A New Highly Effective Synthetic Pheromone Mimic for *Lobesia botrana* (Lepidoptera: Tortricidae)

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Dedicated to Professor Helmut Dörfel on the occasion of his 60th birthday

Pheromone, Attractant, Mimic for (*E,Z*)-7,9-dodecadien-1-yl acetate, (*Z*)-9-Dodecenyl acetate

(*Z*)-9-Dodecen-7-yn-1-yl acetate (y7*Z*9-12Ac) (**3**) is suggested as a pheromone mimic for *Lobesia botrana*. It was synthesized in two different ways and its activity was demonstrated electrophysiologically and in behavioural laboratory and field experiments.

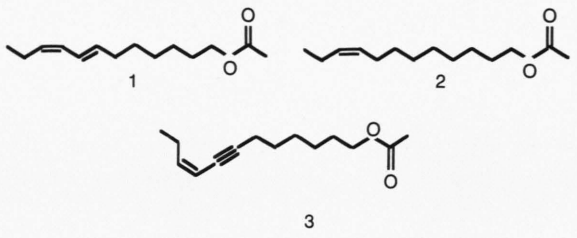
Introduction

In order to avoid problems arising with classical insecticides in some cultures pheromones may be used as a method for integrated pest insect control. The level of control by pheromones may be optimized by further investigation of the effects of naturally occurring pheromone blends or by investigating distinct modification of the chemical structure of pheromone components [1–3].

In this study we report on some work concerning the control of the European grapevine moth, *L. botrana* [4], the main pest in European viticulture.

Although the molecular structure of the main component, (*E,Z*)-7,9-dodecadien-1-yl acetate (*E*7*Z*9-12Ac) (**1**), is not too complicated and in spite of the numerous synthetic routes described in the literature [4–7] there has been to date no industrially practicable way of synthesizing it on a large scale. In order to avoid this problem we looked for a suitable mimic.

Male *L. botrana* were attracted and disorientated by (*Z*)-9-dodecenyl acetate (*Z*9-12Ac) (**2**), the pheromone of *Eupoecilia ambiguella* [8], too, but the required dosages to achieve this were in comparison with **1** higher [9]; (*Z*)-9-dodecen-7-yn-1-yl acetate



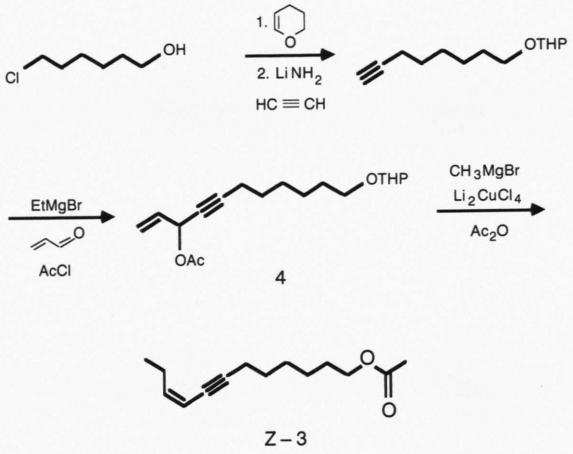
3

(y7*Z*9-12Ac) (**3**) appeared to be much more attractive. This paper describes the synthesis and biological results of **3** used as attractant and in orientation disruption experiments.

Chemical aspects

While looking for an industrially practicable synthesis of the original pheromone the following reaction sequence was developed (Scheme 1):

Scheme 1 :



Starting with chlorohexanol which was protected with dihydropyran and added to acetylene, the resulting 2-(7-octyn-1-oxy)-tetrahydropyran was metalated, hydroxyalkylated with acroleine and, in the same step, acetylated to give **4**. The conjugated system of the product **3** is built up by a S_N2'-type reaction using methylmagnesium bromide. It is known in the literature [10, 11] that these conditions exclusively lead to a (*Z*)-configurated product. In these investigations, however, it was found that the halide used for the Grignard reagent could influence the stereochemical outcome: only if bromide was used, was the pure (*Z*)-olefin formed, both chloride and iodide also yielding amounts (20%) of (*E*)-configurated product.

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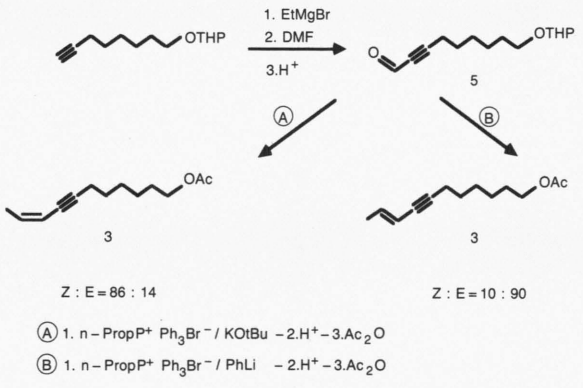
Another route is also possible (Scheme 2): starting from the tetrahydropyranyl ether of octynol the addition of dimethylformamide [12] leads to the 2-alkynal **5**, which can be olefinated in a Wittig reaction to form the desired en-yn system. If suitable conditions are chosen [13], this pathway leads to the (*E*)-isomer for spectroscopic and biological comparisons.

A reduction of (*Z*)-**3** yielding the natural pheromone could not be established.

Scheme 2 :



Chemical experiments

Analytical methods: The purity of the final products was assessed by gas chromatography and ^{1}H NMR spectroscopy. GC analyses were carried out on a CARLO-ERBA instrument equipped with an OV 17 fused silica capillary column under a temperature programme. ^{1}H NMR spectra were taken on a BRUKER AM 270 spectrometer; TMS was used as internal standard.

*2-(9-Acetoxy-undec-10-en-7-yn-1-oxy)-tetrahydropyran (**4**):* To 1.80 mol EtMgBr in 600 ml THF a solution of 340 g (1.60 mol) 2-(7-octyn-1-oxy)-tetrahydropyran in 720 ml THF is slowly added. After heating under reflux for 1 h 87.4 g (1.56 mol) acroleine in 180 ml THF is added at −30 °C, and the mixture is stirred for 2.5 h at 0 °C. Finally, after addition of 122.4 g (1.56 mol) acetic chloride in 400 ml THF at −20 °C and stirring for 1 h, hydrolysis and work-up, distillation at 170 °C/0.4 mm gives 336 g (40%) **4**.

*(Z)-9-Dodecen-7-yn-1-yl-acetate Z-(**3**):* To a solution of 5.40 g $CuCl_2$, 3.40 g LiCl and 308 g (1.00 mol) **4** in 1 l Et_2O and 1 l THF a suspension of 1.5 mol CH_3MgBr in 1 l THF is slowly added at −25 °C. After stirring at room temperature for 12 h the reaction mixture is treated with 800 ml acetic acid and 408 g (4.00 mol) acetic anhydride, and heating under reflux for 20 h follows. After aqueous work-up distillation at 115 °C/0.5 mm gives 160.2 g (72%) **3** as colourless oil. ^{1}H NMR: ($CDCl_3$): $\delta = 5.82$ (dt, $J_1 = 11$ Hz, $J_2 = 7$ Hz, 1H, 10-H), 5.41 (d, $J = 11$ Hz, 1H, 9-H), 4.05 (t, $J = 7$ Hz, 2H, 1-H), 2.5−2.2 (m, 4H, 11-, 6-H), 2.05 (s, 3H, CH_3CO), 1.7−1.3 (m, 8H, 2-, 3-, 4-, 5-H), 1.0 (t, $J = 8$ Hz, 3H, 12-H).

*2-(9-Oxo-non-7-yn-1-oxy)-tetrahydropyran (**5**):* A solution of 52.5 g (0.250 mol) 2-(7-octyn-1-oxy)-tetrahydropyran in 50 ml THF is slowly added to 0.275 mol EtMgBr in THF and heated under reflux for 1 h. After cooling, the reaction mixture is added dropwise to 58.0 ml (0.750 mol) DMF in 100 ml THF and stirred 5 h at room temperature. After hydrolysis with 300 ml 5% H_2SO_4 and extraction with ether 36.3 g (GC: 63%, 38% yield) **5** is obtained. ^{1}H NMR ($CDCl_3$): $\delta = 9.19$ (s, 1H, CHO), 4.55 (br. s, 1H, 2-H), 3.80, 3.45 (2 m, 4H, 6-H, 1′-H), 2.41 (t, $J = 7$ Hz, 2H, 6′-H), 1.90−1.30 (m, 14H).

*(Z)-9-Dodecen-7-yn-1-yl acetate Z-(**3**):* To a suspension of 44.2 g (0.114 mol) propyl-triphenylphosphonium bromide in 1 l THF 12.8 g (0.114 mol) KOtBu is added in portions. After 30 min stirring at room temperature 22.8 g (0.096 mol) **5** is added dropwise, and the reaction mixture is stirred for 45 min. Aqueous work-up and extraction with *n*-hexane afford 25.6 g (GC: 68%, 69% yield) 2-(*Z*-9-dodecen-7-yn-1-oxy)-tetrahydropyran containing 14% (*E*)-isomer. Subsequent treatment with $CH_3COOH/(CH_3CO)_2O$ for 16 h at reflux, aqueous work-up and distillation at 96−97 °C/0.1 mm give 9.12 g *Z*-**3**. The total yield referring to the aldehyde amounts to 24%.

*(E)-9-Dodecene-7-yn-1-yl acetate E-(**3**):* A solution of 0.150 mol phenyl lithium in Et_2O is added dropwise to a slurry of 57.7 g (0.150 mol) propyl triphenylphosphonium bromide in 250 ml THF and 250 ml Et_2O. After cooling to −70 °C the mixture is treated with 35.7 g (0.150 mol) **5**. Again 0.150 mol phenyl lithium in Et_2O is added, and the resulting dark-red betain-ylide solution is kept 30 min at −30 °C. Protonation with 0.165 mol HCl in Et_2O causes decolorization. After addition of 25.2 g (0.225 mol) KOtBu the reaction mixture is stirred for 2 h. Finally aqueous work-up gives the tetrahydropyranyl protected product; the calculated yield amounts to 50%, the *E*/*Z*-ratio being 90:10. Heating with 100 ml 1 M

H_2SO_4 followed by treatment with 10.5 ml (0.112 mol) acetic anhydride gives after work-up and distillation at 96–104 °C/0.2 mm 7.3 g *E*-**3** as a colourless oil. The total yield referring to the aldehyde is 22%. ^1H NMR ($CDCl_3$): $\delta = 6.08$ (dt, $J_1 = 7$ Hz, $J_2 = 17$ Hz, 1H, 10-H), 5.45 (d, $J = 17$ Hz, 1H, 9-H), 4.05 (t, $J = 7$ Hz, 2H, 1-H), 2.30 (m, 2H, 11-H), 2.12 (t, $J = 7$ Hz, 2H, 6-H), 2.05 (s, 3H, CH_3CO), 1.7–1.3 (m, 8H, 2-, 3-, 4-, 5-H), 1.00 (t, $J = 8$ Hz, 3H, 12-H).

Biological experiments

Different lab and field tests were performed out to demonstrate the high efficacy of the mimic. Electrophysiological measurements were carried out to examine the sensory response of the insect antenna to the stimulus. We used the electroantennogramme (EAG) technique [14]: Air currents (100 ml/s) were passed over pheromone-loaded filter paper and directed to an antenna, the measured potential is proportional to the amount of odourant. By comparing the dose-response curves of the natural pheromone **1** and the mimic **3** it can be seen that the new synthetic compound is only about ten times less effective than the original one (Fig. 1). As expected the stereoisomer *E*-**3** which was synthesized for comparison does show a dramatically lower activity (factor 10^4–10^5 weaker).

In a laboratory experiment **3** was tested as a disruptant: according to the method of Vita *et al.* [15],

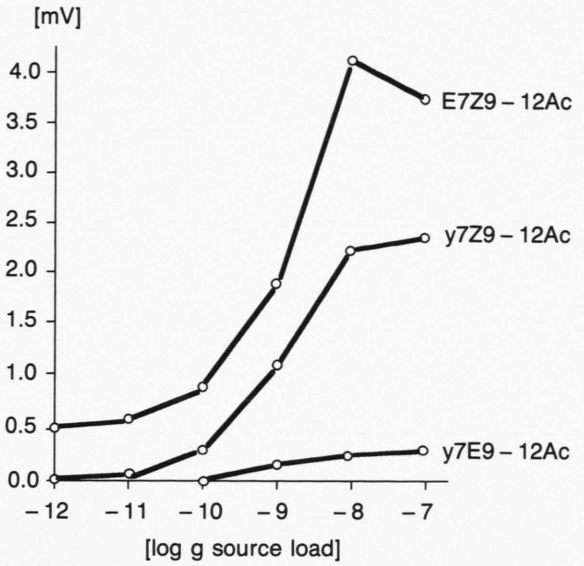


Fig. 1. EAG responses [mV] of *L. botrana* to *E*7*Z*9-12Ac, *y*7*Z*9-12 Ac and *y*7*E*9-12 Ac [log g].

the mating status was investigated under the influence of a saturated pheromone atmosphere. Here 3 females and 3 males of *L. botrana* were incubated in a pheromone-treated 1-l jar. After incubation (3 days, 16:8 light cycle) the females were dissected and the mating status determined by a spermatophore analysis. The result is expressed in percent inhibition in comparison to the untreated control (*n*-hexane) and summarized in Table I.

In a field experiment the activity of **3** as an attractant was tested. Pheromone soaked rubber tubes were placed in delta-type pheromone traps and placed in an area infested by *L. botrana*. The capture of moths was counted. In Table II the results of Wachenheim and Mußbach (Palatinate, West Germany) are listed.

Table I. %-Inhibition of mating of female *L. botrana* after incubation (3 d) in glass jars ($n = 5$). Rate of mated females in untreated control higher than 80%.

Substance	0.5	0.2	0.1	conc. [µl/l]
*E*7*Z*9-12Ac	100	78.5	74.6	
*y*7*Z*9-12Ac	100	85.7	50.8	

Table II. Catches of *L. botrana* males in delta-type pheromone traps ($n = 2$), 1985.

Substance	Amount per bait [mg]	Wachenheim May 16th to June 15th	Wachenheim July 14th to July 30th	Mußbach May 12th to June 6th	Mußbach July 16th to July 30th
*E*7*Z*9-12Ac	1.5	239	198	272	4
		228	91	318	5
*y*7*Z*9-12Ac	0.8	143	94	282	–
		160	192	269	–
	3.0	164	326	230	6
		190	295	243	8

From Table II it may be concluded that the attractivity of the natural pheromone and the mimic are in a similar range.

In addition, a disorientation experiment in the field was carried out. For this purpose within a homogeneous winegrowing area a number of plots (30 × 30 m) were treated with the pheromone mimic, formulated in a matrix dispenser (Hercon). The number of pheromone sources was 90 per plot (1000 per ha), the initial doses of pheromone employed were 50, 100 and 250 g/ha. The result is summarized in Table III and demonstrates the high disorientation effect.

Table III. Orientation disruption of *L. botrana* using *y*7*Z*9-12Ac, % reduction of moth catches in traps, 2. generation, Wachenheim 1986.

Dosage [g/ha] 1000 sources/ha	Catches in traps (n = 3)	% Reduction[a]
50	89	66
125	29	88
250	6	97
Control	271/195/316	

[a] Refering to centre trap within a treated plot compared with moth catches in untreated controls.

Furthermore flight observations were performed: in both a treated and untreated field some *Lobesia* males were released during their active phase at a distance of 1.5 m downwind of a lure trap and take-off, flight and landing at the trap were monitored.

While in the untreated field the majority of the moths was able to locate the attractive source and flew straight against the wind, with some males even landing at the trap, the treatment with *y*7*Z*9-12Ac (250 g/ha) only caused activation, but none of these males located the trap.

Discussion

The precursor of the original *Lobesia botrana* pheromone, the alkyne **3**, is a potent and highly active pheromone mimic with reference to the European grapevine moth. The activity is confirmed by electrophysiological measurements which suggest the mimic is only about 10 times less active. This is remarkable because from classical research on *e.g.* silk moths and the chemically very simple alarm pheromone of ants [16], it is known that substitutes were always 100 times less effective [17].

Under field conditions **3** proved to be a strong attractant; no significant difference to the natural pheromone **1** could be seen. For disorientation an efficacy of 97% could be reached; the required dosage of 250 g/ha, however, is considered to be relatively high.

These results lead to the conclusion that a transconfigurated double bond can obviously be substituted by a triple bond without causing a significant modification of the geometry and, thus, of the activity towards the target insect. This conclusion is, to our knowledge found in only a few other examples [18, 19], and may contribute to a further understanding of pheromone reception mechanisms.

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