Reports

Isolation, Identification, and Synthesis of the Sex Attractant of Gypsy Moth

Abstract. The extremely potent sex attractant of the female gypsy moth has been isolated in pure form and identified as dextrorotatory 10-acetoxy-1-hydroxy-*cis*-7-hexadecene. The *dl*-form of the attractant has been synthesized.

For almost 30 years, investigators at the Department of Agriculture have been trying to isolate and characterize the substance secreted by the female gypsy moth (*Porthetria dispar* L.) and used to lure the male for purposes of mating (*1*). A crude extract containing this attractant is used on a large scale in survey traps to locate areas infested with this destructive pest of forest and shade trees (*2*). We now wish to report the successful isolation, identification, and synthesis of the pure, extremely potent attractant.

The benzene extractive prepared from the last two abdominal segments of 500,000 virgin female gypsy moths (*3*) was saponified with boiling ethanolic alkali, the neutral fraction was dissolved in acetone, and the solid that separated at room temperature, at 5°C, and at −5°C was filtered off and washed with a little cold acetone. Evaporation of the acetone filtrate gave 75 mg of a pale yellow, viscous oil highly attractive to male moths in bioassay and field tests (*4*). Reverse-phase chromatography of the active oil by the ascending technique on silicone- or 30 percent polyethylene-impregnated Whatman No. 1 filter paper sheets with methanol-benzene-water (5:1:1) as solvent gave five spots (as previously determined by spraying a sample chromatogram with Sudan Black), only one of which (R_F 0.0) was attractive. This zone was cut out and extracted repeatedly with cold 95 percent ethanol and then with petroleum ether (hexane). In this way there were obtained 3.4 mg of white waxy crystals soluble in ethanol and 20 mg of colorless, blue-fluorescing, petroleum ether-soluble liquid that solidified in the cold but remelted at room temperature. The latter was attractive to males in field tests in quantities less than 10^{-7} μg; the waxy solid was approximately 25 percent as active.

Gas chromatography of the liquid attractant on Craig polyester succinate showed it to be a pure material, which analyzed most closely for $C_{17}H_{32}O_3$ or $C_{18}H_{34}O_3$. Its infrared spectrum showed the presence of primary hydroxyl, secondary acetoxyl, an unbroken chain of at least four methylene groups, and a probable *cis* double bond; aromatic rings, *trans* unsaturation, acetylenic bonds, and branched methyl groups were absent. The attractant was optically active, $[\alpha]_D^{23} + 7.9°C$ (*c*, 1.0; $CHCl_3$). Catalytic hydrogenation of 2 mg of the attractant with platinum oxide catalyst resulted in an uptake of hydrogen sufficient for almost exactly one double bond, giving an oil whose infrared spectrum indicated complete saturation; it was one-third as attractive to male moths as the natural attractant. Saponification of 1.8 mg of the saturated compound required refluxing with diethylene glycol-potassium hydroxide mixture at 120°C for 3 minutes, showing a saponification equivalent of 314 and yielding 1.45 mg of an unattractive, crystalline diol and an acid identified by its infrared spectrum as acetic acid. Oxidation of 4 mg of the natural attractant with periodate-permanganate reagent (*5*) gave 2.4 mg (92 percent) of a pale yellow oil identified as 3-acetoxy-1-nonanoic acid [bp 120°C (0.2 mm-Hg); n_D^{25}, 1.4470] and 1.6 mg (83 percent) of a viscous oil which was oxidized with potassium permanganate in acetone to 1.2 mg (71 percent) of colorless crystals, mp 104–105°C, identified as pimelic acid by mixed melting point, infrared spectrum, and paper chromatography with a pure synthetic sample.

The major attractant of the female gypsy moth is therefore (+)-10-acetoxy-1-hydroxy-*cis*-7-hexadecene (I).

$$CH_3(CH_2)_5\underset{\underset{O}{\|}}{\underset{|}{C}}HCH_2CH{=}CH(CH_2)_5CH_2OH \qquad \text{I}$$
$$OCCH_3$$

The *dl*-form of compound I was synthesized in 0.2 percent over-all yield by the following procedure. Dec-1-yn-4-ol, prepared by condensing *n*-heptaldehyde and propargyl bromide in the presence of zinc (*6*), was converted to its tetrahydropyranyl ether (55 percent; bp, 142° at 0.5 mm; n_D^{25}, 1.4506). Condensation of the sodium derivative of the latter with 1-chloro-5-iodopentane by the method of Taylor and Strong (*7*) gave 1-chloro-9-(tetrahydro-2-pyranyloxy)-pentadec-6-yne (35 percent; bp, 183–185°C at 0.1 mm; n_D^{25}, 1.4753). Refluxing this compound with sodium cyanide in ethanol and hydrolysis of the crude product with alcoholic alkali, followed by removal of the tetrahydropyranyl group with 2*N* sulfuric acid, gave 10-hydroxy-7-hexadecynoic acid in 15 percent yield as colorless crystals, mp 51–52.5°C. Selective hydrogenation of this acid with quinoline-poisoned Lindlar catalyst (*8*) gave 81 percent 10-hydroxy-*cis*-7-hexadecenoic acid, white waxy solid, mp 28–29°C, which was reduced with lithium aluminum hydride to 1,10-dihydroxy-*cis*-7-hexadecene (98 percent), a pale yellow oil. The undistilled diol was converted to the diacetate (75 percent; bp, 172°C at 1.4 mm; n_D^{25}, 1.4525) with acetyl chloride in anhydrous benzene. Refluxing the diacetate with sufficient ethanolic alkali to saponify the primary acetoxyl group gave (±)-I (92 percent) as a colorless liquid, bp 169°C at 0.2 mm, identical in every respect save optical activity with the natural gypsy moth attractant.

The *d*- and *dl*-forms of compound I show approximately the same attractiveness to male gypsy moths in the field. To our knowledge, this is the first reported synthesis of a naturally occurring insect sex attractant.

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References and Notes

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Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper.

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

3. Of this total, 200,000 were collected in Connecticut and 300,000 were collected in Spain.
4. Bioassay tests were carried out by the method of B. C. Block [*J. Econ. Entomol.* **53**, 172 (1960)]. Field tests were carried out as described by J. M. Corliss ["Insects," *U.S. Dept. Agr. Yearbook Agr.* (GPO, Washington, D.C., 1952), p. 694]. The assistance of Mr. E. C. Paszek, U.S. Department of Agriculture, Nashua, N.H., in carrying out these tests is gratefully acknowledged.
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Expanding Shoals in Areas of Wave Refraction

Abstract. Shoals and spits may grow seaward, despite powerful refracted wave attack, where the wave energy level is too low to handle the supply of sand introduced by the littoral drift. Spits and shoals of this type have distinctive shapes. They may support ripple marks which do not parallel the crests of the waves which form them.

Wave refraction around a headland or point results in a concentration of wave energy, and hence a heightened level of activity. This fact is well known. The result is not, however, invariably erosion. Under certain conditions, concentrations of sand may occur, and be maintained, in areas of wave refraction. Such sand masses may appear as points, spits directed seaward, and near-shore shoals.

One requirement is that the coastal energy level be too low to handle the supply of sand (or other detritus) available. It may also be true that the unidirectional energy be essentially zero, as a result of cancellation of the littoral drift vectors.

Shoals, spits, and points of this type may appear on lagoonal shores, where wave energy is generally low, or on open, unprotected beaches. Each shoal commonly consists of several hummocks or faint swells, separated by gentle furrows, all arranged essentially parallel to the shore (see Fig. 1, *E*, for example). Such shoals may be rather large (that is, miles across; see Fig. 1, *A* and *B*) or small (tens or hundreds of feet across; Fig. 1, *C* and *D*).

The examples reported here occur along a coast of zero to moderate energy. This classification is based on a scale on which an average breaker height of 1 to 10 cm indicates low energy, 10 to 50 cm, moderate energy, and more than 50 cm, high energy. The average breaker at Cape San Blas, Fla. (Fig. 1, *A*), is about 13 cm (*1*). The energy level decreases from there toward the east. At Keaton Beach (Fig. 1, *D*), no long-term average is available, but breakers commonly do not form along the beach at all, and much of the time only small ripples, millimeters high, disturb the surface near the water's edge.

The breaker height at Cape San Blas indicates an energy level sufficient to handle (in terms of net littoral drift, past a given point) only about 2.5×10^4 m^3 of sand per year. Over the years, the Apalachicola (that is, Chattahoochee) River has delivered a greater load than that. Hence the large shoal areas at Cape San Blas and Cape St. George (Fig. 1, *B*) are related to the moderate level of coastal energy, coupled with the relatively large available supply of beach and near-shore sand.

Near Bald Point (Fig. 1, *C*) and Keaton Beach the annual accretion of sand is almost negligible, but the energy level is also very low. The small supply of sand is therefore piled into similar spit-and-shoal areas. These smaller features are in no sense beach cusps, although beach cusps commonly form in the same areas. (Beach cusps do not extend, below water, as shoals.)

Fair weather wave refraction patterns, on the smaller shoals, are quite clear (Fig. 1, *F*), and indicate a piling up of sand in these localities. During storms some of this sand is probably moved seaward, only to be worked landward again during the next period of long-term, essentially calm weather. Refraction of the waves produces two apparently dissociated wave sets, which cross each other at acute angles. The ripple marks produced by these two sets are more-or-less regular in general plan, but parallel neither wave set. Instead, they bisect the acute angle between the two sets, and approach an angle of roughly 45° with either set. Ripple marks of this type seem to be characteristic of the smaller shoals, where the water is very shallow (that is, less than 10 ft). Ripple marks on the larger shoals, which occur in deeper water, are of the more conventional types.

In some instances large shoal areas may result from an abundance of river sediment (as in Fig. 1); in others, a similar pattern may be due to decreasing energy, in the down drift direction (for example, the shoals off of Cape Hatteras, Cape Lookout, and Cape Fear, N.C., where the average breaker heights are about 85 cm, 75 cm, and perhaps 55 cm, respectively).

Sand concentrations of the variety described here are indications that the locality studied either now has, or recently has had, a supply of sand greater than that required by the prevailing wave energy level. The beach at such points must be, in general, either stable in position or prograding. This inter-

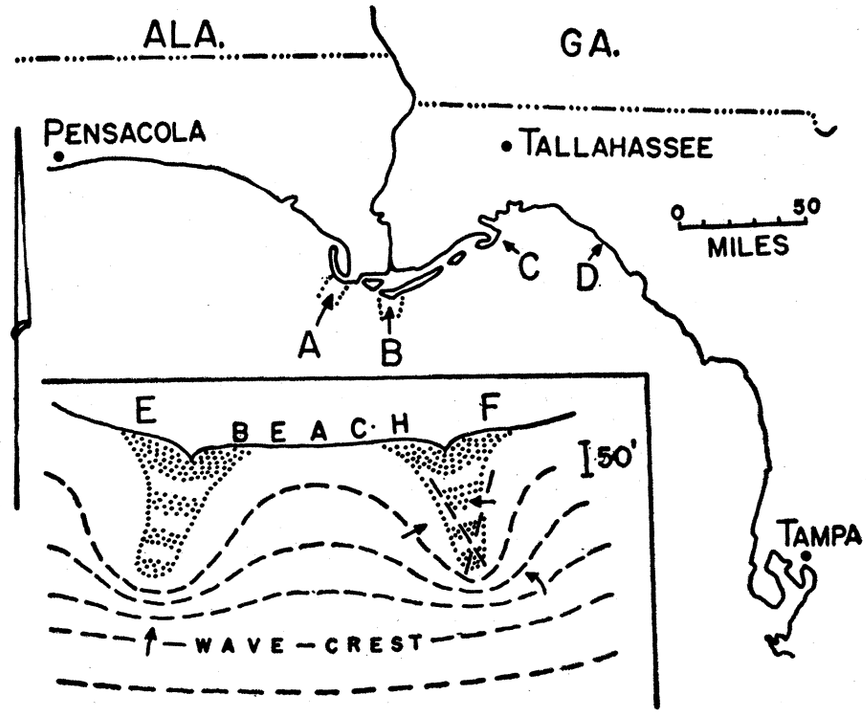

Fig. 1. Map of the Apalachicola River delta and adjacent parts of Florida, showing the location of shoals where available littoral materials are excessive, in the light of the current wave energy levels. Large shoals occur at Cape San Blas (*A*) and Cape St. George (*B*); many small shoals, closely spaced, occur at *C* and *D*. Inset: diagram showing details of two typical small shoals, with wave refraction patterns, such as those near *C*.