IDENTIFICATION OF TWO COMPONENTS OF THE SEX PHEROMONE OF THE MOTH, *Epiphyas postvittana* (LEPIDOPTERA, TORRICIDAE)

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(Received February 26, 1981; revised August 23, 1982)

Abstract—Two compounds, \((E)-11\text{-tetradecen-1-yl acetate}\) and \((E,E)-9,11\text{-tetradecadien-1-yl acetate}\), have been identified in extracts of females of the lightbrown apple moth, *Epiphyas postvittana* (Walker). The two compounds are active as a coalitative pair and are present in extracts of females in a ratio of about 20:1.

Key Words—*Epiphyas postvittana*, Lepidoptera, Tortricidae, lightbrown apple moth, sex pheromone, \((E)-11\text{-tetradecen-1-yl acetate}\), \((E,E)-9,11\text{-tetradecadien-1-yl acetate}\).

INTRODUCTION

The lightbrown apple moth, *Epiphyas postvittana* (Walker), is a pest of fruit trees and of grape vines in southeastern Australia. The insect has a wide range of host plants (Danthanarayana, 1975), and it is a common inhabitant of suburban gardens. It has become established as a pest in New Zealand and is also present in southwest England (Baker, 1968) and in Hawaii (Zimmerman, 1978). The presence of a pheromone in the female has been established and behavioral studies have been conducted (Bartell and Lawrence, 1977, and references therein). This paper describes the isolation and identification of two components, \((E)-11\text{-tetradecen-1-yl acetate}\) (I) and \((E,E)-9,11\text{-tetradecadien-1-yl acetate}\) (II), which are shown to be responsible for the activity of extracts of females in eliciting sexual behavior in males.

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METHODS AND MATERIALS

The insects were from a laboratory strain [defined as the Bartell strain (Geier and Springett, 1976)] which has been maintained for several years. Virgin female moths were collected after emergence and kept under a 14.5 hr:9.5 hr light-dark regime for three days. The last three or four segments of the abdomen were cut off and accumulated and stored under ether.

The pheromone was extracted by the following procedure. About 6000 tips plus the ether solvent were placed in a blender with sufficient pentane to cover the tips by 2-3 cm, and mashed for about 1 min. The slurry was poured into a flask and ethanol (100 ml) added. After addition of NaOH (30 g) in water (50 ml), the mixture was heated under reflux for several hours. The hydrolysate was then continuously extracted with pentane for 10 hr. The pentane extract (ca. 80 ml) was stirred with acetic anhydride (3 ml) and ether (10 ml) which contained one drop of concentrated H₂SO₄. After 30 hr, the solution was washed with water and then dried over Na₂SO₄. The dry solution, which was active on bioassay, was concentrated to about 5 ml by slow distillation of the solvent through a short fractionating column. This extract was sufficiently free of fatty acids to enable direct isolation of the active components by preparative GLC. Two further batches of tips were treated in the same way. In all about 27,000 female moths were used.

The bioassay was that described by Bartell and Shorey (1968) as later modified by Bartell and Lawrence (1973). The stimulus was presented for 20 sec in the stream of air. The numbers of males which responded by performing the wing fanning behavior were counted and averaged for each treatment.

Gas chromatographic analyses and collections were conducted on a Varian Aerograph model 1200 instrument which had been fitted with an annular splitter (Brownlee and Silverstein, 1968). Samples were collected in glass capillaries cooled with solid CO₂. The helium gas flow was about 20 ml/min. All columns were made of stainless steel (2 m × 2.3 mm) and contained the stationary phase at 5% on Gas-chrom Z, 80/100 mesh, unless otherwise stated. Retention index measurements were made under the same conditions with the splitter usually removed. Calculations of Kovats' indices were carried out by computer (Bellas, 1975).

Separations were first conducted on an OV-1 column using a temperature program of 6° or 8°/min from 80° to 200°. Initially the acetates of saturated alcohols containing 12-18 carbon atoms were coinjected as markers but, as these proved almost coincident with the homologous series of normal hydrocarbons already present from the insect, in later runs the hydrocarbons were used as internal markers.

The method of Beroza and Bierl (1967) was followed for ozonolysis experiments using spectrograde CS₂ as the solvent and the phase OV-101, 10% on Gas-chrom Q at 190° for gas chromatography. The samples for ozonolysis