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Sex propheromone of the pine tree lappet moth *Dendrolimus pini* and its use in attractant-based monitoring system

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Abstract: The study presents a new strategy of synthesis of the pine-tree lappet moth sex pheromone *via* hydrolysis of the (5*Z*,7*E*)-1,1-diethoxy-5,7-dodecadiene (propheromone) and their using in biological tests with pheromone traps. The field trials showed that a sticky delta traps with propheromone at dose of 2.4 mg was the most suitable for monitoring of the population of the pine tree lappet moth.

Keywords: cross-coupling reaction, stereoselective reduction, acetal, aldehyde, attractant, *Lepidoptera*, *Lasiocampidae*, rubber dispensers, *Pinus sylvestris* L., field experiments

INTRODUCTION

Pine-tree lappet moth caterpillars, *Dendrolimus pini*, are major defoliators of Scots pine (*Pinus sylvestris* L.) forests in Poland and other countries of Europe. Its populations can increase significantly from time to time in parts of its range in Europe, leaving large areas of pine woodland stripped of foliage as the caterpillars feed on the needles. Many of the trees die during severe outbreaks because the defoliated trees become susceptible to diseases, bark beetles and wood-boring insects as a consequence. The populations of the *D. pini* are usually treated by chemical insecticides during outbreak periods. Application of synthetic pheromones is one of the available methods of the pine lappet detecting that enable limiting of insecticides in protection of pine trees

[1]. The female sex pheromone was isolated and identified as (5*Z*,7*E*)-dodeca-5,7-dienal (**1**) in 1984 [2]. Many synthetic methodologies of the compound **1** are reported [3-7]. This aldehyde was also synthesized at the Institute of Industrial Organic Chemistry (IPO) using a new and simple method from the commercially available 6-hydroxy-1-heksyne [8]. (5*Z*,7*E*)-1,1-Diethoxy-5,7-dodecadiene (**2**) obtained during the synthesis is an immediate precursor of the final pheromone. This compound can be a useful tool for controlled release of the pheromone of pine lappet to the environment. The acetal **2** became the object of biological investigations as a lure in traps for monitoring this pest.

The aim of this paper was to evaluate the effectiveness of the aldehyde **1** and acetal **2** in the monitoring of *D. pini* populations. These field trials were carried out in Poland during 2010.

MATERIALS AND METHODS

Attractant: (5Z,7E)-1,1-Diethoxy-5,7-dodecadiene (acetal **2**) and pheromone **1** were prepared according to the method developed in IPO [8]. This route is outlined in Figure 1.

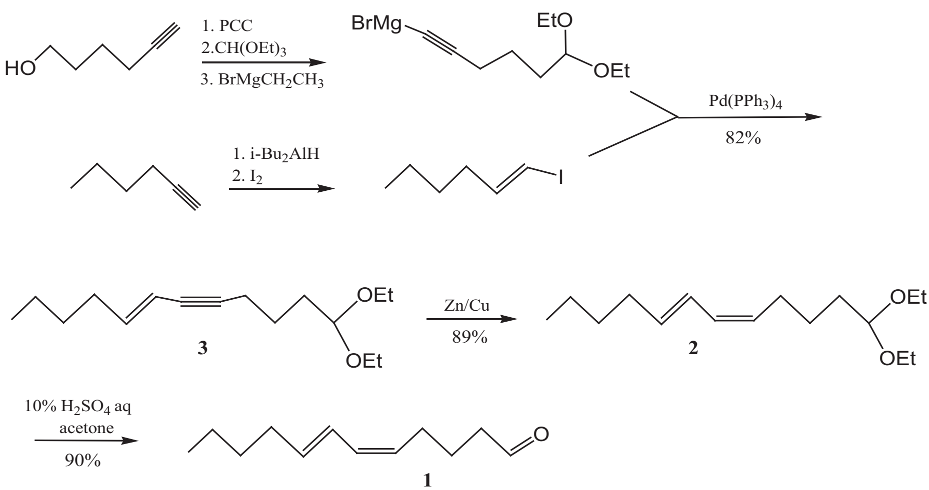

Figure 1. Synthesis of the pheromone **1** and the propheromone **2** (the spectroscopic date for the new compounds **2** and **3** as well as pheromone **1** are reported in [9]).

The stereocontroled introduction of double bonds in this synthesis was achieved by the methodology based on the palladium-catalyzed cross-coupling between suitable Grignard reagent (generated in situ from 6,6'-dietoxy-1-hexyne [10]) and the known (*E*)-1-iodo-1-hexene [11]. The obtained enyne **3** was converted by *cis* reduction with activated Zn (zink galvanized with cooper [12]) in dioxane-water to diene **2**. Compound **2** showed a high stereoisomeric purity (>99%) established by the ^{1}H NMR spectroscopy and GC analysis. This acetal (without purification) was hydrolyzed in 90% yield to the aldehyde **1** by 10% H_2SO_4 – acetone mixture (method reported by Hase et al. [13]). The isomeric purity of the pheromone **1** oscillated between 98 and 99% (mainly the content of the *E-E* dienic isomer slightly increased).

Trapping tests: The synthetic attractant **1** and its propheromone **2** were dissolved in hexane and the solutions were applied to "grey rubber caps" [14] at doses 1.2 mg (dispensers F) and 2.4 mg (dispensers A), respectively [15]. The part of dispensers F was treated with 10 mg of 2,6-di-*tert*-butyl-4-methylphenol (Topanol O) – antioxidant for hydrocarbon-based products, (dispensers FT). Moreover, the hexane solution of propheromone **2** was also added to rubber caps which were earlier washed with 0.5% H_2SO_4 (dispensers AK). The acid catalyst in the dispenser should accelerate process of hydrolysis of the acetal to pheromone. In the investigations, the commercially available pheromone lure from Pherobank (dispenser DEPI) was used as reference control. The all dispensers were placed in traps in the area of experiments. The sticky delta traps (Lunamelt PS 3199 glue) with sticky insert (19.5 x 20 cm) were employed. The field trials were done on two locations in Sława Śląska Forest District (Regional Directorate of the State Forests in Zielona Góra) in 2010 during the *D. pini* flight period. The sites were located relatively close to each other (about 4 km). The traps were suspended on branches (with the aid of long strings) at 7-9 m above the ground and spaced about 50 m apart (one trap per tree). Sticky inserts and lures were not replaced during experiments. In time of the study, the traps could not be checked because string, which fixed the trap to the tree, has undergone damage (the strings were tangled by strong wind and rain).

RESULTS AND DISCUSSION

The attractant **1** and propheromone **2** were obtained with isomeric purity not less than 99%.

In the pheromone trapping study the males of *D. pini* were trapped during flight moths from 5 July to 13 August 2010. The study results are presented in Table 1. In the experimental woodland location (sub-compartment 146, Forestry

Dąbrówno) the pheromone traps with dispensers (3 dispensers F, 2 dispensers FT, 2 dispensers DEPI and 1 dispenser AK) were suspensed on the 50-year-old pine trees (experiment A). The second experiment (B) was located at the pine forest in Strzeszków (sub-compartment 192). The pheromone traps contained: 2 dispensers F, 2 dispensers AK, 1 dispenser A, 1 dispenser FT and 1 dispenser DEPI. One trap was without lure (blank).

Tabela 1. Captures of *D. pini* in delta traps with sticky insert baited with different lures during the flight period in 2010: F - aldehyde **1** at the dose 1.2 mg, FT - aldehyde **1** (1.2 mg) and Topanol O (10 mg), A - acetal **2** at the dose 2.4 mg, AK - acetal **2** (2.4 mg) and a few of sulphuric acid, DEPI - standard pheromone lure from Pherobank, blank – trap without lure; Sława Śląska Forest District, 5 July to 13 August, 2010

Dispenser		Total catch
Experiment A	DEPI	0
	F	0
	DEPI’	0
	FT	0
	F’	0
	AK	0
	F”	1
	FT’	0
Experiment B	DEPI	0
	F	0
	AK	2
	F’	0
	A	4
	AK’	3
	FT	0
	blank	0

The first results of the above studies showed considerable difference in numbers of caught moths in traps with propheromone **2** in comparison with traps containing pheromone **1** and commercial lure DEPI. In the experiment A, only 1 trap with dispenser F caught one male moth of *D. pini*. The remaining traps caught no moth of pest. This fact can be explained by very low population densities of pine lappet in the location. Searching in the ground litter on the area of sub-compartment 146 (November), specialised staff of Forestry have confirmed the existence of slight amounts of over-wintering larvae. In case of the research done in the experiment B, the effectiveness of the traps baited with acetal **2** (dispenser A and AK) was noted (captured 9 moths – all were males). The traps with dispenser F, FT and DEPI did not capture pine lappet adults during the active flight period. The weak or none attractiveness of dispensers with pheromone **1** may be connected, among others, with a worse stereoisomeric purity of attractant than its propheromone. However, the slight increase of (*E*,*E*)-isomer in aldehyde **1** should not influence the effectiveness of the traps (earlier research did not prove that (*E*,*E*)-isomer is a powerful attraction inhibitor for *D. pini* [2]).

The disappearance of attractiveness properties of aldehyde **1** could also be attributed to extremely adverse weather conditions under of the pine lappet flight period (sweltering days and rain were alternate). In this conditions the pheromone **1** can easily undergo decomposition [2]. Probably this fact could also be main reason of disadvantageous result for commercial dispensers DEPI.

The properties of propheromone lures will be further examined during the next seasons.

CONCLUSIONS

- The new methodology based on the palladium-catalyzed cross-coupling with Grignard reagent and consecutive activated zinc reduction is efficiently applied in preparation of pine lappet sex pheromone and its propheromone with excellent or high stereoisomeric purity.
- The study with rubber dispensers containing acetal **2** and aldehyd **1** showed their attractiveness for *D. pini* male moths in field trials. However, the attractiveness of lures with pheromone **1** (dispenser F) was weak or completely disappeared.
- The traps with commercial pheromone lure DIPI and dispenser FT (pheromone **1** and antioxidant) did no capture moth *D. pini*. This may be connected with disappearance of attractiveness of the lures.
- Addition of small amounts of sulfuric acid to dispenser with propheromone **2** did not improve effectiveness of moths caught in the trap.
- For weak or none activity of dispensers with aldehyde **1** as well as commercial lures DEPI the extremely adverse weather conditions under investigations could have influence, favouring the processes of attractant degradation. In the tested dispensers comparable amounts of attractants were used (in dispensers F and FT as well as dispensers A and AK amount of active substance – counting

on (5*Z*,7*E*)-dodeca-5,7-dienal, was 1.2 and 1.7 mg, respectively). Therefore, this factor should not influence the results of field trials, because only at big differences of doses of aldehyde **1** (at least an order of magnitude) considerable changes in the effectiveness of pest attraction were observed [2].

- The acetal form of pheromone **1** (compound **2**) can be a useful tool for the biotechnical techniques used to monitoring/control of adults *D. pini*. This propheromon is more stable then aldehyde **1** and it should be more resistant to adverse processes of degradation in the natural environments.

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[9] **1** ^{1}H NMR ($CDCl_3$) δppm : 0.90(t, 3H, J= 7.2 Hz, C**H$_3$**), 1.20-1.82(m, 6H, 3C**H$_2$**), 2.02-2.30(m, 4H, 2CH=CHC**H$_2$**), 2.46(dt, 2H, J= 1.6, 7.2 Hz, C**H$_2$**CH**O**), 5.25(dt, 1H, J= 7.6, 10.4 Hz, C**H**=CH), 5.71(dt, 1H, J= 7.0, 14.8 Hz, C**H**=CH), 6.00(bt, 1H, J= 10.6 Hz, C**H**=CH), 6.25(ddq, 1H, J= 1.4, 11.0, 14.8 Hz, C**H**=CH), 9.78(t, 1H, J= 1.6 Hz, C**H**O); HRMS (EI) : $[M]^{+\cdot}$ *m/z* 180.15157, calcd for $C_{12}H_{20}O$ 180.15142.
2 ^{1}H NMR ($CDCl_3$) δppm : 0.90(t, 3H, J= 7.1 Hz, C**H$_3$**), 1,20(t, 6H, J=7.1 Hz, 2OCH$_2$C**H$_3$**), 1.24-1.72(m, 8H, 4C**H$_2$**), 2.02-2.26(m, 4H, 2CH=CHC**H$_2$**), 3.40-3.74(m, 4H, 2OC**H$_2$**), 4.49(t, 1H, J= 5.6 Hz, CH$_2$C**H**), 5.28(dt, 1H, J= 7.6, 10.8 Hz, C**H**=CH), 5.67(dt, 1H, J= 6.8, 15.2 Hz, C**H**=CH), 5.96(bt, 1H, J= 10.8 Hz, C**H**=CH), 6.28(ddq, 1H, J= 1.2, 10.8, 15.8 Hz, C**H**=CH); HRMS (EI) : $[M]^{+\cdot}$ *m/z* 254.22546, calcd for $C_{16}H_{30}O_2$ 254.22458.
3 ^{1}H NMR ($CDCl_3$) δppm : 0.89(t, 3H, J= 7.0 Hz, C**H$_3$**), 1.20(t, 6H, J=7.1 Hz, 2OCH$_2$C**H$_3$**), 1.23-1.43(m, 4H, 2C**H$_2$**), 1.48-1.82(m, 4H, 2C**H$_2$**), 2.00-2.16(m, 2H,

C≡CC**H₂**), 2.32(td, 2H, J=1.8, 7.2 Hz, CH=CHC**H₂**), 3.40-3.78(m, 4H, 2OC**H₂**), 4.51(t, 1H, J= 5.5 Hz, CH_2C**H**), 5.45(dq, 1H, J= 1.7, 15.8 Hz, C**H**=CH), 6.04(dt, 1H, J= 7.0, 15.8 Hz, CH=C**H**); HRMS (ESI) : $[M + Na]^{+\cdot}$ *m/z* 275.19929, calcd for $C_{16}H_{28}O_2Na$ 275.19815.

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[14] Grey rubber caps (commercial penicilinum vial caps LK-7/1, purchased with German-Polish Corporation "PASS-STOMIL") were extracted with acetone in a Soxhlet extractor prior to use.

[15] Amount of the (5*Z*,7*E*)-dodeca-5,7-dienal (1.2 mg per dispenser) was based on the earlier research results described by Preisner (Ref. 2), in which the greatest number of *D. pini* was caught on trees, where the sticky-traps contained dispenser (rubber cup) with 1 mg of aldehyde **1** (isomeric purity >99%). Acetal **2** was added to the dispenser in 2.4 mg *i.e*. 1.7 mg, counting on clean aldehyde **1**, what is an insignificant excess of the attractant (~40%) in comparison with dispensers including the (5*Z*,7*E*)-dodeca-5,7-dienal.