Anal. Calcd. for $C_{23}H_{30}N_2O_5$ (414.51): C, 66.65; H, 7.30; N, 6.76. Found: C, 66.55; H, 7.54; N, 6.73.

Methyl 3-isoreserpate 3′,4′,5′-trimethoxybenzoate,[9] m.p. 151–153°, $[\alpha]_D$ −164.0°; λ_{max} 215–217 mμ (ε 59,000), 224–230 (sh., 43,800), 265–269 (15,700), 291–295 (9,750), 327–333 (plateau, 330), 363–375 (117); previously reported[10]: m.p. 150–155°, $[\alpha]_D$ −164°. *Anal.* Calcd. for $C_{33}H_{40}N_2O_9 \cdot {}^1/_2CH_3OH$ (624.21): C, 64.41; H, 6.78; N, 4.48. Found: C, 64.27; H, 6.50; N, 4.63.

Methyl reserpate, m.p. 243–244.5°, $[\alpha]_D$ −99.5°; previously reported[11]: m.p. 235–240°, $[\alpha]_D$ −106°. *Anal.* Found: C, 66.38; H, 7.34; N, 6.63.

(10) H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, *J. Am. Chem. Soc.*, **77**, 4335 (1955).

Methyl reserpate 3′,4′,5′-trimethoxybenzoate (reserpine), m.p. 267–268°, $[\alpha]_D$ −119.3°; previously reported[11]: m.p. 277–277.5° (vacuum), $[\alpha]_D$ −118°. λ_{max} 216–218 mμ (ε 58,500), 226–228 (sh., 43,200), 266–268 (16,300), 292–296 (10,100). *Anal.* Calcd. for $C_{33}H_{40}N_2O_9$ (608.58): C, 65.12; H, 6.62; N, 4.60. Found: C, 65.54; H, 6.63; N, 4.99.

Methyl Neoreserpate and Methyl Neoreserpate 3′,4′,5′-trimethoxybenzoate.—The physical properties of these samples were previously described.[1] The methyl neoreserpate was anhydrous, and the methyl neoreserpate trimethoxybenzoate contained a half mole of water.

(11) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

[CONTRIBUTION FROM THE ENTOMOLOGY RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MD.]

Insect Sex Attractants. I. The Isolation, Identification, and Synthesis of the Sex Attractant of the Gypsy Moth[1]

BY MARTIN JACOBSON, MORTON BEROZA AND WILLIAM A. JONES

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The extremely potent sex attractant secreted by the female gypsy moth (*Porthetria dispar* (L.)) to lure the male has been isolated in pure form and characterized as dextrorotatory 10-acetoxy-*cis*-7-hexadecen-1-ol. A second, as yet unidentified component, has also been isolated and shows attraction of a lower order. The optically inactive form of the major attractant has been synthesized and found to be as attractive as the natural isomer.

The gypsy moth (*Porthetria dispar* (L.) is one of the most serious pests of fruit, shade and woodland trees in New England and eastern New York State. If left uncontrolled, the insect would threaten hardwood forests from northern Maine to the Ozark Mountains. Based on a 20-year study, losses caused by defoliation have been estimated to run in the tens of millions of dollars.

The female gypsy moth does not fly and the male is attracted to the female by scent.[2] As early as 1913 it was determined that an extract prepared from the last two abdominal segments of virgin females could be used in traps as a powerful male attractant, and a benzene extract of these tips has been used for many years in U. S. Department of Agriculture survey traps.[3] Although an effective lure is a practical necessity in a gypsy moth control or eradication program, the collection of female pupae and the clipping and processing of tips from the emerged adults are time-consuming and costly. Investigations had therefore been under way for some time to isolate and identify the attractant with a view toward possible synthesis of this or a related active compound.[4]

The pioneering detailed chemical studies on the problem were conducted by Haller and Acree,[4,5] who soon determined that the active material was a lipid residing in the unsaponifiable neutral fraction of the extract. It was partially volatile with steam, reacted with phthalic anhydride, and could be recovered from the phthalic acid ester upon saponification. Considerable concentration of the attractant was obtained by chromatography of the neutral fraction in sequence on columns of magnesium carbonate and magnesium oxide.[5c] These fractionations gave definite indications of the presence of more than one substance attractive to males. From the abdominal glands of 100,000 female moths, Acree obtained about 12 milligrams of an active impure fraction which he designated "gyptol." Saponification of "gyptol" with potassium hydroxide in diethylene glycol at 120–130° gave a few milligrams of a solid acid, which has since been identified as palmitic acid,[6] and an unidentified alcohol fraction. Lack of a suitable bioassay method made it necessary for Acree to confine the testing of chemical fractions to the 2–3-week period of natural flight each summer.

A benzene extract of the abdominal tips of 200,000 virgin female gypsy moths was made available for our investigations in 1956, and the extract of an additional 300,000 females was prepared in 1958; these were obtained from pupae collected in Connecticut and in Spain, respectively. In the process of isolating the unsaponifiable fraction, large quantities of free and esterified fatty acids unattractive to male moths[7] were obtained. Butenandt,[8] who attempted to isolate and identify the sex attractant of the silkworm moth (*Bombyx mori* (*L.*)), reported

(1) Reported in part as a note in *Science*, **132**, 1011 (1960).

(2) E. H. Forbush and C. H. Fernald, "The Gypsy Moth, *Porthetria dispar* (Linn.)," Massachusetts State Board of Agriculture, Boston, Mass., 1896, p. 345.

(3) R. F. Holbrook, M. Beroza and E. D. Burgess, *J. Econ. Entomol.*, **53**, 751 (1960).

(4) See H. L. Haller, F. Acree, Jr., and S. F. Potts, *J. Am. Chem. Soc.*, **66**, 1659 (1944), and references cited therein for a history of the problem and the early chemical studies.

(5) (a) F. Acree, Jr., *J. Econ. Entomol.*, **46**, 313 (1953); (b) **46**, 900 (1953); (c) **47**, 321 (1954).

(6) M. Jacobson and M. Beroza, unpublished investigations.

(7) Laboratory bioassay tests were carried out by an unpublished modification of the method of B. C. Block, *J. Econ. Entomol.*, **53**, 172 (1960). Field tests were carried out as described by J. M. Corliss, "U. S. Dept. Agr. Yearbook," Govt. Printing Office, Washington, D. C., 1952, p. 694. The assistance of E. C. Paszek, U. S. Department of Agriculture, Nashua, N. H., in carrying out these tests is gratefully acknowledged.

(8) A. Butenandt, *Nova Acta Leopoldina*, **17**, 445 (1955).

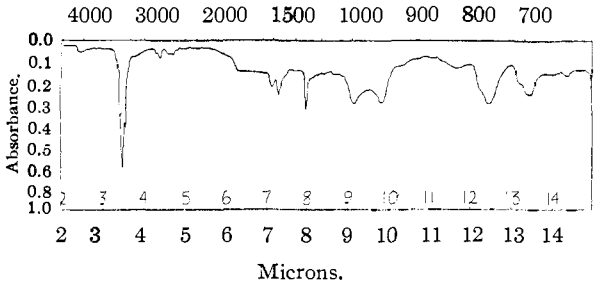


Fig. 1a.—Infrared spectrum of solid gypsy moth attractant.

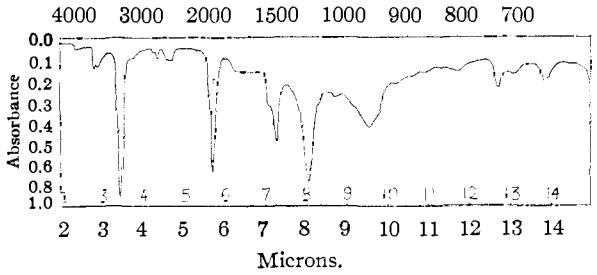


Fig. 1b.—Infrared spectrum of liquid gypsy moth attractant.

that reduction of the inactive free and combined fatty acids gave a mixture of alcohols attractive to the male silkworm moth. However, lithium aluminum hydride reduction of the gypsy moth fatty acids (consisting mainly of palmitic and stearic acids) to the corresponding alcohols failed to give active material.

Chromatography of the unsaponifiable fraction from 200,000 female moths on successive columns of magnesium carbonate and magnesium oxide by Acree's method,[5c] with absorbancy ratios at 249 and 285 mμ, gave a yellow oil which was highly attractive to males. Subsequent investigations showed that these time-consuming operations (at least 3 days per column per 20,000 tips) could be avoided and approximately equal fractionation obtained by repeatedly dissolving the crude, unsaponifiable fraction from 300,000 Spanish tips in acetone and filtering off the solid that separated at room temperature, 5°, and −5°. The large amount of inactive solid thus obtained consisted of orange pigments, sterols (mainly cholesterol), and hydrocarbons; the acetone filtrates were highly active. In this way, a total of 75 mg. of attractive oil was obtained from 500,000 female moths.

An inactive crystalline material, m.p. 59.5–60.0°, obtained from the acetone mother liquors proved to be identical with crystals from fraction BI reported by Acree[5c] to be attractive to male moths. His material was shown to be contaminated with a trace of attractant which was apparently resistant to washing. Gas chromatography showed the solid to be heptacosane with traces of nonacosane and hentriacontane. A crystalline solid, m.p. 61.0–61.5°, isolated in large amount by Stefanović, *et al.*,[9] from a hydrogenated extract of female gypsy moth tips, was identified by them as a triglyceride α-palmitodistearate.

Reversed-phase chromatography of the active oil by the ascending technique on silicone- or polyethylene-impregnated filter paper sheets gave five spots, only one of which was attractive in field tests. Successive extraction of this active zone with cold ethanol and with Skellysolve B gave 3.4 mg. of white, waxy crystals, m.p. 37.0–37.5°, soluble in ethanol, and 20 mg. of colorless, Skellysolve-soluble liquid that solidified in the cold but remelted at room temperature. The crystals were volatile with steam and attractive to male moths in the field at 10^{-2} microgram; the liquid showed blue fluorescence in ultraviolet light and was attractive in the field at 10^{-7} microgram. Neither material showed absorption in the ultraviolet range.

An infrared spectrum (Fig. 1a) of the crystals shows no readily recognizable functional groups and indicates the absence of hydroxyl and carbonyl groups. The strong bands at 1252 and 800 cm.$^{-1}$ suggest the possibility of an epoxy linkage, although the latter band is at a somewhat lower frequency than that usually found with epoxide. The lack of readily recognizable functional groups, together with the extremely small quantity available and its lower activity, caused us to lay aside temporarily the crystalline attractant in favor of a detailed study of the extremely potent liquid attractant.

Gas chromatography of the liquid attractant showed it to be a pure material, whose analysis agreed most closely with $C_{17}H_{32}O_3$ or $C_{18}H_{34}O_3$. It possessed optical activity, $[\alpha]^{23}D$ +7.9°. Its infrared spectrum (Fig. 1b) showed doublet bands at 3580 and 3450 cm.$^{-1}$, that indicated the presence of hydroxyl, which was certainly primary from the broad band at 1042 cm.$^{-1}$. The strong sharp band at 1740 cm.$^{-1}$ indicated ester carbonyl stretching vibrations and the band at 1234 cm.$^{-1}$ earmarked this ester as an acetate, since formates and higher esters absorb at considerably lower frequencies. This secondary ester grouping would account for the failure of the attractant to be saponified by ethanolic alkali.[5a] The shoulder at 1660 cm.$^{-1}$ (unsaturation) and the band at 783 cm.$^{-1}$ suggested a *cis* double bond, and the absence of a band at 967 cm.$^{-1}$ precluded the presence of *trans* unsaturation. The band at 720 cm.$^{-1}$ indicated an unbroken chain of at least four methylene groups. Spectra determined in carbon tetrachloride showed the absence of aromatic rings, acetylenic bonds, and branched methyl groups.

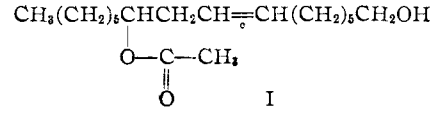
On catalytic microhydrogenation with platinum, the attractant absorbed an amount of hydrogen equivalent to one double bond for a compound of molecular weight 298. The saturated compound attracted male moths in the field at 10^{-2} microgram/trap. The double bond thus hydrogenated was shown to possess the *cis* configuration by the absence of the band at 783 cm.$^{-1}$ in its infrared spectrum. The saturated compound was saponified in 3 minutes at 120–125° with potassium hydroxide in diethylene glycol, conditions found to be sufficient for the saponification of esters of most secondary alcohols. Microtitration showed a saponification equivalent of 314 (theory for $C_{18}H_{36}O_3$ is 300). Acetic acid was identified by means of infrared spectra, and the spectrum of the crystalline, inactive diol obtained was similar to those of sample

(9) D. Stefanović, B. Grujić and P. Prekajski, *Plant Protection (Belgrade)*, No. **52–53**, 176 (1959).

long-chain lipids containing both a primary and a secondary hydroxyl group. The attractant thus appeared to be a 16-carbon straight-chain primary alcohol possessing a secondary acetoxyl group and a *cis* double bond, and only the determination of the positions of this bond and of the acetoxyl group remained.

Lack of absorption in the ultraviolet precluded the 2-position for the double bond, and the optical activity of the attractant showed that the acetoxyl group did not lie on an unsaturated carbon atom, since an asymmetric carbon atom would not then exist. Stability of the attractant to high-temperature vapor phase chromatography on Craig polyester succinate indicated the probability that at least one methylene group separated the acetoxyl group from the double bond, since Stoffel, *et al.*,[10] have shown that the common methylene-interrupted polyunsaturated esters may be safely analyzed by gas chromatography. (This has since been substantiated for unsaturated hydroxy acetates by Morris, *et al.*,[11] who found that hydroxy esters not vicinally unsaturated are stable under gas chromatography on polyester resins at high temperature whereas vicinally unsaturated hydroxy esters are dehydrated under these conditions).

Oxidation of the attractant with periodate–permanganate reagent[12] cleaved the double bond only, leaving the hydroxyl and acetoxyl groups intact. A 92% yield of 3-acetoxy-1-nonanoic acid was obtained together with an ω-hydroxy acid which was further oxidized in 71% yield with alkaline permanganate to pimelic acid. The only structure for the attractant consistent with the foregoing data is (+)-10-acetoxy-*cis*-7-hexadecen-1-ol (I).



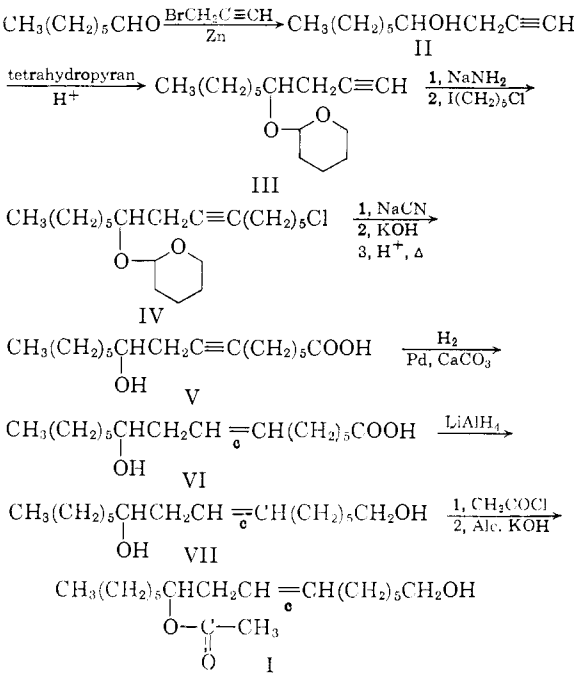
I

The structure I of the major sex attractant of the gypsy moth is quite similar to that of the silkworm moth sex attractant, recently identified by Butenandt, *et al.*,[13] as 10,12-hexadecadien-1-ol having the *trans*-10-*cis*-12 configuration.[14]

The reason for the secretion of two sex attractants by the female gypsy moth is not yet apparent. Perhaps one of these serves to lure the male moth from a distance, the other being mainly responsible for initiating the copulatory response. Behaviorial tests to be conducted shortly may shed light on this question. It is interesting to note that gas chromatography of an extract of the abdominal tips of virgin female silkworm moths indicated the presence of more than one sex attractant.[15]

The *dl*-form of I was synthesized in 0.2% over-all yield by the steps shown below. 1-Decyn-4-ol[16] (II), prepared in 18% yield by condensing *n*-heptaldehyde with propargyl bromide in the presence of zinc, was converted in 55% yield to its tetrahydropyranyl ether (III) to protect the secondary hydroxyl group. Chain extension by condensation of the sodio derivative of III with 1-chloro-5-iodopentane[17] in liquid ammonia gave a 35% yield of 1-chloro-9-(tetrahydro-2-pyranyloxy)-pentadec-6-yne (IV). Refluxing this compound with sodium cyanide and hydrolysis with alcoholic alkali, followed by removal of the pyranyl grouping, gave 10-hydroxy-7-hexadecynoic acid (V) in 15% yield. Semihydrogenation of V, with quinoline-poisoned palladium as catalyst, gave 81% of 10-hydroxy-*cis*-7-hexadecenoic acid (VI), which was quantitatively reduced to 1,10-dihydroxy-*cis*-7-hexadecene (VII). The diol VII was converted to the diacetate in 75% yield, and this diacetate was refluxed with sufficient ethanolic alkali to saponify the primary ester grouping, giving the desired product, *dl*-I, in 92% yield. The *dl*-form of the attractant was a colorless liquid, b.p. 169° at 0.2 mm., identical in every respect save optical activity with the natural gypsy moth attractant. Both isomers showed approximately the same attractiveness to male gypsy moths in the field.

To our knowledge, this procedure represents the first reported synthesis of a naturally occurring insect sex attractant.



Experimental[18]

Preparation and Preliminary Fractionation of the Crude Extract.—The starting material used for extraction consisted of the clipped abdominal tips of 200,000 virgin female

(10) W. Stoffel, W. Insull, Jr., and E. H. Ahrens, Jr., *Proc. Soc. Expil. Biol. Med.*, **99**, 238 (1958).

(11) L. J. Morris, R. T. Holman and K. Fontell, *J. Lipid Research*, **1**, 412 (1960).

(12) E. von Rudloff, *J. Am. Oil Chem. Soc.*, **33**, 126 (1956); F. D. Gunstone and L. J. Morris, *J. Chem. Soc.*, 2127 (1959).

(13) A. Butenandt, R. Beckmann, D. Stamm and E. Hecker, *Z. Naturforsch.*, **14b**, 283 (1959).

(14) E. Hecker, paper presented at the 11th International Congress of Entomology, Vienna, Austria, Aug. 17–25, 1960.

(15) E. Bayer and F. Anders, *Naturwissenschaften*, **46**, 380 (1959).

(16) L. Crombie and A. G. Jacklin, *J. Chem. Soc.*, 1740 (1955).

(17) W. R. Taylor and F. M. Strong, *J. Am. Chem. Soc.*, **72**, 4263 (1950).

(18) Melting points are corrected and boiling points are uncorrected. Infrared spectra were measured, as 1 or 2% solutions in carbon disulfide or carbon tetrachloride, with a Perkin–Elmer model 21 spectrophotometer with rock-salt optics. Ultraviolet spectra were determined in ethanol solution with a Beckman model DK-2 spectrophotometer. The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

gypsy moths collected in Connecticut in 1956 and of 300,000 virgin female moths collected in Spain in 1958; these tips were received in thiophene-free benzene. The abdominal tips were separated from the benzene by pressing in a fruit press and washed successively with two fresh portions of benzene. Combined extract prepared from Connecticut tips was not mixed with that prepared from Spanish tips. The benzene extracts were freed of most of the solvent by distillation on the water aspirator (bath temperature below 45°, p = *ca.* 20 mm.), and the brown residual oil was dissolved in ether to make stock solutions containing the equivalent of 100,000 tips/1500 milliliters.

A 1500-ml. aliquot of the stock solution of domestic (Connecticut) extract was shaken with two 1-liter portions of 5% sodium hydroxide (the emulsions formed separated extremely slowly, even after the addition of small amounts of isoöctane or ethanol), and the combined aqueous layers were extracted with one portion of ether which was then added to the main ethereal solution of neutral fraction.

Free fatty acids (44 g.) were obtained as a viscous yellow oil by acidification of the alkaline liquor and extraction with ether. The neutralization equivalent was 311. Lithium aluminum hydride reduction of a portion of these unattractive acids gave a 91% yield of the corresponding alcohols as a viscous yellow oil that solidified in the cold but melted again at room temperature. These mixed alcohols were likewise unattractive to male gypsy moths. In this way, there were obtained from the extracts of 200,000 domestic and 300,000 Spanish tips total free fatty acids weighing 88 g. and 105 g., respectively.

The ethereal solution of neutral fraction obtained from 100,000 domestic tips was freed of solvent, and the residue was dissolved in 300 ml. of benzene and refluxed for 4 hours, in a nitrogen atmosphere, with a solution of 50 g. of potassium hydroxide in 50 ml. of water and 250 ml. of 95% ethanol. The benzene and ethanol were removed under reduced pressure and 1 l. of ether and 300 ml. of water were added to the residue. The layers separated upon addition of a few milliliters of isoöctane. Evaporation of the ethereal layer gave 14.0 g. of unsaponifiable fraction as an orange oily solid that was highly attractive to male moths.

Combined (esterified) fatty acids (53 g.) were obtained as a viscous yellow oil by acidification of the aqueous layer and extraction with ether. The neutralization equivalent was 284. Lithium aluminum hydride reduction of a portion of these unattractive acids gave a 99% yield of the corresponding alcohols as a viscous yellow oil that solidified in the cold but melted again at room temperature. These alcohols did not attract male moths. In this way, there were obtained from the total domestic extract 105 g. of combined fatty acids and 25.8 g. of unsaponifiable fraction; the corresponding weights of these fractions from 300,000 Spanish tips were 121.2 g. and 45.6 g., respectively.

Isolation of the Mixed Attractants. A. By Chromatography.—Unsaponifiable fraction equivalent to 20,000 domestic tips was chromatographed on two successive 1-lb. batches of magnesium carbonate[19] (column dimensions 2 × 34-inches) with Skellysolve B,[20] by the method of Acree,[5c] with absorbancy ratios at 249 and 285 mμ. The active fraction obtained was then further chromatographed on 200 g. of magnesium oxide[21] (column dimensions 2 × 12-inches) with 0.3% of absolute ethanol in Skellysolve B, at the same wave lengths. Practically all the activity was shown by the fraction corresponding to Acree's fraction B1. In similar manner, the corresponding fractions were obtained from the unsaponifiable fraction of the remaining 180,000 domestic tips. The combined active fraction obtained consisted of 120 mg. of yellow semi-solid, which resisted further fractionation by reversed-phase partition chromatography on columns of cellulose acetate.

This semi-solid was dissolved in 1.5 ml. of acetone and cooled overnight at −30°. The colorless solid that separated was filtered off by suction on silk, washed on the funnel with a little ice-cold acetone, and dried; it weighed 92.7 mg. Treatment with concentrated sulfuric acid and repeated recrystallization from ice-cold acetone gave a mixture of hydrocarbons melting sharply at 59.5–60.0°. Gas chromatography[22] on an SE 30 column showed it to consist of heptacosane, with traces of nonacosane and hentriacontane.

Evaporation of the combined acetone filtrates left 27 mg. of a pale yellow, viscous oil highly attractive to male gypsy moths. It showed blue fluorescence in ultraviolet light but did not absorb in the ultraviolet range.

B. **By Fractional Crystallization.**—The unsaponifiable fraction (45.6 g.) obtained from 300,000 Spanish tips was dissolved in 125 ml. of acetone and the orange precipitates that separated successively at room temperature, 5°, and −5° were filtered off and washed with small volumes of acetone cooled to −20°. Repeated applications of this procedure with increasingly concentrated volumes of the mother liquors resulted in the separation of 44.9 g. of an orange, solid mixture of inactive hydrocarbons, pigments and sterols that were not further identified. Evaporation of the acetone mother liquors to dryness gave 48 mg. of highly active, pale yellow viscous oil showing blue fluorescence. It was combined with the corresponding fluorescent oil obtained by chromatography to give a total yield of 75 mg.

Isolation of the Pure Attractants.—Strips of Whatman No. 1 filter paper (1 × 12-inch) were drawn through a 5% (by volume) solution of Dow Corning Silicone No. 550 in acetone, dried in air, rinsed with 95% ethanol, blotted between sheets of absorbent paper, and again dried in air. Other untreated strips of the same size were immersed for 2 minutes in a hot saturated solution of polyethylene[23] in xylene, dried in a stream of air, rinsed with 95% ethanol, blotted lightly, and dried in air. The polyethylene content of these strips was 30%.

A solution of 1 mg. of the fluorescent, active oil in 0.5 ml. of Skellysolve B was prepared, and 10 microliters of this solution (containing 20 micrograms of active oil) was transferred to each of several treated strips by means of a micropipet. The strips were spotted about 1 inch from one end and hung in individual graduate cylinders by the other end from tightly fitting cork stoppers. Chromatograms were developed by the ascending technique at 25–27° with the solvent mixture methanol–benzene–water (5:1:1) for 4 hours, during which time the solvent front traveled 22 cm. The strips were then removed, dried in air, soaked for 1 hour in sudan black reagent prepared according to Swahn,[24] washed for 5 minutes with 50% ethanol, and air-dried. Chromatograms obtained on silicone-impregnated strips showed four black spots on a white background, at R_f 0.0, 0.07, 0.27 and 1.0, whereas chromatograms obtained on polyethylene-impregnated strips showed five spots, at R_f 0.0, 0.2, 0.34, 0.73 and 1.0. When the chromatography was repeated without staining and the spots corresponding to these R_f values were cut out and tested in the laboratory, only the point of origin (R_f 0.0) was attractive to male moths. The point of origin was extracted repeatedly with 95% ethanol at 5° and then with Skellysolve B; bioassay showed that each of these solvents removed a different material attractive to males. Since Skellysolve B also removed small amounts of impurities from the silicone-impregnated paper, polyethylene-impregnated papers were used in the subsequent large-scale paper chromatography.

The total amount (75 mg.) of active, fluorescent oil was chromatographed on sheets (8 × 8-inch) of Whatman No. 1 paper impregnated with 30% polyethylene in the above manner and pre-extracted with 95% ethanol. Concentrated solutions of the oil in Skellysolve B were applied with a pipet as a streak across the sheet, about 0.5 inch from the bottom edge; when the streak had dried a new streak was applied directly to the preceding one to double the amount of sample. The chromatograms were developed for 3 hours in stainless steel tanks (9 × 9 × 3.5-inch) at 25–27° with methanol–benzene–water (5:1:1), and then air-dried. The section of the sheets corresponding to the original streak was cut out and extracted successively with cold 95% ethanol and Skellysolve B. Evaporation of the combined ethanol extracts to dryness under reduced pressure gave 3.4 mg. of white, waxy crystals, m.p. 37.0–37.5°; infrared spectrum

(19) Special, low in chlorine, powdered C.P. magnesium carbonate, J. T. Baker Chemical Co., Phillipsburg, N. J.

(20) Spectroscopically-pure solvent prepared by the method of M. M. Graff, R. T. O'Connor and E. L. Skau, *Ind. Eng. Chem., Anal. Ed.*, **16**, 556 (1944).

(21) Powdered magnesium oxide No. 2642 or Sea Sorb 43, Westvaco Chlorine Products Co., Newark, Calif., mixed 2:1 with Hyflo Super-cel.

(22) Kindly carried out by Dr. S. Louloudes, Pioneering Research Laboratories, Entomology Research Division, Beltsville, Md.

(23) Polyethylene DYLT (molecular weight 12,000), Union Carbide Chemicals Co., New York, N. Y.

(24) B. Swahn, *Scand. J. Clin. Lab. Invest.*, **4**, 247 (1952).

(1% in carbon disulfide): 2900s, 1400w, 1370w, 1254m, 1092m, 1015m, 860w, 803m, 741m cm.$^{-1}$.

Evaporation of the combined Skellysolve B extracts *in vacuo* left 20.0 mg. of colorless oil (I) that solidified to a waxy solid at 5° but remelted at room temperature; it showed strong blue fluorescence in ultraviolet light but no absorption in this region; $[\alpha]^{23}D$ + 7.9° (*c* 1.0, $CHCl_3$); infrared spectrum (2% in carbon disulfide): 3580w, 3450w, 2900s, 1740s, 1362s, 1234s, 1124w, 1042s, 955w, 757w, 719m cm.$^{-1}$.

Anal. Calcd. for $C_{17}H_{32}O_3$: C, 71.78; H, 11.34. For $C_{18}H_{34}O_3$: C, 72.41; H, 11.50. Found (single determination): C, 71.76; H, 11.05.

A single peak (retention time, 42 minutes) was obtained by vapor phase chromatography of I, using a 4.5-foot column packed with 20% Craig polyester succinate on firebrick, temperature 206°, helium flow 75 ml./min., filament current 130 milliamp., sample size 10λ of a 10% solution in benzene. Compound I was attractive to male gypsy moths in field tests at concentrations less than 10^{-7} μg.; the waxy solid was far less active.

Hydrogenation of Attractant I.—Attractant I (2.00 mg.) was dissolved in 0.5 ml. of dioxane and hydrogenated in a micro-hydrogenator at 23.5° and 1 atmosphere with 5 mg. of reduced platinum oxide catalyst. In 35 minutes 0.13 ml. (cor.) of hydrogen was taken up, and the reaction then ceased. (The theoretical requirement for 1 mole of hydrogen for the above weight of a substance of molecular weight 298.3 is 0.15 ml.) The catalyst was removed by filtration, and the solution was treated with 2 drops of water and extracted with two 2-ml. portions of Skellysolve B. The extract was dried and freed of solvent *in vacuo*, leaving 2.0 mg. of colorless liquid that solidified in the cold. Its infrared spectrum no longer showed a peak at 757 cm.$^{-1}$, which indicated that a *cis* double bond originally present had been hydrogenated.

Vapor phase chromatography under the conditions described above gave one peak, with a retention time of 39.5 minutes.

Saponification of Hydrogenated Attractant.—Hydrogenated attractant (1.8 mg.) was saponified for 3 minutes with 8.2 mg. of diethylene glycol–potassium hydroxide reagent according to the procedure of Schneider,[25] modified by extraction of the neutral fraction with 3 portions of warm Skellysolve B prior to back-titration. The Skellysolve B solution gave on evaporation 1.45 mg. of colorless crystals, m.p. 63–64°, whose infrared spectrum was indicative of a diol.

Titration of the aqueous reaction mixture with phenolphthalein as indicator required 0.326 ml. of 0.02 *N* hydrochloric acid and the saponification equivalent was calculated to be 314 (theory for $C_{18}H_{36}O_3$ is 300). The acidified solution was extracted with three small portions of chloroform and the extract was dried over sodium sulfate and concentrated to 0.5 ml. The infrared spectrum of this solution was identical with that of authentic acetic acid run in the same solvent.

Periodate–Permanganate Oxidation of Attractant I.—The stock solution used was prepared by dissolving 2.24 g. of potassium periodate and 0.04 g. of potassium permanganate in 500 ml. of distilled water with slight warming.

A mixture of 4 mg. of attractant I, 1.8 ml. of pure *t*-butyl alcohol, 3 ml. of the oxidation stock solution and 7.2 ml. of water was brought to approximately *p*H 8–9 by addition of powdered potassium carbonate and stirred on a magnetic stirrer for 17 hours. The mixture was acidified with a drop of 10% sulfuric acid and treated with powdered sodium metabisulfite to convert all the periodate, iodate and iodine into iodide (the dark red color that formed initially soon disappeared and the solution became completely colorless). The solution was made alkaline with 5% potassium hydroxide, the butanol was distilled off on the water-pump, and the remaining solution was acidified and continuously extracted with ether for 19 hours. The extract was dried over sodium sulfate and freed of solvent, and the pale yellow, oily residue was triturated with several small portions of cold Skellysolve B, in which 1.6 mg. of viscous, colorless oil remained insoluble.

Evaporation of the Skellysolve solution *in vacuo* left 2.4 mg. (92%) of very pale yellow oil, whose infrared spectrum showed the presence of acetoxyl and carboxyl, but not hydroxyl. It was shown to be identical with 3-acetoxynonanoic acid by comparison of its infrared spectrum and paper chromatographic behavior with those of a synthetic sample prepared by the following procedure.

To 38.7 g. (0.6 mole) of pure zinc dust in a 500-ml. three-necked flask provided with stirrer, reflux condenser and dropping funnel was added 10 ml. of a mixture of 77 g. (0.5 mole) of methyl bromoacetate, 68.4 g. (0.6 mole) of *n*-heptaldehyde, 80 ml. of dry benzene and 20 ml. of dry ether. After a little warming a vigorous reaction started and stirring was begun. The remainder of the mixture was added at such a rate as to cause gentle refluxing. The mixture was refluxed for an additional 30 minutes after addition was complete, cooled in an ice-bath, and treated with 200 ml. of cold 10% sulfuric acid solution. The upper layer was washed successively with 50 ml. of 10% sulfuric acid, two 25-ml. portions of 10% sodium carbonate solution, and 25 ml. of water, and dried over magnesium sulfate. The solution was evaporated to dryness *in vacuo* and the residual orange oil (63 g.) was refluxed for 3 hours with an alcoholic solution of 26 g. of potassium hydroxide. The cooled solution was poured into several volumes of water, extracted once with ether, and acidified with 20% hydrochloric acid. Extraction of the acid solution with ether and evaporation of the ethereal extract yielded a solid which was recrystallized from Skellysolve B to give 17 g. of 3-hydroxynonanoic acid as colorless, shining crystals, m.p. 60–61°.

Anal. Calcd. for $C_9H_{18}O_3$: neut. equiv., 174. Found: neut. equiv., 179.

To a solution of 16 g. of this acid in 50 ml. of anhydrous benzene and 20 ml. of pyridine was added slowly, with shaking and ice-cooling, a solution of 8.8 g. (10% excess) of acetyl chloride in 20 ml. of benzene. The mixture was refluxed for 3 hours and then cooled, washed with 10% hydrochloric acid and then with water, and dried over sodium sulfate. Removal of solvent and distillation of the residue gave 8.8 g. of 3-acetoxynonanoic acid as a colorless liquid, b.p. 120° (0.2 mm.), $n^{25}D$ 1.4470.

Anal. Calcd. for $C_{11}H_{20}O_4$: neut. equiv., 216. Found: neut. equiv., 215.

Paper chromatography of this acid as well as of the Skellysolve-soluble acid obtained by oxidation of the attractant I was carried out by the method of Stark[26] with chloroform–ethanol (2:1) containing 2% of 90% formic acid as developer. The chromatograms were visibilized with 0.04% alcoholic brom phenol blue as yellow spots on a blue background. Identical spots (R_f 0.93) were obtained in each case, whereas a synthetic sample of 2-acetoxynonanoic acid gave a spot showing R_f 0.85.

The Skellysolve-insoluble oxidation product (1.6 mg.) obtained above was dissolved in 3 ml. of acetone and treated at 50°, with magnetic stirring, with 4 mg. of finely-powdered potassium permanganate. As soon as the reaction mixture had become colorless, the manganese dioxide was filtered and washed thoroughly with a little warm water. The filtrate was evaporated to about 1 ml. on the water-pump, acidified with sulfuric acid, and extracted with ether in a continuous extractor for 20 hours. The ether solution was dried over sodium sulfate and freed of solvent, giving 1.2 mg. (71%) of colorless crystals, m.p. 104–105°. It was identified as pimelic acid by a mixture melting point determination with an authentic specimen (m.p. 104–105°) and a comparison of their infrared spectra.

Paper chromatography of these acids was carried out by the method of Cheftel[27] with ethanol–ammonia–water (80:5:15) as developer. The chromatograms, treated with brom cresol green and lead acetate, appeared as greenish-yellow spots on a violet background. Identical spots (R_f 0.38) were obtained in each case.

Dec-1-yn-4-ol (II).—Reformatski reaction between propargyl bromide and heptaldehyde by the procedure of Crombie and Jacklin[18] gave the desired product, b.p. 82° (2 mm.) (lit. b.p. 57° (0.06 mm.)), in 18% yield.

4-(Tetrahydro-2-pyranyloxy)-dec-1-yne (III).—To 28.6 g. of dec-1-yn-4-ol (II) were added 33 g. of distilled dihy-

(25) F. Schneider, "Qualitative Organic Microanalysis," John Wiley and Sons, Inc., New York, N. Y., 1946, p. 161.

(26) J. B. Stark, A. E. Goodban and H. S. Owens, *Anal. Chem.*, **23**, 413 (1951).

(27) R. I. Cheftel, R. Munier and M. Macheboeuf, *Bull. soc. chim. biol.*, **35**, 1085 (1953).

dropyran (b.p. 88–90°) and 3 drops of concentrated hydrochloric acid, and the slightly warm solution was set aside overnight at room temperature. Potassium hydroxide pellets were added and the liquid was decanted off and distilled over potassium carbonate (foaming was extremely troublesome) to give, after a small forerun, 24.2 g. (55%) of colorless liquid, b.p. 142° (0.5 mm.), $n^{25}D$ 1.4506.

Anal. Calcd. for $C_{15}H_{26}O_2$: C, 75.57; H, 11.00. Found: C, 75.39; H, 10.93.

1,5-Dichloropentane.—Commercial 1,5-pentanediol (60 g.) and dry pyridine (10 ml.) were placed in a 3-necked flask immersed in an ice-bath and equipped with a stirrer, reflux condenser and dropping funnel. Thionyl chloride (275 g., 100% excess) was added dropwise with stirring at such a rate that the temperature remained at about 25°, and the mixture was then heated at 70° for 2 hours with continuous stirring. After cooling, ice-water was cautiously added, and the dense, precipitated orange oil was dissolved in petroleum ether (b.p. 37–48°) and shaken successively with concentrated sulfuric acid, 5% sodium bicarbonate solution, and water. The solution was dried over magnesium sulfate, freed of solvent, and distilled to give 70.2 g. (74%) of colorless oil, b.p. 73° (15 mm.), $n^{25}D$ 1.4540.

Anal. Calcd. for $C_5H_{10}Cl_2$: C, 42.55; H, 7.09. Found: C, 42.38; H, 7.12.

1-Chloro-5-iodopentane.—A mixture of 76.5 g. of sodium iodide, 70 g. of 1,5-dichloropentane and 400 ml. of dry acetone was heated on the steam-bath for 2.5 hr. Violent bumping occurred as sodium chloride precipitated, precluding longer refluxing for fear of breaking the reaction flask. The mixture was poured into 600 ml. of water and the precipitated oil was taken up in petroleum ether, washed with water, and dried over magnesium sulfate. Removal of solvent and distillation of the residue through a 15-inch Vigreux column gave 43.0 g. (37%) of pale yellow liquid, b.p. 105–107° (14 mm.), $n^{25}D$ 1.5280 (lit.[17] b.p. 104–105° (14 mm.), $n^{25}D$ 1.5282)).

1-Chloro-9-(tetrahydro-2-pyranyloxy)-pentadec-6-yne (IV).—Sodamide was prepared from 5.3 g. (0.23 gram atom) of sodium in liquid ammonia,[28] and 24.0 g. (0.1 mole) of III was added to the mixture with continuous stirring over a period of 45 minutes. After stirring 1 hour longer, 25.8 g. (0.11 mole) of 1-chloro-5-iodopentane was added dropwise. Ammonia lost by evaporation was replenished at this point, stirring was continued for an additional 4 hours, and the ammonia was allowed to evaporate overnight. About 300 ml. of cold water was cautiously added, and the organic layer was collected in ether, washed once with water, and dried over sodium sulfate. Removal of the solvent and distillation of the residue gave 10.2 g. (35%) of pale yellow liquid, b.p. 183–185° (0.1 mm.), $n^{25}D$ 1.4753.

Anal. Calcd. for $C_{20}H_{35}ClO_2$; C, 70.01; H, 10.30. Found: C, 69.37; H, 10.21.

10-Hydroxy-7-hexadecynoic Acid (V).—A mixture of 10 g. (0.03 mole) of IV, 4.4 g. (0.09 mole) of sodium cyanide and 75 ml. of 95% ethanol was refluxed for 27 hours, the solution was decanted, and the insoluble salts washed thoroughly with ethanol. The combined ethanolic solutions were refluxed for 4 days with 2.8 g. (0.07 mole) of sodium hydroxide and 20 ml. of water, and the product was evaporated to dryness, redissolved in 20% ethanol, and twice extracted with ether. After acidification of the aqueous layer with dilute sulfuric acid and extraction of the mixture with ether, the ether-soluble oily liquid obtained was refluxed for 30 minutes with 2 *N* sulfuric acid and extracted with ether. Drying of the solution and evaporation of the ether left a yellow-white solid which, on recrystallization from Skellysolve B, yielded 1.2 g. (15%) of colorless solid, m.p. 51.0–52.5°.

Anal. Calcd. for $C_{16}H_{28}O_3$: C, 71.59; H, 10.51; neut. equiv., 268.2. Found: C, 71.52; H, 10.39; neut. equiv., 265.8.

10-Hydroxy-*cis*-7-hexadecenoic Acid (VI).—A solution of 1.1 g. of 10-hydroxy-7-hexadecynoic acid (V) in 10 ml. of methanol was hydrogenated at room temperature with 0.5 g. of Lindlar catalyst[29] and a few drops of quinoline. When the required amount of hydrogen for one double bond had been absorbed (92 ml. at 30° and 761 mm.), the reaction was interrupted and the mixture was extracted with ether. The extract was washed with 5% hydrochloric acid and water, dried over sodium sulfate, and freed of solvent. The residual oil crystallized at acetone–Dry Ice temperature, giving 0.9 g. (81%) of white waxy solid, m.p. 28–29°.

Anal. Calcd. for $C_{16}H_{30}O_3$: C, 71.06; H, 11.17; neut. equiv., 270.2. Found: C, 71.21; H, 11.02; neut. equiv., 272.8.

1,10-Dihydroxy-*cis*-7-hexadecene (VII).—A solution of 0.81 g. of 10-hydroxy-*cis*-7-hexadecenoic acid (VI) in 5 ml. of ether was added dropwise with stirring to a suspension of 0.15 g. of lithium aluminum hydride in 10 ml. of ether and the mixture was worked up in the usual way to give 0.75 g. (98%) of viscous yellow oil, which was used without further purification.

Anal. Calcd. for $C_{16}H_{32}O_2$: C, 74.91; H, 12.60. Found: C, 74.72; H, 12.49.

1,10-Diacetoxy-*cis*-7-hexadecene.—To a solution of 0.7 g. of the diol VII and 1 ml. of dry pyridine in 15 ml. of anhydrous benzene was added, with stirring and ice-cooling, a solution of 0.46 g. (10% excess) of acetyl chloride in 5 ml. of benzene. The mixture was refluxed on the steam-bath for 2 hours, cooled, washed successively with dilute hydrochloric acid, dilute potassium hydroxide, and water, and dried over sodium sulfate. Removal of solvent and distillation of the residual oil gave 0.7 g. (75%) of colorless, mobile liquid, b.p. 172° (1.4 mm.), $n^{25}D$ 1.4525.

Anal. Calcd. for $C_{20}H_{36}O_4$: C, 70.52; H, 10.67. Found: C, 70.60; H, 10.59.

(+)-10-Acetoxy-*cis*-7-hexadecen-1-ol (I).—A solution of 0.63 g. of 1,10-diacetoxy-*cis*-7-hexadecene, 0.12 g. of potassium hydroxide, 0.5 ml. of water and 2 ml. of 95% ethanol was refluxed on the steam-bath for 1.5 hours, then diluted with several volumes of water and extracted with ether. The ether solution was washed with water, dried, and evaporated to dryness, and the residue was distilled to give 508 mg. (92%) of colorless, somewhat viscous liquid, b.p. 169° (0.2 mm.).

Anal. Calcd. for $C_{18}H_{34}O_3$: C, 72.41; H, 11.50. Found: C, 71.91; H, 11.26.

The infrared spectra of the *dl*-form and the natural (*d*−) form of the attractant were superimposable, and both showed the same attractiveness to male moths in the field.[7]

(28) K. Ahmad and F. M. Strong, *J. Am. Chem. Soc.*, **70**, 1699 (1948).

(29) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).