Original Research

The Effect of α-Methylenelactone Group on the Feeding Deterrent Activity of Natural and Synthetic Alkenes Against Colorado Potato Beetle, *Leptinotarsa decemlineata* Say

M. Szczepanik[1]*, A. Szumny[2], C. Wawrzeńczyk[2]

[1]Nicolaus Copernicus University, Institute of General and Molecular Biology,
Department of Invertebrate Zoology, Gagarina 9, 87-100 Toruń, Poland
[2]Wrocław University of Environmental and Life Sciences, Department of Chemistry,
Norwida 25, 50-375 Wrocław, Poland

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Abstract

Studies on the feeding deterrent activity of some natural, cyclic terpenes (myrcene, (+)-3-carene, (+)-limonene, (±)-camphene), synthetic alkenes (2-methyl-1-pentene, 2,4,4-trimethyl-1-pentene, 1-methylcyclohexene, 1-tetradecene) and their derivatives α-methylenelactones in choice and no-choice tests against Colorado potato beetle with potato, *Solanum tuberosum* L. leaf discs as consuming food were carried out. Deterrent indexes determined in the tests show that the strongest antifeedant to larvae and adults of *Leptinotarsa decemlineata* was α-methylenelactone obtained from 2,4,4-trimethyl-1-pentene. The structure modification of (+)-3-carene and (±)-camphene via introduction of α-methylenelactone moiety increased their deterrent activity, especially against larvae. Other starting alkenes and α-methylenelactones obtained from them were weak deterrents to both developmental stages of *L. decemlineata.*

Keywords: insect antifeedants, terpenes, lactones, Colorado potato beetle, *Leptinotarsa decemlineata*

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say, is one of the most damaging insect pests of potato, *Solanum tuberosum* L. in Europe and North America. Chemical control of this insect has become very difficult due to the rapid development of resistance of the beetle population to most insecticides, including some biological insecticides such as δ-endotoxin of *Bacillus thuringiensis* Berliner [1-5]. Applying chemical insecticides is also often harmful to beneficial organisms and the environment, which is why these problems require a search for alternative control methods. One of the solutions of these difficult problems could be to use insect feeding deterrents (antifeedants), often in combination with other methods within the integrated pest management (IPM). Antifeedants are defined as natural or synthetic chemical compounds limiting or completely inhibiting the insects′ feeding by acting on the taste organs [6]. These compounds are produced probably by all plants as secondary metabolites and play a decisive role in food-plant selection by insects because herbivores avoid plants containing these phytochemicals. Unlike conventional pesticides, antifeedants are often highly pest-specific in their action, and being closely related to natural products are highly likely to undergo biodegradation. In spite of these favorable considerations,

*e-mail: mszczep@umk.pl

antifeedants are not yet in common use except for extracts of the neem tree (*Azadirachta indica*), which are currently marketed. The reason for this is that isolation of pure, naturally occurring antifeedants is difficult and expensive, and active compounds are not available in quantities necessary for agricultural application. On the other hand, total synthesis of naturally occurring antifeedants is usually multi-step and economically unprofitable. The isolation of compounds (e.g. terpenes) occurring in appropriate amounts in plants and simple modification of their structure into highly active compounds could be a good way to obtain environmentally friendly insecticides.

Here we present a possible way to obtain active antifeedants - α-methylenelactones as a result of chemical transformation of simple compounds, mainly monoterpenes and commercially available synthetic alkenes. The feeding deterrent activity of the starting compounds and α-methylenelactones synthesized from them against larvae and adults of *Leptinotarsa decemlineata* Say was comparably studied.

The inspiration for synthesis of α-methylenelactones was the observation that α-methylenelactone moiety is an integral part of many natural products, especially the sesquiterpene lactones that exhibit a broad spectrum of biological activities. This group of compounds represents approximately 10% of all structurally elucidated natural products [7]. Their biological activity could be divided into several main types: antitumoral and cytotoxic activity, e.g. vernolepin [8], and parthenin [9], probably due to the inhibition of DNA synthesis and transcription, antimicrobial, e.g. helenalin [10], or parthenolide [11] feeding deterrent activity against pest insects, e.g. lactucine, and encelin [12]. The strong biological activity of α-methylenelactones is presumably due to a high reactivity of α-methylene group with biological nucleophiles such as thiol-containing important enzymes [13].

Materials and Methods

Chemicals

All terpenes and other alkenes were purchased from Fluka (Poznań, Poland). α-Methylenelactones were obtained in 35-50% yield via reaction of alkenes with Meldrum's acid leading to α-carboxylactones, (A) which in the next step, decarboxylation-methylenation, were transformed into final products (Fig. 1). The syntheses were carried out according to the procedure described earlier by Szumny and Wawrzeńczyk [14].

The crude products were purified by column chromatography on silica-gel with mixture of hexane-ethyl ether as an eluent. The purity of samples was determined by gas chromatography using HP-5 column. Compounds subjected to tests were above 95% purity.

Bioassays

Insects

In our experiments, the adults of the first generation of Colorado potato beetle, *L. decemlineata,* collected from an unsprayed potato field situated near Toruń, Poland, and newly ecdysed third instar larvae selected from the laboratory colony were used.

Choice and No-Choice Feeding Assays

For the feeding assays 1% alcohol solutions of the tested compounds were prepared. Leaf discs uniform in size (40 mm in diameter) were cut from potato leaves, dipped in the experimental solutions for a few seconds to assure accurate cover of the leaf surface and left to air-dry. The control leaves were immersed in alcohol only. After complete evaporation of the solvent the discs were placed in the feeding arena (Petri dishes lined with moistened filter paper) together with 6 adults or 10 newly ecdysed third – instar larvae of *L. decemlineata*. In the choice-test two treated and two control discs were placed in Petri dishes, in the no-choice test only three treated or three control discs were offered to insects. Four replicates of choice and no-choice test for each compound were conducted on each insect life stage. The dishes were transferred into a rearing chamber and kept there for 24h under a constant temperature of 24ºC and 16:8 (L:D) photoperiod. After ending the experiments the remaining uneaten area of each potato leaf disc was measured using a scanner (Microtec Scan Maker 3800) and software programmed by A. Zienkiewicz (Department of Biophysics, Nicolaus Copernicus University, Toruń). The amount of ingested food in all variants was calculated as the difference between the surfaces before and after feeding. Based on the amount of food consumed in both tests, the three deterrence coefficients – relative (R), absolute (A) and total (T) – were calculated using the formulae according to Nawrot et al. [15].

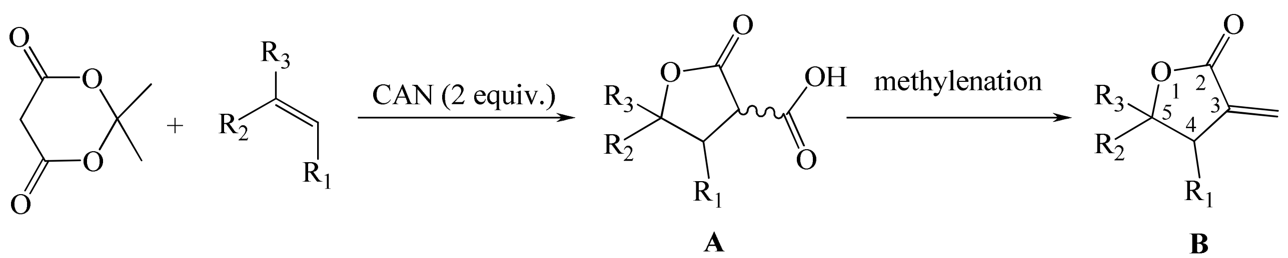


Fig. 1. Scheme of synthesis of compounds studied.

$$R = C - E/C + E \times 100 \text{ (choice test)}$$
$$A = CC - EE/CC + EE \times 100 \text{ (no-choice test)}$$

...where C and CC are the leaf area consumed in the control treatments for choice and no-choice tests, respectively; E and EE are the treated leaf area consumed in choice and no-choice tests, respectively;

The measure of the deterrent activity of the tested compounds is the total coefficient of deterrence:

$$T = A + R$$

The total of the deterrence coefficient, which ranged from -200 to +200, served as the activity index. The compounds with T values ranging from 151 to 200 are very good deterrents, those with values of 101-150 are good deterrents, and those with values of 51-100 are only medium deterrents. Compounds with T values lower than 50 are weak deterrents. Negative T values indicate that the compound has attractant properties.

In practice, the deterrent activity of the chemical compounds is significant in the no-choice situation. To estimate and compare larval and adult feeding levels in the no-choice tests, the amount of treated food consumed was expressed as a percentage of the consumption in the control according to the formula: EE/CC x 100, where EE and CC are the areas of the treated leaf and the control leaf consumed in no-choice tests, respectively. The data were show in Fig. 2.

Statistical Analysis

The deterrence coefficients were statistically analyzed by means of one-way analysis of variance ANOVA and Tukey test. The mean values of consumption in no-choice tests were compared using Student t-test.

Results

The deterrence indexes presented in Tables 1 and 2 show the diverse feeding deterrent activity of the compounds under study. Natural monoterpenes: (+)-3-carene, (+)-limonene, (±)-camphene and myrcene in most tests stimulated feeding

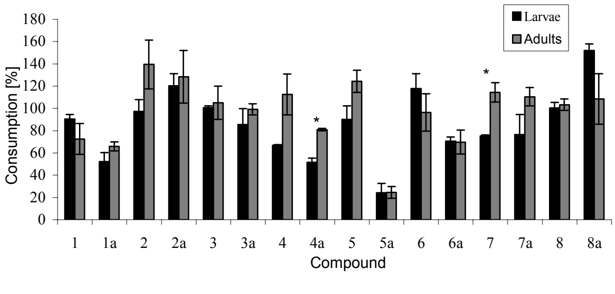
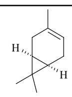
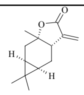
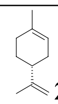
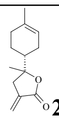
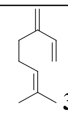
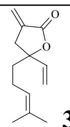
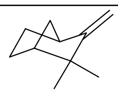
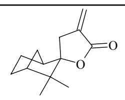


Fig. 2. Consumption of the treated food (mean ± SE) in *Leptinotarsa decemlineata* in the no-choice tests. Data are expressed as percentage of control consumption (EE/CC x 100). *P<0.05 (Student t-test).

in both development stages of the Colorado potato beetle. The only exception was (+)-3-carene, which showed weak deterrent properties towards adult individuals. The strongest attractant for both development stages was (+)-limonene. A change in structure of those hydrocarbons by introducing the α-methylenelactone group was favorable only in the case of (+)-3-carene and (±)-camphene. The derivatives of those hydrocarbons showed stronger deterrent properties compared with their precursors. Particularly pronounced changes of those properties were noticeable with the pair of compounds 1 and 1a. α-Methylenelactone obtained from (+)-3-carene considerably reduced the larvae's feeding, particularly in choice tests. The differences between relative and total coefficients were substantial (Table 1). In no-choice tests the observed increase in activity was not as high as in the choice tests. For the beetles the total deterrence coefficient T for the derivative of (+)-3-carene was also higher, but that increase was associated with the higher activity of this compound in choice tests. In no-choice tests the beetles' feeding levels in the presence of compounds 1 and 1a were very close (Fig. 2). A similar increase in deterrent activity was also characteristic for the α-methylenelactone obtained from (±)-camphene. That racemic hydrocarbon 4 showed attractant properties towards both developmental stages of the pest (Table 1). α-Methylenelactone 4a reduced the feeding level of larvae and adults in both tests. In the no-choice test the amount of food consumed by larvae relative to control decreased from 66.91 to 51.5%, while in the case of the adults that decrease was higher – 31.66% (Fig. 2). The other two natural hydrocarbons, limonene (2) and myrcene (3), showed attractant properties toward both developmental stages of *L. decemlineata*. The functionalization of those compounds did not significantly affect their deterrent properties. α-Methylenelactone 2a obtained from limonene, like limonene itself, remained an attractant toward larvae and adults. The derivative of myrcene, α-methylenelactone 3a, was an attractant towards beetles. It also showed very weak deterrent properties towards larvae (Table 1).

The synthetic alkenes 5, 6, 7 and 8 under study showed, similarly to the terpene hydrocarbons, attractant or very weak deterrent properties. The introduction of α-methylenelactone moiety to their structures change the attractant properties in deterrent activity. The highest increase in deterrent activity toward both developmental stages of the pest was observed in comparison of test results of trimethylpentene (5) and synthesized from it lactone (5a). The hydrocarbon (5), which considerably stimulated the insects´ feeding after transformation, became a good feeding deterrent toward larvae and beetles with deterrence coefficients of 159.7 and 136.0, respectively (Table 2). The area consumed in the leaves treated with α-methylenelactone 5a constituted only 25.0% of the area consumed in the control (Fig. 2). A considerable increase in activity toward adults, mainly in the choice test, was also observed in the case of introducing lactone moiety into 2-methyl-1-pentene (6). The two next synthetic alkenes, 1-methylcyclohexene (7) and tetradecene (8), however, lost their attractant properties and became very weak deterrents against adults.

Table 1. Feeding deterrent activity of natural terpenes and their derivatives with α-methylenelactone group against *L. decemlineata*.

Compound	Deterrence coefficients[a]					
	Larvae			Adults		
	Absolute	Relative	Total	Absolute	Relative	Total
 **1**	5.10 defgh	- 12.50 cdef	- 7.40 def	17.44 bc	4.20 d	21.64 c
 **1a**	31.58 bc	86.40 a	117.98 b	20.70 b	36.82 bc	57.52 b
 **2**	1.91 efgh	- 34.27 efg	- 32.36 fgh	- 15.13 e	- 9.87 d	- 24.91 d
 **2a**	- 8.71 hi	- 40.00 fgh	- 48.71 gh	- 10.38 e	8.31 cd	-2.05 cd
 **3**	- 0.42 fgh	- 14.87 cdef	- 15.29 efg	- 1.42 de	- 4.76 d	- 6.18 cd
 **3a**	9.17 defg	- 4.44 bcde	4.73 def	0.57 cde	- 5.03 d	- 4.46 cd
 **4**	19.79 bcd	- 26.03 efg	- 6.22 def	- 4.42 de	- 6.75 d	- 11.17 cd
 **4a**	32.18 b	17.59 bc	49.77 c	10.52 bcd	47.97 ab	58.49 b

[a]Values are the means of the four replications.
Values within a column followed by the same letter are not significantly different at p<0.05 (Tukey test).

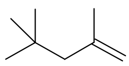
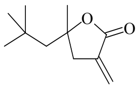
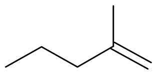
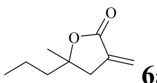
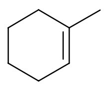
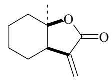
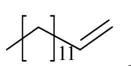
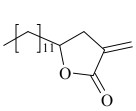
They did not have any major effect on the adults´ feeding compared with control (Fig. 2). The tests showed that α-methylenelactones 6a, 7a and 8a were more attractive to larvae than the substrates from which they had been synthesized. A particularly strong increase in the larvae´s feeding level was observed for lactone 8a (Fig. 2).

Discussion

The results presented above point out that the introduction of the lactone ring with the α-methylene group at C-3 into the structure of natural or synthetic alkenes and cycloalkenes affected the activity of the compounds thus obtained in different ways. Only one of the eight compounds with modified structure showed strong deterrent properties toward both developmental stages of the Colorado potato beetle. The strongest reduction of both larvae and adult feeding was observed in the presence of α-methylenelactone 5a being derivative trimethylpentene (5). The high values of deterrence coefficients obtained for that compound in both tests exclude the occurrence of habituation. These values also suggest deterrent modes of action of compounds rather than toxic mode of action. Significant suppression of feeding in the choice and no-choice assays but only low discrimination between treated and control food in the choice tests suggest that compounds operate as antifeedants with a post-ingestive toxic mode of action [16]. In these cases the relative coefficient is much lower than the absolute coefficient. This low value is the result of low consumption of control food by poisoned insects. In our study we did not observe a like situation.

An important factor contributing to the high activity of that compound can be the presence of methyl groups at C-2´. Lactones containing accessory methyl groups, both in the lactone ring and in the alkane chain joined to the lactone ring show as a rule a higher deterrent activity toward various insect species compared with lactones with less number

Table 2. Feeding deterrent activity of synthetic alkenes and their derivatives with α- methylenelactone group against *L. decemlineata*.

Compound	Deterrence coefficients[a]					
	Larvae			Adults		
	Absolute	Relative	Total	Absolute	Relative	Total
 **5**	6.05 defgh	- 55.88 gh	- 49.83 gh	- 10.52 e	- 10.95 d	- 21.47 d
 **5a**	62.57 a	97.13 a	159.77 a	61.04 a	74.97 a	136.01 a
 **6**	- 7.48 ghi	21.98 b	14.50 cde	3.41 b	6.73 d	10.14 c
 **6a**	17.37 bcde	- 40.83 fgh	- 23.46 fgh	18.72 b	56.39 ab	75.11 b
 **7**	14.06 cdef	9.53 bcd	23.59 cd	- 6.40 de	- 11.38 d	- 17.78 d
 **7a**	15.87 bcdef	- 25.49 efg	- 9.62 def	- 4.67 de	13.98 cd	9.31 cd
 **8**	- 0.06 efgh	- 21.89 def	- 21.95 efgh	- 1.49 de	- 5.59 d	- 7.08 cd
 **8a**	- 20.58 i	- 37.73 fgh	- 58.31 h	- 2.04 de	4.39 d	2.35 cd

[a]Values are the means of the four replications.
Values within a column followed by the same letter are not significantly different at p<0.05 (Tukey test).

of methyl groups [17-19]. That is confirmed by the low activity of the lactone derivative of methylepentene (6a) compared with 5a. It seems interesting that the most active compounds 1a and 5a, are the only ones having two or three *gem*-dimethyl groups four carbon atoms away from the α-methylene group and a methyl group at C-5 of the γ-lactone ring.

As demonstrated by studies carried out by Dancewicz et al. [20], lactone 5a is characterized by a diversified deterrent activity toward different species of aphids. It is also a very good antifeedant against the pea aphid, *Acyrthosiphon pisum*. (Harris) but shows no activity at all toward the green peach aphid, *Myzus persicae* (Sulzer). Our earlier studies have also demonstrated that functionalization of trimethylpentene intensifies the deterrent properties of its derivative, α-methylenelactone 5a against the lesser mealworm, *Alphitobius diaperinus* Panzer. That increase in activity, however, is not as significant as in the case of the Colorado potato beetle [21].

A substantial increase in activity was also observed as a result of chemical modification of (+)-3-carene and (±)-camphene. Colorado potato beetle larvae consumed about 50% less food treated with those compounds in no-choice tests compared with the control (Fig. 2). The lactone derivative of (+)-3-carene (1a) is also a very good feeding deterrent against *M. persicae* [22]. Studies carried out by Lochyński [22] show that spirolactones with the carene system considerably reduce feeding of *Trogoderma granarium* Ev. larvae.

Natural terpenes can then be a rich source of substrates for the synthesis of active antifeedants. That is confirmed by the high deterrent activity of lactone derivatives of monocyclic terpenes, pulegone and isopulegol as well as of diterpene derivatives of neo-clerodanes isolated from *Teucrium chamaedrys* L. against the Colorado potato beetle [16, 24]. The diversified activity of the compounds studied toward different insect species is associated with the high specificity characterizing food deterrents. Numerous studies show that chemical compounds that are a strong deterrent against one species can be neutral toward another one [6, 15, 23]. Those differences in activity among species also show that the dependence structure-activity cannot be generalized, and the deterrent activity of a chemical compound depends on the whole structure of the molecule. Our studies on the effect of the α-methylenelactone group on the deterrent activity of natural or synthetic alkenes demonstrated that the introduction of that group into their structure only in some cases has a positive effect on the reduction of the Colorado potato beetle´s feeding level.

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