FEEDING DETERRENT ACTIVITY OF LACTONES WITH DI – AND TRIMETHYLCYCLOHEXANE SYSTEM AGAINST LESSER MEALWORM, *ALPHITOBIUS DIAPERINUS* PANZER AND COLORADO POTATO BEETLE, *LEPTINOTARSA DECEMLINEATA* SAY

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Abstract: The antifeedant activity of lactones with di- and trimethylcyclohexane system was assessed in choice and no-choice bioassays against *Leptinotarsa decemlineata* and *Alphitobius diaperinus* larvae and beetles. The results showed that feeding deterrent activity depends on the structure of the tested compounds. Additional methyl group in the molecule of some compounds influences their activity increase or changes their properties from attractant to deterrent ones. The present results also demonstrate the species and developmental stage dependence of antifeedant activity.

Key words: lactones, antifeedants, lesser mealworm, *Alphitobius diaperinus*, Colorado beetle, *Leptinotarsa decemlineata*

INTRODUCTION

The limitation of losses caused by insect predation is one of the basal aims of plant protection. The application of classical, chemical insecticides attacking mainly insect nervous system causes relatively quick cessation of predation but is accompanied by threats related with the resistance rise and environment pollution. Today, when integrated methods of insect pest control are widely introduced, there occurs an increase of interest in natural chemicals produced by plants. Many of them of antifeedants character often decide about host plant choice by phytophagous insects. Polyphagous insects have an ability to tolerate many plant secondary compounds due to various behavioral and anatomical adaptations. Oligophagous insects have a relatively high level of sensitivity to the taste of many secondary compounds (Eichenseer and Mullin 1997; Bernays and Chapman 2000). Lactones play a significant role among secondary plant compounds with inhibitory influence on insects predation. Their synthetic analogues are of increasing interest and can be included into integrated methods of plant protection, which are particularly recommended for insect pest control, in which quickly developing resistance against commonly used insecticides is observed. These chemicals protect plants by insect predation stopping on one hand, and on the other hand they cause weaker development or starvation death of the insect pests which do not accept food containing antifeedants.

The aim of the present studies is searching for the chemicals limiting predation of those insects, which control is particularly arduous. Colorado potato beetle is the insect pest in which quickly appearing resistance toward newly introduced insecticides is commonly known (Wegorek et al. 2001; Noronha et al. 2001; Loseva et al. 2002). The control of lesser mealworm is based mainly on the use of an adulticidal compounds (pyrethroids) and a larvicidal compound - insect growth regulators (Ignatowicz 1997; Salin et al. 2001). Application of these insecticides often fails to provide satisfactory control of this pest and re-establishment of the insect population was observed during the second broiler or turkey growing period. The studies conducted concerning azadichratin influence on A. diaperinus development showed that this substance with strong deterrent activity inhibits the growth and development of the pest and with 0.01% concentration in the food it completely eliminated the pest under laboratory conditions (Szczepanik 2001). The results obtained in our laboratory and the lack of literature data concerning the substances inhibiting lesser mealworm predation encouraged us to undertake the studies of searching the compounds with deterrent activity against this pest.

The antifeedant activity and high selectivity of lactones is connected with their structure. Some of them are active deterrents, and the others have attractant properties. This fact demands many chemicals examination because even small structural changes resulted in drastic differences in antifeedant activity (Ley et al. 1989; Blaney et al. 1990; Olejniczak et al. 2000; Gonzalez–Coloma et al. 2002). The choice of lactones with gem – dimethylcyclohexane system was based on the fact that this system is structural element of natural, biologically active compounds, including insect food deterrents. Besides, the biological activity examination of compounds with different type of cyclohexane ring substitution enables the studies of moiety structure influence on the compound activity.

MATERIALS AND METHODS

Lactones 1–9 (Fig. 1) which were tested for feeding deterrent activity, were obtained in three step syntheses from known γ , δ -unsaturated esters ethyl (5,5-dimethyl-2-cyclohexen-1-yl) acetate (Wawrzeńczyk and Lochyński 1985), ethyl (1.5.5-trimethyl-2-cyclohexen-1-yl)acetate (Jones et al. 1993) and ethyl (1,5-dimethyl-2-cyclohexen-1-yl)acetate (Grabarczyk et al. 2003). Hydroxy lactones 1a and 1b were obtained as a result of acidic lactonization of corresponding epoxy esters obtained from starting esters (Olejniczak et al 2001, Wawrzeńczyk et al. 2002). Hydroxy lactones 1a and 1b were substrates in synthesis of acetoxy lactones 3a and 3b and 2-oxolactones 2a and 2b (Wawrzeńczyk et al. 2003). Acetoxy lactones 3a and 3b were obtained in a standard manner as products of the reaction of hydroxy lactones 1a and 1b with CrO₃ pyridine complex gave the oxolactones 2a and 2b respectively. The reaction was carried out in methylene chloride.

Epoxy esters were also starting compounds in synthesis other 2-substituted lactones 4a–b, 5a–b and 6a–b. All of them were products of reaction of epoxy esters with equimolar amount of p-toluenosulphonic acid monohydrate (Wawrzeńczyk et al. 2003). Tosyloxy lactone 4a and 4b were obtained when the reaction with p-toluenosulphonic acid was carried out in benzene or methylene chloride. Etoxy 5a and 5b or metoxy 6a and 6b lactones were obtained in the case when this reaction was carried in ethanol or methanol respectively.

Esters were also substrates in syntheses of saturated (8a–c) and unsaturated (9a–c) lactones. The key step of these syntheses was iodolactonization of γ , δ -unsaturated carboxylic acids, which were obtained as a result of basic hydrolysis of esters. Iodolactonization was carried out by method described by Mori and Nakazano (Mori and Nakazano 1986). Saturated lactones 8a–c were the products of reductie dehalogenation of iodolactones 7a–c by means of tributyltin hydride. Lactone 8c was also obtained in the reaction of (1,5-dimethyl-2-cyclohexen-1-yl) aceticacid with iodine in acetic acid. Unsaturated lactones 9a–c were obtained by dehydrohalogenation of corresponding iodolactones with 1,8-diazabicyclo[5.4.0]undece-7-ene (DBU).

The purity of synthesized products was determined by thin layer and gas chromatography. The tested compounds were higher than 95% pure.

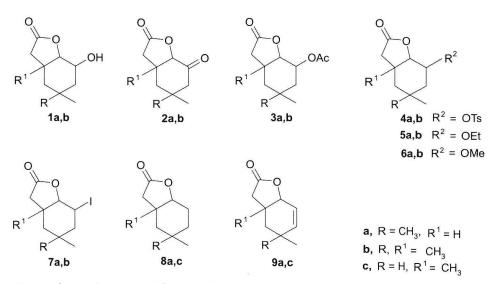


Fig. 1. Chemical structure of lactones used in this study

The feeding deterrent activity of 18-th lactones against the lesser mealworm and 12-th lactones against the Colorado beetle was determined. For *A. diaperinus wheat* wafer disk bioassay described by Nawrot et al. (1982) was used. The wafer disks were saturated by dipping in either solvent only (control) or in 0.1% solution of lactones. The disks were then air-dried for 1 h and weighed before offering them to the 10 adults or 10 larvae (30-ty days old) over the following three-day period in choice and no-choice tests.

In the experiments with *L. decemlineata* 3-rd instar larvae and adults were used. For the feeding assays 0.1% alcohol solutions of compounds were prepared. Disks (4.0 cm in diameter) were cut from potato leaves and were coated with either appropriate solution of lactone or alcohol. After the solvent evaporated the disks were offered to 10 larvae or 6 adults (3 pairs): solvent-control and treated disks were placed at alternate corners in Petri dishes lined with moistened filter paper (choice test). In no-choice test only control or only treated disks were placed in the dishes. In each four replicates, the insects were allowed to feed *ad lib.* for 24h at 24°C under a 16:8 light-dark photoperiod.

After the completion of the experiments, the wafer disks were reweighed, the areas of remaining uneaten potato leaf disks were measured using a scanner and special software. Based on the amount of food consumed in control, choice and no-choice test, three coefficients for feeding deterrent activity were calculated: absolute A (no-choice test), relative R (choice test) and total T, which is a sum of these two mentioned. Values of the total deterrence coefficient served as an index of feeding deterrence activity expressed on a scale between 0 and 200. The compounds for which the total coefficient amounted 150–200 were very good antifeedants. Good deterrents had this coefficient in the range 101–150. The compounds with coefficient below 100 were medium (51–100) or poorly active (0–50).

Data were analyzed by analysis of variance (Duncan 1951).

RESULTS

Alphitobius diaperinus

Table 1 list of the total deterrence coefficient values indicated the differential activity of the chemicals tested against the lesser mealworm. Coleopterons predation was inhibited the most strongly by lactone 7b. This compound activity was similar in the both assays what was showed by similar values of A and R coefficients. In the no-choice test, no increase in the treated food acceptance was observed. Lactone 3a was also good deterrent, though its activity in no-choice test decreased. Somewhat more poor deterrent properties were shown by the lactones 4b 5b, 6b and 9c. However, they were characterized by equal activity level in the both tests. Lactones 1b and 5a with an average deterrent properties and similar values of coefficient T showed higher activity in the no-choice tests. However, they were easily accepted in no-choice tests and were without greater effect on predation in comparison with the control. Very poor deterrents were 2b and 8a lactones. The three compounds among the studies ones were attractants. The strongest attractant was lactone 4a. Poor attractant properties were also shown by lactone 6a and 7a.

Compound	Deterrence coefficients								
	larvae			adults					
	А	R	Т	А	R	Т			
1b	31.46def	-15.55bc	15.91cde	63.06c	23.04bcdef	86.10de			
2a	7.54bc	30.06def	37.60efg	50.09c	7.93bcd	58.02cd			
2b	13.70bcd	49.62f	63.32gh	-6.58a	31.39cdef	24.81bc			
3a	36.18ef	44.54ef	80.72h	48.33c	80.11g	128.44e			
3b	30.82def	-28.31b	2.51bcd	12.76ab	61.35fg	74.11d			
4a	-5.96ab	-66.56a	-72.52a	-3.07a	-49.32a	-52.40a			
4b	39.13f	-30.49b	8.64bcde	39.24 bc	43.78defg	83.02de			
5a	12.95bcd	-24.28b	-11.33bc	63.44c	17.85bcde	81.29de			
5b	36.95ef	15.19cde	52.14fgh	48.42c	51.31efg	99.74de			
6a	-18.13a	-0.26bcd	-18.39b	-4.14a	-12.76b	-16.91ab			
6b	41.39f	39.52ef	80.93h	33.81bc	53.27efg	87.08de			
7a	49.80f	30.76def	80.56h	5.22a	-12.21b	-6.99b			
7b	1.65bc	65.2g	66.85gh	65.88c	68.88fg	134.76e			
8a	16.78cde	14.82cde	31.60def	17.46ab	4.34bc	21.80bc			
8c	6.01bc	29.7def	35.71efg	13.52ab	62.81fg	76.33d			
9a	39.99f	-13.12bc	26.88def	27.15bc	53.89efg	81.04de			
9c	4.04bc	23.14def	27.18def	46.70c	41.53defg	88.23de			

Table 1. Deterrent activity of lactones studied against Alphitobius diaperinus

Values followed by the same letter within a column are not significantly different at the 0.05 level

The compounds examined were moderately active against *A. diaperinus* larvae. Medium deterrent activity was shown by 2b, 3a, 5b, 6b, 7a and 7b lactones. Coefficient T values were not significantly different for these compounds. Lactone 4a, similarly as in the case of the lesser mealworm adult individuals resulted in an increase in predation level in comparison with the control. The lactones 5a and 6a were also attractants for these larvae.

Leptinotarsa decemlineata

The studied lactones more strongly inhibited the predation of *L. decemlineata* larvae comparing to adult individuals (Tab. 2). The highest activity against larvae was shown by lactone 3a. The food treated with this compound was consumed reluctantly in the both tests. Larvae predation level decreased by 92.7% in relation to the control in the choice test and by 66.8% in the no-choice test. This compound was the most poorly accepted in no-choice test and its coefficient A value was significantly different from the others (P<0.05). Lactone 7b was also good deterrent for the larvae. This compound significantly lowered the insect predation level, however in the choice tests mainly. Coefficient A low values point to the treated food easy acceptance in the no-choice test. Lactones 3b, 5b, 7a, 8c and 7b with middle antifeedant properties were also much more active in the choice tests than in the no-choice tests.

The lactones tested were poor deterrents against *L. decemlineata* beetles. Among on an average active antifeedants only the lactones 6a, 7b and 8c can be ranked.

Compound	Deterrence coefficients								
	larvae			adults					
	А	R	Т	А	R	Т			
lb	12.98abcd	-9.65a	3.34a	1.30 abc	5.17ab	6.48ab			
2b	12.13abc	37.51ab	49.64bc	2.21abcd	23.02abc	25.23abc			
3a	52.13e	87.33d	139.46f	-6.74ab	22.19abc	15.45abc			
3b	9.73ab	80.25cd	89.98cde	29.5de	9.49ab	38.99bcd			
5b	12.46abc	61.12bc	73.58cd	5.52abcde	26.65abc	42.17bcde			
6a	nt	nt	nt	23.94cde	30.26abc	54.21cdef			
6b	26.05d	41.12bc	67.17cd	nt	nt	nt			
7a	1.48a	61.47bcd	62.95cd	-10.65a	2.02 a	-8.63a			
7b	18.28bcd	85.19cd	103.47def	37.40ef	42.2 bc	79.60def			
8a	-0.25a	53.35bcd	53.09c	17.06bcd	-0.61 a	16.44abc			
8c	1.98a	68.60bcd	70.58cd	42.19ef	22.41 abc	64.60def			
9c	10.67ab	74.51bcd	85.18cde	10.92abcd	25.81 bcd	36.73bc			

Table 2. Deterrent activity of lactones studied against Leptinotarsa decemlineata

Note - see table 1

nt – not tested

DISCUSSION

The highest values of coefficient T were obtained during our studies for acetoxy lactone 3a and iodolactone 7b. Those lactones were particularly good deterrents for A. diaperinus imago and L. decemlineata larvae. Acetoxy lactone 3a showed also relatively high activity against the lesser mealworm larvae in comparison with other compounds studied. Similarly, iodolactone 7b was the best deterrent against L. decemlineata imago on the background of the remaining lactones, low active against coleopterons of this species. Acetoxy lactone 3a is a derivative of racemic, bicyclic hydroxy lactone 1a, which activity against L. decemlineata larvae was tested in our previous studies (Szczepanik et al. 2000). This chemical lowered larvae predation level by 43.5%. The replacement of lactone 1a hydroxyl group by an ester one (3a) resulted in an increase in the activity of the compound obtained. The amount of food treated with lactone 3a consumed by Colorado beetle larvae decreased by 59.3% in the no-choice test and by 92.7% in the choice test. Lactone 3b was also much more active against *L*. decemlineata larvae in comparison with lactone 1b. With reference to this species adult individuals an increase in lactone 3b activity in comparison with its precursor was not significant. In the case of lesser mealworm – both, hydroxy lactone 1b and acetoxy lactone 3b were poor deterrents against the larvae and middle against the adult specimens. These compounds structure changes did not influence their activity against the both developmental stages of A. diaperinus. On the other hand oxolactone 2b obtained as a result of hydroxy lactone 1b oxidation showed higher activity against the both species larvae. Deterrency coefficient T values of oxolactone 2b increased and were statistically different (P< 0.05) from hydroxy lactone 1b. Oxolactone 2b activity was clearly decreasing against A. diaperinus coleopterons in comparison with hydroxy lactone 1b. The replacement of hydroxylic group by carbonyl group did not influence the change in these compounds activity against Colorado beetle adult individuals.

The second group of the compounds examined by us were iodolactones. Iodolactone 7b was, along with acetoxy lactone 3b, the most active compound against lesser mealworm coleopterons and Colorado beetle larvae. It also revealed moderate deterrent properties against L. decemlineata coleopterons and A. diaperinus larvae. On the other hand iodolactone 7a was characterized by differential activity against the insects studied. In comparison with other compounds studied it was relatively good deterrent against larvae of the both species, but was an attractant against coleopterons. The addition of one methyl group in 7a molecule causes this compound attractant properties change into deterrent properties of lactone 7b against the both species coleopterons and also increases its activity against L. decemlineata larvae. Iodine atom removal from lactone 7a molecule in the case of A. diaperinus larvae influenced the lowering of deterrent properties of the saturated lactone 8a obtained by this way. With reference to the remaining insects, T coefficient values for 7a and 8a lactones do not differ in statistically significant way. Another structural modification, elimination of hydrogen iodide from 7a molecule and forming double bond in cyclohexane ring influenced significantly the properties of unsaturated lactone 9a. Deterrent activity of this compound against A. diaperinus coleopterons increased significantly, however was lowered against larvae of this species.

Lactones 8c and 9c activity differed only against adult specimens of Colorado beetle. The both compounds were moderate deterrents against lesser mealworm coleopterons and Colorado beetle larvae, but were poor against *A. diaperinus* larvae.

Some of the 2-substituted lactones studied revealed, in contrast to hydroxy-, acetoxy- and iodolactones, food attractant properties. Tosyloxy lactone 4a the most strongly stimulated predation of *A. diaperinus* both developmental stages. Attractants were also etoxy lactone 5a and metoxy lactone 6a. The presence of additional methyl group in molecule of these compounds caused a change in their properties from attractant into deterrent properties of lactones 4b, 5b and 6b.

The studies performed concerning deterrent activity of the tested lactones confirmed marked influence of molecule structure upon the compound properties. The clearest example of such influence is the activity of lactones differing in one methyl group in their structure. In many cases methyl group presence completely changed the examined compounds properties from attractant into deterrent ones. The presented results also demonstrate the species-dependent antifeedant activity of these compounds. Most of them had various activities against *A. diaperinus* and *L. decemlineata*. Lactone activity depended also on the examined insects developmental stages. These compounds were stronger deterrents against Colorado beetle larvae in comparison with adult individuals. On the other hand the coleopterons of lesser mealworm were more sensitive than their larvae. The relationship: developmental stage-activity was confirmed by the results of other studies. According to Nawrot et al. (1986), Wawrzeńczyk et al. (1997) and Olejniczak et al. (2000) higher sensitivity to antifeedant was shown by adult individuals belonging to the same family *Tenebrionidae*, the confused flour beetle,*Tribolium confusum* Dav., than their larvae.

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POLISH SUMMARY

ANTYFIDANTNA AKTYWNOŚĆ LAKTONÓW Z UKŁADEM DI – I TRIMETYLOCYKLOHEKSANU WOBEC PLEŚNIAKOWCA LŚNIĄCEGO, ALPHITOBIUS DIAPERINUS PANZER I STONKI ZIEMNIACZANEJ, LEPTINOTARSA DECEMLINEATA SAY

Zbadano w warunkach laboratoryjnych aktywność antyfidantną bicyklicznych laktonów z układem cykloheksanu podstawionego grupami metylowymi, które otrzymano w trójetapowych syntezach ze znanych cyklicznych γ, δ-nienasyconych estrów. Testy biologiczne przeprowadzono na larwach oraz osobnikach dorosłych pleśniakowca lśniącego, *Alphitobius diaperinus* Panzer i stonki ziemniaczanej, *Leptinotarsa decemlineata* Say. Zastosowano metodę testów "z wyborem" i "bez wyboru", w których badane związki podawano owadom w postaci 0,1% roztworów alkoholowych.

Przeprowadzone badania wykazały wyraźny wpływ struktury cząsteczki na właściwości związku. W wielu przypadkach obecność dodatkowej grupy metylowej całkowicie zmieniała właściwości badanych związków, które z atraktantów stawały się deterentami. Zamiana grupy hydroksylowej na grupę estrową lub karbonylową również powodowała wzrost aktywności deterentnej laktonów. Aktywność tych związków zależna była także od gatunku i stadium rozwojowego owadów. Były one silniejszymi deterentami dla larw stonki ziemniaczanej w porównaniu z osobnikami dorosłymi, natomiast chrząszcze pleśniakowca lśniącego były bardziej wrażliwe niż ich larwy.