several species of* Neodiprion *through the electroantennographic technique, it was determined that four species responded to the acetate and six to the propionate.*

The sawflies, although hymenopterans, have interesting similarities to the moths. Phytophagous cocoon-spinning larvae and nonfeeding adults highly specialized only for reproduction are common to both groups. The females of both groups produce long-range sex attractants (*1*); also, both groups show a multiplicity of closely related species or races adapted to different plant hosts and environments (*2*). In moths, differences in the sex pheromones of closely related species are based on, and restricted to, a few well-defined chemical changes (*3*). The acetate moiety is a conservative feature, although it is sometimes exchanged for an alcohol or aldehyde group. Changes from acetate to propionate or higher esters have not been reported (*4*).

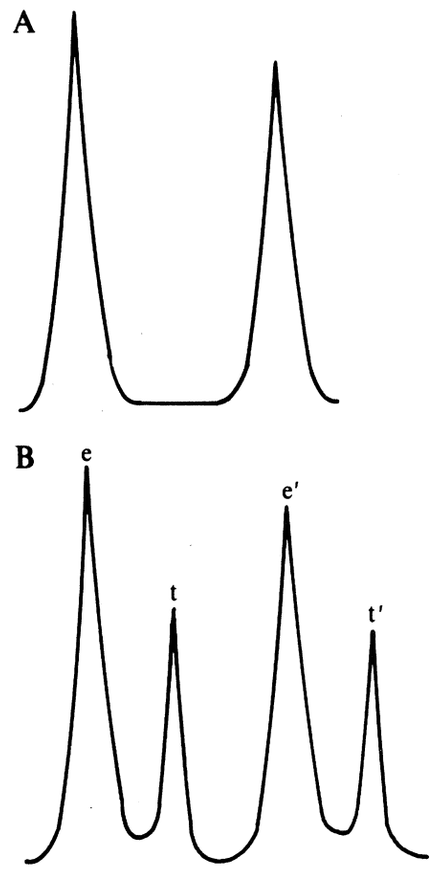

Fig. 1. Juxtaposition of the proton NMR signals for the C-1 methyl group of (A) natural (from *N. sertifer*) and (B) synthetic 3,7-dimethylpentadecan-2-ol acetate, showing that the doublet for the natural product corresponds to the *erythro* diastereoisomer (*ee'*) of the synthetic. The chemical shift, τ, for the latter is 8.91 parts per million, and the coupling constant is 6.5 hertz. The solvent is deuterobenezene.

A genus of pine sawflies, *Neodiprion*, shows exceptional evolutionary diversity; in North America about 30 species or races are adapted to different host conifers (*2*). This diversity provides an opportunity to find out how a group of insects, taxonomically distinct from the Lepidoptera, has solved the common problems of long-range sex attraction and species specificity. The pine sawflies, while conservative in the use of 3,7-dimethylpentadecan-2-ol as the common alcohol moiety, have exploited the interchange of acetate and propionate groups as one means of achieving specificity. Evidence for this is based on chemical identification and on responses by the insects to natural and synthetic esters.

An ether extract was obtained from virgin female *Neodiprion lecontei* by a method already described for *Diprion similis* (*5*). This extract was subjected to short-path distillation, exclusion from a urea complex (*6*), and preparative thin-layer and gas-liquid chromatography (TLC and GLC) (*7*). These procedures, which were monitored by electroantennograms (EAG) (*8*), resulted in the isolation of an active compound that shows a single peak by GLC (*9*). About 4 μg of this compound were obtained from 27,000 insects. Treatment of this compound with potassium hydroxide in methanol produced an inactive neutral compound that has a shorter retention time than the original compound by GLC on an apolar column. Samples of the inactive compound were esterified with formic acid and with the anhydrides of acetic, propionic, butyric, and isobutyric acids in pyridine. After removal of the reagents the samples were tested by EAG. Of the esters so produced, only the acetate was identical to the original active compound as shown by GLC position and EAG activity.

When the remaining (inactive) TLC fractions were acetylated one fraction

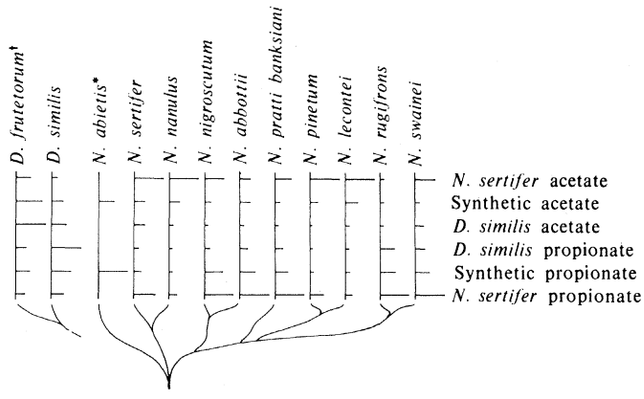

Fig. 2. Relative responses by different species of pine sawfly to acetates and propionates of 3,7-dimethylpentadecan-2-ol obtained from insects of different genera and by synthesis. Horizontal bars represent the magnitude of the EAG response in arbitrary units. The branching pattern indicates proposed phylogenetic relationships (*18*). *For *N. abietis* only synthetic esters were tested. †Males from one field collection of *D. frutetorum* responded most strongly to isobutyrates rather than to acetates. *Neodiprion abietis* were collected in Ontario; all other species were collected in Wisconsin. *Neodiprion lecontei* from Florida showed the same response as those from Wisconsin. Responses to esters obtained from *N. lecontei* are not shown since they were identical to those for esters from *N. sertifer*.

became intensely active. Preparative GLC of the acetylated material yielded an active compound that showed a single peak by GLC, corresponding to about 50 μg. According to the GLC position and the EAG activity, this compound was identical to the active acetate isolated previously and to a compound obtained in the same way from *N. sertifer*. The mass spectrum showed a strong mass to change ratio (*m/e*) 238, indicating the loss of acetic acid from an acetate with a molecular weight of 298; an abnormally strong *m/e* 87 ($CH_3COOCHCH_3$), indicating that the acetate moiety was at the C-2 position; and a prominent *m/e* 116, characteristic of a methyl branch in the C-3 position. These assignments were based on comparisons with spectra of appropriate reference compounds. A portion of the acetate was hydrolyzed back to the alcohol and treated with chromium trioxide–pyridine complex in methylene chloride (*10*). The mass spectrum of the oxidation product showed a strong *m/e* 254 (molecular ion), a base peak at *m/e* 72 that is characteristic of the $-CH(CH_3)-C(O)-CH_3$ fragment, and a relatively intense *m/e* 141 consistent with the presence of a methyl branch at the C-7 position (*11*).

Comparison of the natural product with synthetic compounds by GLC indicated a total chain length for the alcohol moiety of 15 carbons with a methyl branch near the middle of the chain in addition to that at the C-3 position. Integration over the methyl and methylene regions of the nuclear magnetic resonance (NMR) spectrum (*12*) confirmed this general structure. Compounds with a second methyl group in all possible branch positions were synthesized and compared to the natural product (as the acetate and corresponding methyl ketone) by GLC and mass spectra. Of these only 3,7-dimethylpentadecan-2-ol was identical to the natural alcohol by all criteria. Comparison of the synthetic compounds by EAG further confirmed the assignment of the second methyl branch to the C-7 position.

Comparison of the NMR spectra for the natural and synthetic compounds showed extra doublets for the latter, corresponding to the methyl groups attached to the asymmetric carbons at the C-2 and C-3 positions. The signal for the C-1 methyl group was completely resolved (Fig. 1), facilitating application of the rule governing the steric control of asymmetric induction (*13*). Since the asymmetric center at C-2 was produced by the addition of methyl magnesium iodide to an aldehyde (*14*), the doublets *ee'* and *tt'* should correspond to the *erythro* and *threo* disastereoisomers, respectively. Thus, although absolute configuration is unknown, the natural product has the *erythro* form.

The first evidence that propionates were used as sex attractants by the pine sawflies came from attempts to identify the pheromone from *D. similis*. Since there is a well-studied field population of this species, each step of the isolation could be monitored by responses under natural conditions as well as by EAG. For the natural pheromones good agreement was found between the two assays (*15*). An active ester and a corresponding inactive alcohol were isolated from *D. similis*, by the procedure described above for *N. lecontei*. The ester was hydrolyzed and reesterified with different acid groups (see above). The propionate obtained in this way was highly active for *D. similis*; the acetate was less active than the propionate by EAG and inactive in the field. The propionate of the free alcohol was likewise active, and it was identical by TLC and GLC to the original active ester. It could not be distinguished by mass spectrometry or by GLC position from the propionate of the synthetic 3,7-dimethylpentadecan-2-ol (*16*). The original active ester had positions in TLC and GLC corresponding to the propionate of the synthetic alcohol but not to the acetate. Its mass spectrum showed a strong *m/e* 238, indicating loss of propionic acid from an ester with a molecular weight of 312, and an extraordinarily strong *m/e* 101 that is characteristic of a propionate moiety at the C-2 position and a branch at the C-3 position.

The acetate and propionate of synthetic 3,7-dimethylpentadecan-2-ol were compared by EAG with esters made from the free alcohols isolated from *N. lecontei*, *N. sertifer*, and *D. similis* (Fig. 2). For all 12 species tested the specificity for either acetate or propionate was apparent. This specificity was confirmed in the field for *N. lecontei*, *N. sertifer*, and *D. similis*. In addition, several individuals that were collected in southern Wisconsin and identified as *D. frutetorum* responded to isobutyrates rather than to acetates.

While the choice of the acid moiety is the most important discriminating factor, a more subtle chemical specificity, based on the difference in alcohol moieties, exists at the level of the genus (Fig. 2). For instance, all acetate-preferring species of *Neodiprion* (*N. swainei*, *N. lecontei*, *N. pinetum*, *N. nanulus*, and *N. sertifer*) always responded better toward the acetate of *N. sertifer* alcohol than to the acetate of *D. similis* alcohol, while *D. frutetorum* males preferred the acetate of *D. similis* alcohol to that of *N. sertifer*. The same relationship also exists for propionate-preferring groups. There are three logical alternatives for the chemical basis for this preference: a difference in carbon skeleton, the presence of contaminants, or the presence of different optical isomers in the two genera. In view of the exhaustive chromatographic and spectroscopic data on the chemical identicalness of the alcohol moieties from these three species, the first alternative can be eliminated. The second alternative, although it is unlikely, cannot easily be dismissed, since no preparations can be ascertained to be 100 percent pure. The third possibility of the involvement of optical isomers is most attractive to us, but at present we have no supporting evidence for it. In the field the synthetic acetate was attractive to *N. sertifer*, *N. lecontei*, and *D. frutetorum* males, and the synthetic propionate was attractive to *D. similis* and *N. swainei* at various test levels (*17*).

The lack of a simple pattern of specificity for acetate or propionate esters vis-à-vis the phylogenetic scheme proposed by Ross (Fig. 2) (*18*) suggests that the interchange of ester groups may have happened more than once during evolutionary history. If such switching back and forth between acetate and propionate esters did actually occur, it indicates that the acid moiety may be a site of biochemical plasticity in pheromone production. Indeed, this possibility is consistent with our observation that the pheromone is stored as the alcohol precursor, which is esterified as the last step before its release from the insect. Such isolation of the esterification step from the other steps in pheromone biosynthesis would make it especially amenable to the changes in the acid moiety that may have contributed to the speciation of the pine sawflies.

DOUGLAS M. JEWETT
FUMIO MATSUMURA
HARRY C. COPPEL

Department of Entomology, University of Wisconsin, Madison 53706

References and Notes

1. M. Jacobson, *Insect Sex Pheromones* (Academic Press, New York, 1972).
2. G. Knerer and C. E. Atwood, *Science* **179**, 1090 (1973).
3. W. Roelofs and R. Cardé, in *Pheromones*, M. Birch, Ed. (Elsevier, New York, 1974), pp. 97–99.
4. A single exception is the pine emperor moth where the sex attractant is reported to be an isovaleric acid ester. However, no interspecific data is available in this case [H. E. Henderson and F. L. Warren, *Chem. Commun.* (1972), p. 507].
5. J. E. Casida, H. C. Coppel, T. Watanabe, *J. Econ. Entomol.* **56**, 18 (1963).
6. E. V. Truter, *J. Chem. Soc.* (1951), p. 2416.
7. For TLC, plates coated with silica gel were developed with a mixture of hexane and ethyl acetate (14 : 1). For preparative GLC a column 6 m by 1 cm, containing 10 percent Dow 11 on Gas-Chrom Q, was used.
8. The system used to measure electroantennograms was similar to that described by W. Roelofs and A. Comeau [*J. Insect Physiol.* **17**, 1969 (1971)].
9. This compound has a retention index of 1.32 compared to the acetate of pentadecan-2-ol, by coinjection on an OV-101 column. After conversion to the corresponding ketone the material had a retention index of 1.23 compared to 2-pentadecanone on the same column. Analytical columns OV-17, XE-60, EGSS-X, NGA, and ECNSS-M were also used.
10. J. C. Collins, *Tetrahedron Lett.* (1968), p. 3363.
11. R. Nishida, H. Fukami, S. Ishii, *Appl. Entomol. Zool.* **10**, 10 (1975); *Experientia* **30**, 979 (1974). The pheromone from the German cockroach has a 2-keto-3,11-methyl substitution pattern and a $CH_3(CH_2)_7(CH_3)CH$– moiety at the end opposite the functional group. These features suggest a common, partially isoprenoid, origin for the sawfly and cockroach pheromones.
12. Proton spectrum, Bruker 90 Mhz, Fourier transform, time averaged; solvent, deuterobenzene.
13. D. Cram and F. Elhafez, *J. Am. Chem. Soc.* **74**, 5828 (1952); K. Maskens and N. Polgar, *J. Chem. Soc. Perkin Trans. 1* (1973), p. 1117. By convention the *erythro* form is defined as follows: viewing the molecule along the C-2, C-3 bond axis, when the two methyl groups are in the eclipsed position, then the two H– groups are also in the eclipsed position.
14. 3,7-Dimethylpentadecan-2-ol was prepared by the following method: methyl magnesium iodide was reacted with 2,6-dimethyltetradecanal obtained by hydride reduction and subsequent oxidation (*10*) of 2,6-dimethyltetradecanoic acid. The latter was obtained by reacting diethyl methylmalonate with 1-chloro-4-methyldodecane [J. Cason, P. Brewer, E. Pippen, *J. Org. Chem.* **13**, 239 (1948); J. Cason and W. Winans, *ibid.* **15**, 143 (1950)].
15. All compounds isolated from *N. lecontei*, *N. sertifer*, and *D. similis* on the basis of EAG activity have subsequently been found active in the field.
16. Although no contaminants could be detected by GLC of the propionate ester from *D. similis*, hydrolysis and subsequent oxidation to the ketone revealed that a second compound was present. Because only about 1 μg of the ester was available, further purification was not attempted.
17. To test the possibility that the genus specificity might result from different optical isomers at the C-2 position, the active alcohols from *N. lecontei*, *N. sertifer*, and *D. similis* were racemized at the C-2 position by oxidation to the ketones (*10*) and reduction back to the alcohols. After this procedure these compounds (as the appropriate esters) retained their genus specificity and their attractiveness in the field.
18. H. Ross, *For. Sci* **1**, 196 (1955).
19. Supported by the College of Agriculture and Life Sciences, University of Wisconsin, Madison, by the Wisconsin Department of Natural Resources through the School of Natural Resources, and by the Research Committee of the Graduate School with funds supplied by the Wisconsin Alumni Research Foundation. Drs. A. Tai, R. Howard, and P. Hart gave us valuable help with chemical problems. We depended on Drs. J. Mertins, R. Wilkinson, L. Lyons, J. M. McLeod, P. Barald, M. Kraemer, and D. R. Wallace for supplying insects and making observations in the fields.

16 December 1975

Nonenzymic Joining of Oligoadenylates on a Polyuridylic Acid Template

Abstract. *3′,5′-Linked hexa-adenylic acid with a 2′,3′-cyclic phosphate terminus [$(A)_5A > p$] couples on a polyuridylic acid template in the presence of ethylenediamine to form the dodecamer (24 percent) and octadecamer (5 percent). The bond produced is largely that of the 2′,5′ isomer.*

The discovery of a nonenzymic system that could carry out the faithful replication of a polynucleotide under plausible prebiotic conditions would have a major effect on contemporary thought concerning the origin of life. Some experiments to find such a system have already been made. The coupling of thymidine oligomers on a polyadenylic acid template (*1*), adenosine derivatives on polyuridylic acid [poly(U)] (*2*), and 2′-*O*-methylinosine oligomers on polycytidylic acid (*3*) have been achieved by means of water-soluble carbodiimides, but the use of this class of coupling reagent would appear to exclude these experiments as models of prebiotic polymerization (*4*). Nucleoside phosphoramidates (*5*) and imidazolides (*4*) have also been investigated and have appeared promising as activated precursors to oligonucleotides. Nucleoside 2′,3′-monophosphates (cyclic nucleoside monophosphates) qualify as plausible prebiotic compounds (*6*), and a driving force for polymerization has been sought in the large negative standard enthalpy of hydrolysis (*7*) and high reactivity (*8*) of these five-membered ring cyclic phosphates. Adenosine 2′,3′-monophosphate forms a triple helix with poly(U) at low temperatures in the presence of various helix stabilizing compounds, which in the case of short chain diaminoalkanes also catalyze the formation of the internucleotide link (*6*). Later, it was shown that the poly(U) can be replaced by poly(vinyluracil) (*9*). The yield of dimer did not exceed about 23 percent maximum and only traces of the trimer were detected; about 97 percent of the internucleotide bonds were the unnatural 2′,5′ isomer (*6*). However, it was considered on the basis of equilibrium calculations that, even if all hydrolysis were suppressed (a competing reaction), the yield of all oligomers higher than the nonamer would total less than 8 percent in a solution that was 1*M* in adenosine base residues, and this appeared to place a severe restriction on the use of cyclic phosphates as activated monomers for template directed polymer formation (*6*).

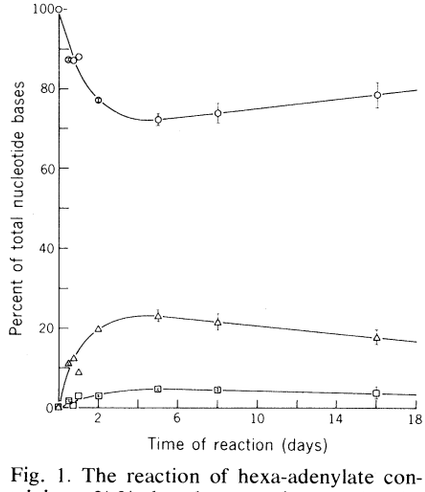

Fig. 1. The reaction of hexa-adenylate containing a 2′,3′-phosphate terminus to give the dodecamer and octadecamer on a poly(U) template. Experimental points: ○, hexamer; △, dodecamer; and □, octadecamer. The curves have no theoretical significance. The reaction mixture was analyzed by means of a diethylaminoethyl-Sephadex column with a triethylammonium bicarbonate gradient. The eluate was monitored at 260 nm with a Zeiss PMQ-H spectrophotometer.