Med. **116**, 207 (1962); S. Cohen, *Biochem. J.* **89**, 334 (1963); G. M. Bernier *et al.*, *Biochemistry* **4**, 2072 (1965).
7. D. W. Talmage, *Science* **150**, 1484 (1965); F. W. Putnam and K. Titani, *ibid.*, p. 1484.
8. K. Titani and F. W. Putnam, *ibid.* **147**, 1304 (1965).
9. Abbreviations for amino acid residues: Lys, lysine; His, histidine; Arg, arginine; Asp, aspartic acid; Asn, asparagine; Thr, threonine; Ser, serine; Glu, glutamic acid; Gln, glutamine; Pro, proline; Gly, glycine; Ala, alanine; Val, valine; Met, methionine; Ilu, isoleucine; Leu, leucine; Tyr, tyrosine; Phe, phenylalanine; Try, tryptophan; CyS, half-cystine.
10. O. Smithies, *Nature* **199**, 1231 (1963).
11. R. Brimacombe, J. Trupin, M. Nirenberg, P. Leder, M. Bernfeld, T. Jaouin, *Proc. Nat. Acad. Sci. U.S.* **54**, 954 (1965).
12. The Inv type of protein Ag was determined by Dr. A. G. Steinberg, Dept. of Biology, Western Reserve Univ., Cleveland. In nomenclature, Inv(2) is equivalent to Inv(a) and Inv(b) to Inv(3).
13. M. Potter, E. Appella, S. Geisser, *J. Mol. Biol.* **14**, 361 (1965).
14. We thank Laurel Bailey and Patricia Tindall for amino acid analyses. Supported by grants HE 10116-01 and CA 08497-01 from National Institutes of Health.

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Sex Attractant of the Pink Bollworm Moth: Isolation, Identification, and Synthesis

Abstract. *The sex attractant produced in extremely small amount by the virgin female pink bollworm moth has been isolated in pure form and identified as 10-propyl*-trans-*5, 9-tridecadienyl acetate. Successful synthesis of this molecule confirms the structure and makes possible its practical use to help control this destructive pest of cotton. The attractant, for which the common name "propylure" is suggested, may be the first naturally occurring compound with propyl branching to be reported.*

The pink bollworm moth, *Pectinophora gossypiella* (Saunders), is one of the most destructive pests in the cotton-growing areas of the world (*1*). Although mention was made in 1957 (*2*) of the presence of a sex attractant in the female insect, no details were given concerning the techniques used for its demonstration. In 1962, Ouye and Butt (*3*) showed that small traps baited with a methylene chloride extract of mating insects lured males, and in 1964, Berger *et al.* (*4*) demonstrated that extracts prepared from the terminal (2 to 3) abdominal segments of virgin female moths elicited excited flight, rapid wing vibrations, and a characteristic upward curving of the abdomen in males. Males readily responded with their characteristic dance to the vapors expelled from a glass pipette contaminated with the abdominal extracts. Field traps baited with a crude methylene chloride extract of the female moths have been used in surveying pink bollworm infestation (*3–7*).

Although some concentration of the sex attractant was accomplished by chromatography prior to 1964 (*4*), and the attractant was thought to be an 18-carbon ester, its isolation in pure form was not realized at that time. We now wish to report the successful isolation, identification, and synthesis of the pure, highly active attractant.

The methylene chloride extractive, prepared from the whole bodies of 850,000 virgin female moths (2 days old), was dissolved in ten volumes of acetone, and the solution was kept overnight at −20°C. The large amount of precipitated white solid was filtered rapidly through a cold Buchner funnel and washed with cold acetone, and the combined mother liquor and washings were freed of solvent at 20 mm-Hg (bath below 40°C). The yellow oily residue was shaken repeatedly with portions of methanol at room temperature and the combined methanol-soluble portions were evaporated to dryness. The resulting oily residue was dissolved in five volumes of acetone, kept overnight at −20°C, and filtered. Evaporation of the acetone filtrate gave an oil that was chromatographed successively on two columns of Florisil (*8*), using a sample-to-adsorbent ratio of 1:30 for the first and 1:200 for the second column. Each column was eluted successively with hexane, 3-, 5-, and 10-percent ethyl ether in hexane (*9*). Only 3-percent ether in hexane removed active material in each case, as shown by exposure of caged male moths to the air from pipettes containing the solution vapors (*4*).

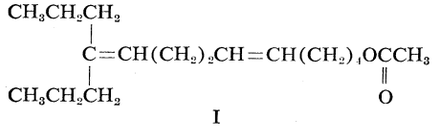
The active fraction was chromatographed on a column of silica gel impregnated with silver nitrate (*10*) and eluted successively with hexane, with 5-, 10-, 25-, and 50-percent ether in hexane, and then with ethyl ether. The active fractions eluted with 25- and 50-percent ether in hexane were combined and rechromatographed on silver nitrate-impregnated silica gel, and eluted successively with 40- and 60-percent benzene in hexane, 5-percent ether in benzene, and finally with ethyl ether. All fractions except that eluted with 40-percent benzene in hexane were active, and these were combined and subjected to preparative gas chromatography (*11*) to give four components with retention times of 8.0, 11.5, 16.5, and 20.0 minutes. Only the component emerging from the column in 11.5 minutes caused a sexual response in caged, male, pink bollworm moths. In this way there was obtained approximately 1.6 mg of the pure attractant as a colorless liquid having no detectable odor. Air expelled from a pipette contaminated with a very dilute solution of the attractant and stored at 5°C for at least 6 weeks was still attractive to caged males in the laboratory.

The infrared spectrum of the attractant showed strong bands at 1755 and 1235 cm^{-1} and a medium band at 1038 cm^{-1}, characteristic of a primary acetate group. The spectrum also showed the presence of unsaturation (1660 cm^{-1}, weak), a *trans* double bond (965 cm^{-1}, medium), and an unbroken chain of at least four methylene groups (723 cm^{-1}, broad). The ultraviolet spectrum showed only end absorption, precluding the presence of conjugation. Hydrogenolytic gas chromatography of the attractant by the method of Beroza and Sarmiento (*12*) established the presence of branching in its structure. Additional support for an acetate group was derived from the fragmentation pattern of the mass spectrum, which showed a large base peak when the ratio of mass to charge, m/e, was 43 (CH_3CO). The mass spectrum showed a molecular weight of 280 for the attractant, and peak matching on a double-focusing high-resolution instrument established the elemental formula as $C_{18}H_{32}O_2$. The above data characterized the attractant as the acetate of a branched-chain, C_{16}, primary alcohol with two double bonds (*13*).

Nuclear magnetic resonance spectra were obtained in deuterochloroform by means of a Varian HR-100 spectrometer equipped with a C-1024 time averaging computer and facilities for proton-proton spin decoupling. Time averages of up to 25 scans were used, with the C^{13}-labeled satellite of tetra-

methylsilane as a reference. The spectra gave evidence (chemical shift in values of δ) for three olefinic protons (δ, 5.40), two methylene protons adjacent to an acetate oxygen atom (δ, 4.06), ten methylene protons adjacent to double-bonded carbon atoms (δ, 2.03), three acetyl methyl protons (δ, 2.03), eight methylene protons adjacent to either methylene or methyl groups (δ, 1.34), and six terminal methyl protons (δ, 0.89) that appeared to be separated from a double-bonded carbon by at least two methylene groups. Double resonance studies indicated at least three methylene groups between the acetate group and a double bond, and only two methylene groups between the double bonds.

The only structure for the attractant consistent with the foregoing data is 10-propyl-*trans*-5, 9-tridecadienyl acetate (I), and this structure was confirmed by an 11-step synthesis.



I

Compound I was synthesized in 0.2-percent overall yield by the following procedure. Condensation of 4-heptanone with ethyl bromoacetate in the presence of zinc (*14*) gave ethyl 3-hydroxy-3-propylcaproate (75 percent; bp, 85°C at 1.5 mm-Hg), which was dehydrated with phosphorus oxychloride in pyridine to a mixture (88 percent; bp, 105° to 109°C at 20 mm; n_D^{25}, 1.4412) consisting of approximately 50 percent of ethyl 3-propyl-2-hexenoate and 50 percent of ethyl 3-propyl-3-hexenoate. Distillation through a spinning band column gave the pure α,β-isomer (bp, 116.5°C at 28 mm) and the pure β,γ-isomer (bp, 113.5°C at 28 mm) as colorless liquids. Ethyl 3-propyl-2-hexenoate was reduced with lithium aluminum hydride to 3-propyl-2-hexen-1-ol (*15*) (80 percent; bp, 125°C at 25 mm; $n_D^{23.5}$, 1.4520), which was converted with phosphorus tribromide in pentane to 1-bromo-3-propyl-2-hexene (72 percent; bp, 105°C at 40 mm and 63°C at 3.3 mm; n_D^{27}, 1.4805). Stirring this compound for 6 days at room temperature with sodium cyanide in ethanol and hydrolysis of the crude product with alcoholic alkali gave crude 4-propyl-3-heptenoic acid, which was reduced with lithium aluminum hydride to 4-propyl-3-hepten-1-ol (*15*) (bp, 108° to 112°C at 15 mm); overall yield from the bromide was 76 percent. Treatment of this olefinic alcohol with phosphorus tribromide gave the unstable 1-bromo-4-propyl-3-heptene (76 percent; bp, 82° to 83°C at 2.5 mm; $n_D^{21.5}$, 1.4716), which was coupled, without delay, with the tetrahydropyranyl ether of 5-hexyn-1-ol (*16*) by means of sodamide in liquid ammonia. The resulting 10-propyl-1-(tetrahydro-2-pyranyloxy)-tridec-9-en-5-yne (23 percent; bp, 175°C at 0.5 mm; n_D^{27}, 1.4729) was reduced with sodium in liquid ammonia to 10-propyl-1-(tetrahydro-2-pyranyloxy)-*trans*-5,9-tridecadiene (85 percent; bp, 135°C at 0.1 mm; n_D^{21}, 1.4721), which was then hydrolyzed at room temperature with methanolic sulfuric acid to 10-propyl-*trans*-5, 9-tridecadien-1-ol (90 percent; bp, 110° to 120°C at 0.08 mm-Hg; n_D^{25}, 1.4715). Refluxing this alcohol with acetyl chloride in anhydrous benzene gave compound I (16 percent) as a colorless liquid (bp, 135°C at 0.1 mm; n_D^{25}, 1.4635) identical in all respects with the natural attractant.

The sex attractant, for which the name "propylure" is suggested, is believed to be the first-reported natural constituent possessing propyl branching.

WILLIAM A. JONES
MARTIN JACOBSON
DIAL F. MARTIN
Entomology Research Division,
U.S. Department of Agriculture,
Beltsville, Maryland

References and Notes

1. Anonymous, "Controlling the pink bollworm on cotton," USDA Farmer's Bull. No. 2207 (1965), 12 pp.
2. B. Flaschenträger, E. S. Amin, H. J. Jarczyk, *Mikrochim. Acta* **1957**, 385 (1957).
3. M. T. Ouye and B. A. Butt, *J. Econ. Entomol.* **55**, 419 (1962).
4. R. S. Berger, J. M. McGough, D. F. Martin, L. R. Ball, *Ann. Entomol. Soc. Amer.* **57**, 606 (1964).
5. *Agr. Res. U.S.* **11**, No. 6, 10 (1962).
6. H. M. Graham and D. F. Martin, *J. Econ. Entomol.* **56**, 901 (1963).
7. J. W. Gentry, *Agr. Chem.* **20**, No. 12, 68 (1965).
8. Mesh 60-100, obtained from the Floridin Co., Tallahassee, Fla., and treated to contain 3 percent water. The mention of trade names or products does not constitute endorsement by the U.S. Department of Agriculture over those not named.
9. The hexane used in these investigations was purified to the equivalent of spectral grade by percolation of reagent-grade hexane through silica gel and distillation. The ethyl ether was distilled and stored over sodium. All other solvents used were reagent grade, unless otherwise specified.
10. Adsorbosil-CABN, 60-100 mesh, containing 10 percent calcium sulfate binder and 25 percent silver nitrate, obtained from Applied Science Laboratories, State College, Pennsylvania.
11. The chromatography was carried out on an Aerography "Autoprep" instrument, model 700, obtained from Varian Aerograph, Walnut Creek, California, with a stainless steel column (2.4 m by 0.63 cm diameter), packed with 5 percent SE-30 on Chromosorb W; column temperature, 185°C; helium flow rate, 33.3 ml/min.
12. M. Beroza and R. Sarmiento, *Anal. Chem.* **35**, 1353 (1963).
13. *Chem. Eng. News* **43**, No. 38, 42 (1965); *ibid.* **43**, No. 16, 39 (1965); *ibid.* **43**, No. 19, 5 (1965); *ibid.* **43**, No. 35, 62 (1965).
14. G. A. R. Kon and C. J. May, *J. Chem. Soc.* **1927**, 1549 (1927).
15. Additional amounts of this compound, prepared by a different method, were later obtained from Midwest Research Institute, Kansas City, Missouri.
16. 5-Hexyn-1-ol, prepared in 75-percent yield by the method of G. Eglinton, E. R. H. Jones and M. C. Whiting, *J. Chem. Soc.* **1952**, 2873 (1952), or purchased from Farchan Research Laboratories, Willoughby, Ohio, was converted to its tetrahydropyranyl ether (bp, 70° to 80°C at 0.3 mm; n_D^{25}, 1.4556) in 76-percent yield.
17. We thank Drs. M. T. Ouye and M. J. Lukefahr and Mr. J. M. McGough, all of the U.S. Dept. of Agriculture, Brownsville, Texas, for supplying the large numbers of insects necessary for the isolation and laboratory bioassay investigations. We thank Dr. H. Fales, NIH, Bethesda, Maryland, for running the mass spectrum; E. Pier and Drs. L. F. Johnson and N. Bhacca, all of Varian Associates, Palo Alto, California, for obtaining and interpreting the nuclear magnetic resonance spectra; and R. Sarmiento, USDA, Beltsville, Maryland, for the hydrogenolytic gas-chromatographic determination.

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Allelic Antigenic Factor Inv(a) of the Light Chains of Human Immunoglobulins: Chemical Basis

Abstract. *Twenty-seven Bence Jones proteins of immunological type K show a common set of peptides. One of the common peptides differs in three of the proteins which are the only ones classified by a serological test as Iav(a+). The difference in the peptide analyzed is caused by a valine-leucine interchange; Inv(a+) proteins have leucine, whereas Inv(a−) proteins have valine in position 189.*

Genetic variants (designated Gm and Inv) of the peptide chains of human immunoglobulins having distinct serological properties have been described (*1*). The Gm factors are present in heavy chains of γG immunoglobulins (*2*), whereas the Inv factor is present in light chains (*2*). The Bence Jones proteins, which correspond to the light chains of immunoglobulins (*3*), offer a