of a major component of purified scarlet oak extracts are identical to those of (*Z*)-3-tetradecenyl acetate. Because of similarities in the mass spectra and GC retention times of tetradecenyl acetate isomers having double bonds in the positions 2 through 8, further analyses of this component are being conducted. This isomer is one of the five most highly attractive isomers in field tests (*8*, *9*).

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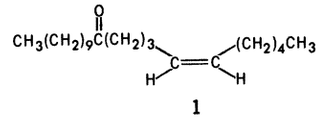
7 November 1974; revised 31 January 1975 ■

Douglas-Fir Tussock Moth: Sex Pheromone Identification and Synthesis

Abstract. *The sex pheromone of the Douglas-fir tussock moth* Orgyia pseudotsugata *(McDunnough) has been isolated and identified as* (Z)*-6-heneicosen-11-one. This compound and its* E *isomer have been synthesized and are highly potent in laboratory bioassays and field trials.*

The Douglas-fir tussock moth (DFTM) *Orgyia pseudotsugata* (McDunnough) (*1*) is a severe defoliator of fir forests of western North America (*2*, *3*). This insect is capable of dramatic population increases, which can result in severe damage to forest resources for a year or two before such populations subside (*2*). The availability of DFTM sex attractant (pheromone) for use in surveillance traps should prove invaluable, as such traps would constitute an early warning system for the detection of increasing moth populations, and would thus identify areas requiring intensified surveillance and possible containment measures.

The sex attractant of the DFTM has been isolated and identified as (*Z*)-6-heneicosen-11-one (**1**).



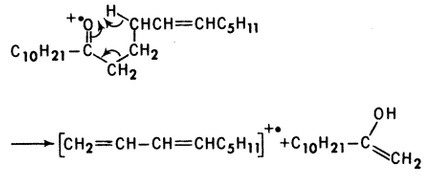
1

This structure, novel among known lepidopteran pheromones (*4*), has been confirmed by chromatographic, chemical, and spectrometric comparisons of the isolated attractant with synthetic **1**, and by moth attraction to the synthetic pheromone.

The isolation and structure elucidation was achieved by using the methylene chloride extract of 6000 female DFTM abdominal tips (each containing about 40 ng of pheromone). Functional groups present in the attractant molecule were established through chemical reactions in combination with bioassay. Alcohol and ester functional groups, two common structural features of known lepidopteran pheromones (*4*), were ruled out when treatment of the crude pheromone extract with alcoholic sodium hydroxide or acetic anhydride under reflux failed to destroy biological activity. Treatment of the crude extract with lithium aluminum hydride in dry ether destroyed the activity, which suggested the presence of an aldehyde, ketone, or epoxide function. A sample of the attractant extract in acetic acid heated at 105°C for 16 hours retained activity; this result excluded an epoxide function for the DFTM attractant, since (*Z*)-7,8-epoxy-2-methyloctadecane (disparlure), the attractant of the gypsy moth *Porthetria dispar* (*5*), reacted under these conditions to yield two products, presumably the two possible hydroxy acetates. Biological activity was destroyed when the DFTM attractant was treated with a standard 2,4-dinitrophenylhydrazine solution—a result consistent with the presence of a carbonyl group. The presence of one or more multiple bonds was inferred when pheromone activity was lost from samples which were catalytically hydrogenated, treated with ozone, or reacted with *m*-chloroperbenzoic acid.

A preliminary separation of the crude extract by "dry-column" chromatography (*6*) on alumina yielded an active fraction which, by gas-liquid chromatography [GLC, with a column 1.2 m by 6 mm (inner diameter) of 3 percent SE-30 on Chromosorb W, AW-DMCS], contained only two major components, one of which was biologically active. A mass spectrum of this purified pheromone exhibited a molecular ion at *m/e* (mass to charge) 308.3074 (the ratio calculated for $C_{21}H_{40}O$ is 308.3079); important fragment ions at *m/e* 169.1587 (calculated for $C_{11}H_{21}O$, 169.1592) and 167.1412 (calculated for $C_{11}H_{19}O$, 167.1435); and the base ion at *m/e* 124.1246 (calculated for C_9H_{16}, 124.1252). A mass spectrum of pheromone subjected to base-catalyzed deuterium exchange (*7*) indicated the presence of four exchangeable protons, consistent with a ketone functional group. The ions arising by the two modes of α-cleavage of the ketone attractant (*m/e* 167 and 169) and the deuterated analog (*m/e* 169 and 171) establish the position of the carbonyl at C-11. Conclusive identification of the carbon skeleton was obtained when catalytic reduction of the pheromone yielded a product that exhibited a mass spectrum and a GLC retention time identical to those of an authentic sample of 11-heneicosanone (*8*).

The base ion in the mass spectrum (*m/e* 124) arises via a McLafferty rearrangement (scheme 1) (*9*).



2

Scheme 1

The fact that most of the charge is retained on the hydrocarbon ion at *m/e* 124 rather than on the oxygen-containing ion at *m/e* 184 (which retains 15 percent as much charge as the base ion) indicates that extra stabilization is associated with the olefin ion radical, possibly because of conjugation with a double bond at position 6, as shown for **2**. This initial suggestion that the double bond of the attractant molecule is at position 6 was confirmed when ozonolysis of the pheromone produced a keto aldehyde which exhibited a GLC retention time identical with that of the product of ozonolysis of 1-hexadecen-6-one (*10*).

To establish the stereochemistry about the C-C double bond of the natural attractant derivatives of the

isolated DFTM attractant and of synthetic (*Z*)- and (*E*)-6-heneicosen-11-ones were prepared by reaction with *m*-chloroperbenzoic acid. By using a 1.2 m by 2 mm (inner diameter) column of 10 percent Apolar 10C on Gas-Chrom Q (Applied Science Laboratories) the isomeric *Z* and *E* derivatives were readily separated; the derivative obtained from the natural attractant corresponded to the *Z* isomer.

To synthesize both isomers of 6-heneicosen-11-one (**1** and the corresponding *E* isomer) we used as starting material 2-decyl-1,3-dithiane prepared from undecanal (*8*) and 1,3-propanedithiol (*11*) by the method of Fieser (*12*). The lithium derivative of this dithiane, prepared by using *n*-butyllithium (*13*), was alkylated with 1-chloro-4-decyne (*8*), and the carbonyl function of the resulting product was regenerated by using cupric chloride and cupric oxide (*14*) to yield 6-heneicosyn-11-one. The acetylenic bond of this compound was reduced over P-2 nickel catalyst (with ethylenediamine) (*15*) to yield (*Z*)-6-heneicosen-11-one. To prepare the *E* isomer from 6-heneicosyn-11-one it was necessary first to prepare the corresponding acetylenic alcohol by lithium aluminum hydride reduction; the acetylenic bond of this compound was then reduced with sodium in liquid ammonia (*16*). Finally, the olefinic alcohol obtained in this manner was oxidized with chromium trioxide–pyridine complex (*17*) to yield (*E*)-6-heneicosen-11-one.

The laboratory bioassay procedure was designed to provide qualitative, yes-or-no evaluations of test samples. A wind tunnel (60 by 60 by 170 cm) containing 0.5-liter cylindrical adhesive traps baited with test samples was used. Usually two but sometimes three traps including a blank control were exposed during a test. Test samples in methylene chloride solution were impregnated on filter paper or small disks of cotton wick for placement in the traps. The rate of air flow through the wind tunnel was regulated at 7 cm/sec.

For a sample to be judged attractive, DFTM males had to fly upwind toward a sticky trap baited with the sample, land on the trap, and enter it through the opening, which was 3 cm in diameter. Positive responses were generally clear-cut, with 50 percent or more of 30 to 50 DFTM males (1 to 5 days old) exhibiting this behavior. Bioassays were carried out between 4 and 6 p.m. to correspond with the period of maximum flight activity in the field (*2*). The caged males were exposed to no more than one attractive sample within a 24-hour period.

Both synthetic *Z* and *E* isomers are strongly attractive to DFTM males in laboratory bioassays. When a choice of a 200-ng sample of the *Z* isomer and an extract of five DFTM female abdominal tips was presented, DFTM males exhibited a 4:1 preference for the synthetic attractant. In a similar test DFTM males showed a 5:1 preference for female extract when the alternative was 200 ng of the synthetic *E* isomer.

Adhesive traps baited with as little as 400 ng of the synthetic *E* isomer have successfully captured DFTM males under field conditions. Owing to initial incorrect assignment of *E* stereochemistry to the natural pheromone, only this isomer was available for field trial during the 1974 flight season. Results of laboratory tests leave little doubt that the synthetic *Z* isomer will be even more effective in the field.

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9 September 1974; revised 13 January 1975 ■

Butylated Hydroxytoluene Inactivates Lipid-Containing Viruses

Abstract. *Butylated hydroxytoluene (BHT) is widely used as a food preservative for its antioxidizing property. This small, hydrophobic molecule has been found to be a potent inactivator of lipid-containing mammalian and bacterial viruses.*

Butylated hydroxytoluene (BHT) (Fig. 1) is one of several antioxidants that are commonly added to foods to maintain freshness and prevent spoilage by oxidation. It is frequently used in dried cereals, cooking oils, canned goods, and various animal foods. The average daily intake of BHT per person in the United States has been estimated at 2 mg (*1*) and in the United Kingdom as 1 mg (*2*). It is not readily excreted however, and measurements on 12 U.S. residents revealed a concentration of 1.30 ± 0.82 parts per million (ppm) in the body fat (*3*). For U.K. residents, the value was 0.23 ± 0.15 ppm. This represents an accumulation factor of approximately 45 over the daily intake in the United States and about 16 in the United Kingdom. Although BHT is generally recognized as safe by the Food and Drug Administration (*4*), there are varying reports as to the effects of this compound on organisms. It has been found that BHT causes bile duct proliferation in mice (*5*), is toxic to developing insect larvae (*6*), and reduces the growth rate of cultured mammalian cells (*7*). Some individuals show chemical intolerance