Sex Pheromone of *Lobesia botrana*: (E,Z)-7,9-Dodecadienyl Acetate in the Female Grape Vine Moth

Hans-Rudolf Buser, Stefan Rauscher, and Heinrich Arn

Swiss Federal Research Station for Arboriculture, Viticulture and Horticulture, Wädenswil

(Z. Naturforsch. **29 c**, 781–783 [1974]; received July 1, 1974)

Sex Pheromone, *Lobesia botrana*, Grapevine Moth, (E,Z)-7,9-Dodecadienyl Acetate

Mass spectrometry in combination with high resolution gas chromatography were used to demonstrate the presence of (E,Z)-7,9-dodecadienyl acetate in *Lobesia botrana* females. The data indicate that this male attractant is a natural sex pheromone of the grapevine moth.

Electroantennograms obtained with model compounds and gas chromatographic fractions of female extracts have recently led to the proposal of (E,Z)-7,9-dodecadienyl acetate (**1**) as a sex attractant of the European grape vine moth, *Lobesia botrana* (Schiff.) [1]:

1

Although the synthetic product proved even more attractive to *L. botrana* males than live females, its identity with the sex pheromone emitted by the females has so far not been established.

The identification of insect sex pheromones has usually been a tedious and laborious process involving the isolation and purification of minute quantities of material from thousands of insects. A reduction of the insect requirement was recently achieved with computerized gas chromatography – mass spectrometry (GC – MS) in the identification of (E,E)-8,10-dodecadien-1-ol in codling moth females [2]. The double bond configuration, however, could be assigned only by additional chromatographic data.

We have now obtained mass spectrometric evidence for the presence of **1** in *L. botrana* females. Using high resolution glass capillary columns and mass specific detection, we have been able to ob-

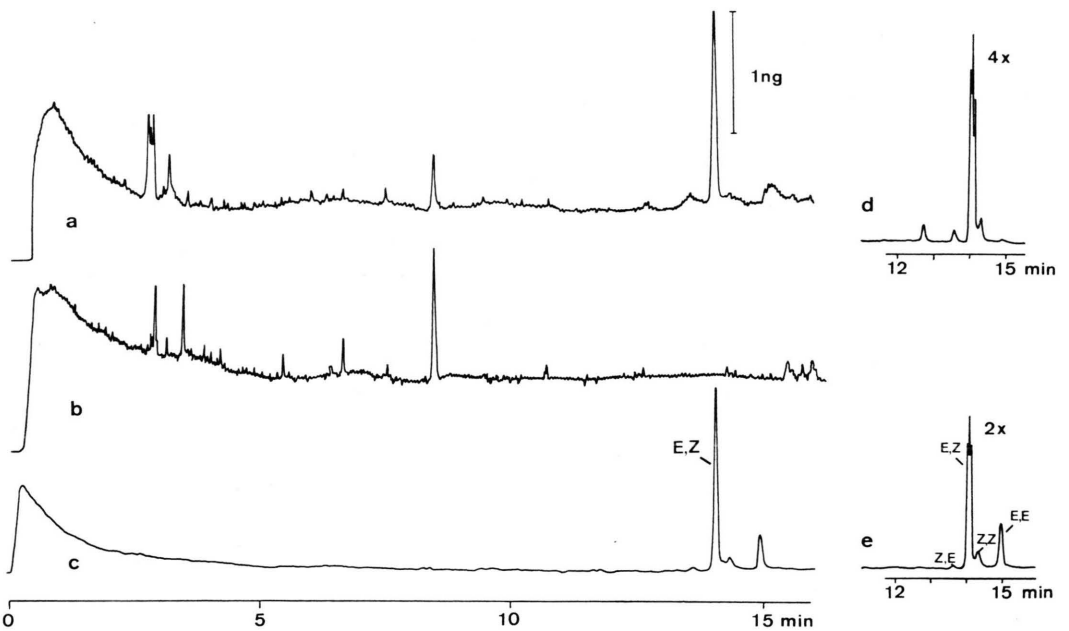

Fig. 1. Mass fragmentograms of partially purified *L. botrana* extracts and (E,Z)-7,9-dodecadienyl acetate (**1**). a: 1-female aliquot, b: 1-male aliquot, c: synthetic sample of **1** showing separation of geometrical isomers, d: 10-female aliquot, expanded scale, e: synthetic sample of **1**, expanded scale. UCON 50 HB 5100 glass capillary column: 50 m × 0.3 mm ID. Splitless injection at ambient temperature followed by ballistic programming to 170 °C, where recording is initiated. Helium carrier gas velocity: 25 cm/sec, column efficiency: 150 000 theoretical plates. Finnigan 1015 D quadrupole MS at *m/e* 164, electron energy 23 eV.

Requests for reprints should be sent to Dr. H. Arn, Swiss Federal Research Station for Arboriculture, Viticulture and Horticulture, *CH-8820 Wädenswil*, Switzerland.

tain information on quantity and double bond geometry of the female component from injections of as little as one moth equivalent.

L. botrana extracts were obtained by grinding and sonifying 500 abdominal tips of 3 to 4 day-old laboratory-reared male or female moths in the presence of 5 ml diethyl ether (Et_2O). After filtration through glass wool, the extract was concentrated, transferred to a 75 × 10 mm silica gel column topped with sodium sulphate and eluted with 15 ml portions of 0, 3, 8 and 20% Et_2O in pentane. Dodecyl acetate and its mono- and diunsaturated analogues eluted with 8% Et_2O; recoveries of **1** were ca. 65 and 95% at levels of 200 and 1000 ng, respectively. The corresponding alcohols eluted with 100% Et_2O. All fractions were concentrated to a final volume of 0.5 ml.

Mass fragmentographic analysis was carried out on a 50 m × 0.3 mm UCON 50 HB 5100 glass capillary column coupled to a Finnigan 1015 D quadrupole mass spectrometer. To take full advantage of the separation power of glass capillaries, the column was mounted in a separate Carlo Erba 2101 oven and was interfaced via a heated platinum capillary through the direct inlet into the ion source. By splitless injection at ambient temperature [3] followed by ballistic programming to 170 °C, up to 10 µl solution could be injected without impairing the vacuum system of the MS. Replacement of the vaporizer glass insert for each injection was imperative to avoid peak-broadening and loss of **1** caused by the presence of non-volatile coextractants.

For use as a mass specific detector, the MS was set at *m/e* 164 with unit resolution and the resulting ion intensities were recorded. While **1** gives a fairly intense molecular ion (Fig. 2 d), the more saturated analogues do not; and to aim at a general application for long-chain acetates, the M^+-CH_3COOH peak was chosen as a good compromise between desired sensitivity and required specificity. Best signal to noise ratio was obtained with a low electron energy of 23 eV. In spite of the use of a low intensity ion, a sensitivity better than 100 pg was obtained.

Mass fragmentograms were obtained of all fractions of moth extract. Only the 8% Et_2O fraction of females showed a peak at the retention time of **1**. Fig. 1 a shows a chromatogram obtained with one female equivalent. The main peak at 14.10 min co-chromatographed exactly with the (E,Z)-isomer. This peak was absent from the male extract (Fig. 1 b). By co-injection, using the (E,E)-isomer content of a synthetic sample as an internal standard, the amount of **1** per female tip was determined as 1.6 ng. Isomeric purity of the female component is high; the (Z,E) and (Z,Z)-isomers may be present at no more than 1.5% each, the E,E-isomer at up to 0.2% (Fig. 1 d).

An entire mass spectrum of the female component was obtained as confirmatory evidence. To remove non-volatile coextractants that would prohibit the required concentration prior to injection and prevent proper vaporization and elution of **1** in the GC-system, the 8% Et_2O fraction was subjected to co-sweep distillation [4]. A 150-female aliquot was injected into a stream of nitrogen at 180 °C, followed by several injections of Et_2O. The volatiles were trapped along with the solvent in a glass capillary kept at −10 °C and the solution concentrated to 5 to 10 µl. One-second MS scans of the GC eluate were obtained at 5 sec intervals. The differential spectrum (Fig. 2 c) obtained from scans during (Fig. 2 a) and immediately following (Fig. 2 b)

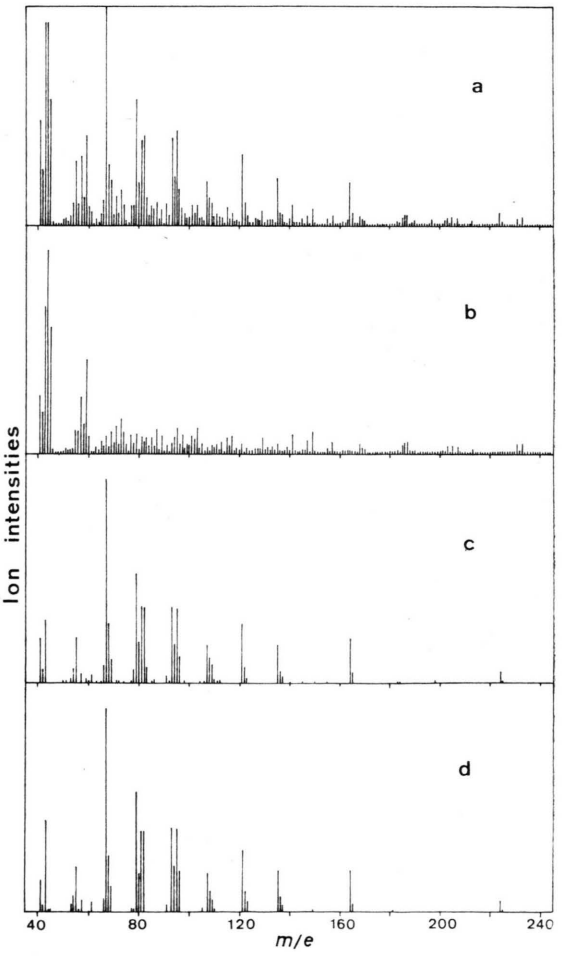

Fig. 2. Mass spectra of *L. botrana* female component and (E,Z)-7,9-dodecadienyl acetate (**1**). a: Composite spectrum at retention time of female component, b: background 10 sec after elution of female component, c: differential spectrum (a−b), d: spectrum of synthetic **1**.

elution of the female component was essentially identical to the mass spectrum of synthetic **1** (Fig. 2 d).

These data are strong evidence for the identity of the female component with (E,Z)-7,9-dodecadienyl acetate. Although co-chromatography and identity of mass spectra do not exclude all positional isomers, the biological activity of **1** and its absence from the male extract strongly suggest that this compound is a sex pheromone of *L. botrana*.

The electroantennogram technique[5] has made it possible to predict sex attractant structures using only a small number of insects, often without the need for mass rearing. The GC-MS technique with glass capillary columns is an analytical method of similar sensitivity. As a rapid analysis involving minimum cleanup and risk of decomposition, it can not only serve to verify the occurrence of proposed pheromones as natural products, but can provide additional information on the possible presence of isomers and analogues. This information can be invaluable for defining pheromone medleys and for optimizing attractants for field use.

We thank Dr. W. Roelofs (N.Y. State Agric. Experiment Station, Geneva, N.Y.) and Dr. C. Henrick (Zoecon Corp., Palo Alto, Calif.) for samples of the 7,9-dodecadienyl acetate isomers.

[1] W. Roelofs, J. Kochansky, R. Cardé, H. Arn, and S. Rauscher, Mitt. Schweiz. Entom. Ges. **46**, 71–73 [1973].
[2] M. Beroza, B. A. Bierl, and H. R. Moffitt, Science **183**, 89–90 [1974].
[3] K. Grob and G. Grob, Chromatographia **5**, 3–12 [1972].
[4] R. W. Storherr and R. R. Watts, Journal of the A.O.A.C. **48**, 1154–1158 [1965].
[5] W. Roelofs, A. Comeau, A. Hill, and G. Milicevic, Science **174**, 297–299 [1971].