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Synthesis of β-damascone derivatives with a lactone ring and their feeding deterrent activity against aphids and lesser mealworms

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Starting from β-damascone, six new lactones were obtained. The Claisen–Johnson rearrangement of allylic alcohol and halolactonization of γ,δ-unsaturated acid were the key steps of the presented synthesis. The structures of the new derivatives were determined by spectroscopic data. The antifeedant activity of β-damascone towards two insect species with different feeding habits and food preferences, *i.e.*, the peach-potato aphid *Myzus persicae* (Sulz.) and lesser mealworm, *Alphitobius diaperinus* Panzer, was studied, as well as the biological consequences of structural modification of the starting substrate. The successive structural modifications of β-damascone that resulted in the greatest antifeedant activity towards *M. persicae* were the incorporation of a lactone moiety and concomitant presence of bromine in the side chain. All β-damascone derivatives with a lactone moiety deterred the feeding of adults and larvae of *A. diaperinus*. Halo-δ-lactones were more active than halo-γ-lactones, and *A. diaperinus* adults were more sensitive to the compounds studied than larvae.

Introduction

β-Damascone (**1**) belongs to the group of norisoprenoids defined as rose ketones, because they were discovered in the 1960s during a quest to identify the characteristic smell of Bulgarian rose oil.[1,2] This compound has been identified not only as a component of rose oil but it also creates tea aroma and occurs in some types of tobacco, wine, and whiskey.[3–7] β-Damascone (**1**) has a pleasant blackcurrant/plum note and is widely used in perfume compositions. Apart from this commercial importance, β-damascone has been shown to have a variety of biological activities. For example, it was discovered that this compound possesses cancer chemopreventive potential[8] and appeared to be toxic towards three species of mosquitoes, *Aedes aegypti* L., *Aedes albopictus* (Skuse), and *Anopheles quadrimaculatus* Say, the housefly, *Musca domestica* L., the stable fly, *Stomoxys calcitrans* L., and the sand fly, *Lutzomyia shannoni* (Dyar).[9–11] Apart from that, information on the effect of β-damascone (**1**) on arthropods and their behavior is very scarce. Our interests in the synthesis of isoprenoid lactones with a damascone skeleton were motivated by the search for new biologically active compounds that can reduce the population of insect pests and can be useful in the protection of crops. Insects are responsive to many plant lower terpenoids and their synthetic derivatives. For example, the repellent properties of linalool and α-terpineol to the peach-potato aphid *Myzus persicae* were reported by Hori,[12,13] and (*S*)-limonene restrained phloem sap ingestion and had other negative effects on the behaviour of this aphid.[14] Citral, linalool, (*S*)-limonene, α-ionone, and camphene reduced the total and mean probing time of aphids and their settling on leaves.[15] Following these studies, several analogues of natural terpenoids, including the lactones, have been synthetized and their biological activity examined. We discovered active feeding deterrents among lactones derived from natural isoprenoids: pulegone,[16,17] piperitone,[18] and farnezol.[19] It appeared that the antifeedant activity of synthetic analogues was highly enantiospecific and depended on various substituents and functional groups.[14,18,20] Chemical transformation of the piperitone molecule by the introduction of a lactone moiety and a halogen atom strongly increased its antifeedant properties against *M. persicae* and the lesser mealworm *Alphitobius diaperinus*.[18,21]

In the present study, we concentrated on two insect pests with different feeding habits and food preferences, *i.e.*, the peach-potato aphid *Myzus persicae* (Sulz.) (Hemiptera: Aphididae) and the lesser mealworm, *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae). Aphids are responsible for at least 2% of all crop losses attributed to insect feeding.[22] Moreover, the indirect damage caused by aphids due to virus transmission exceeds their direct impact on crops.[23] *M. persicae* alone can infest plants of over 40 different families and it is able to transmit over 100 plant viruses.[24] At the same time, it developed

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clones that were resistant to one or more insecticides.[23] The lesser mealworm is a cosmopolitan pest inhabiting chicken and broiler houses in vast numbers. In poultry houses, the beetles consume manure, spilled feed, dead birds, and other organic materials.[25,26] At the same time, the adults and larvae have been reported as potential vectors for the transfer of *Campylobacter jejuni* and *Salmonella enterica* between successive broiler flocks[27] and other pathogens.[28]

Chemicals targeting insect taste receptors are considered potential bioinsecticides to protect crops.[29] Aphids possess piercing–sucking mouthparts, and they feed on the phloem sap of living plants, while the lesser mealworm is a general stored-products pest that feeds using chewing mouthparts. Moreover, aphids lack external taste receptors, which makes the pre-ingestional rejection or acceptance of the food impossible.[30] In contrast, the lesser mealworm's gustatory receptors are located on their mouthparts, which allows a preingestional response to food quality. The different food preferences, modes of food uptake, and preingestional evaluation ability of food chemistry may determine different behavioural responses of these insects. In the present study, we present the synthesis and structural modifications of β-damascone (**1**)-derived compounds, including the lactones, as well as their biological activities expressed as antifeedant properties that affect *M. persicae* and *A. diaperinus*.

Results and discussion

Chemical synthesis

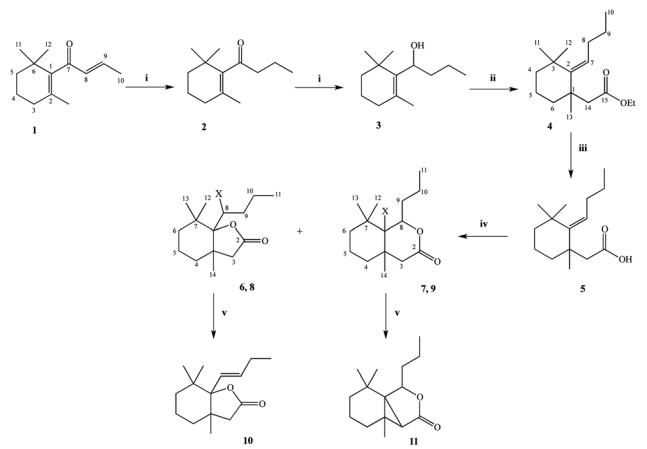
Six new isoprenoid lactones **6–11** were obtained in a six-step synthesis (Scheme 1) from the naturally occurring ketone, β-damascone (**1**). The damascone carbon skeleton consists of a trimethyl substituted cyclohexane ring with a four-carbon α,β-unsaturated ketone side chain. The starting material was commercially available β-damascone (**1**).

The first step of the synthesis was the reduction of the double bond in the side chain of compound (**1**) with lithium aluminium hydride according to standard procedure. The reaction course and purity of the product were monitored by GC on a capillary column (HP-5). Known dihydro-β-damascone (**2**), which was identified in tobacco[31] and previously synthesized by Mori *et al.* in a two-step chemical synthesis from trimethylcyclohexanone,[32] was obtained in high 99% yield. The structure of **2** was confirmed by the comparison of its spectral data with previously published data.

Ketone (**2**) was next transformed in high 89% yield into the corresponding allylic alcohol, dihydro-β-damascol (**3**), by treatment with $LiAlH_4$. Preparation of dihydro-β-damascol (**3**) was patented for the first time in 1973.[33] The crude alcohol (**3**), without further purification (97% purity according to the GC analysis), was subjected to the orthoacetate modification of the Johnson–Claisen rearrangement.[34] The mechanism involves the esterification of alcohol followed by subsequent elimination of ethanol to form ketene acetal. The latter is rearranged to the γ,δ-unsaturated esters in a [3.3] sigmatropic shift during heating. In our case, the reaction afforded the new compound ethyl 2-(2-butylidene-1,3,3-trimethylcyclohexyl)-acetate (**4**) in 98% yield. An absorption band at 1743 cm^{-1} of the carbonyl group and characteristic quartet in the ^1H NMR spectrum from two protons at 4.05 ppm confirmed the presence of the carboethoxy group in the ester (**4**).

The ester (**4**) was subsequently hydrolyzed in ethanolic KOH solution to the corresponding acid (**5**) in 85% yield. This compound has not been obtained before. In the next step, acid (**5**) was subjected to halolactonization reactions, giving access to bromo- and chlorolactones (Scheme 1). The bromolactonization of acid (**5**) was carried out with *N*-bromosuccinimide (NBS) in tetrahydrofuran, affording a mixture of two new products in a ratio of 45% : 55% according to GC. We separated them using column chromatography and established their structure on the basis of spectroscopic data. The products of cyclization were δ-bromo-γ-lactone (**6**) (minor, 17% yield) and γ-bromo-δ-lactone (**7**) (major, 27% yield). The reaction of γ,δ-unsaturated acid (**5**) with *N*-chloro-succinimide (NCS) was carried out to obtain chlorolactones. Using chloride as an electrophilic agent, we observed a similar situation as in the process of bromolactonization. Two new lactones were formed as products of cyclization – δ-chloro-γ-lactone (**8**) and γ-chloro-δ-lactone (**9**). According to GC, the mixture consists of 41% of γ-lactone (**8**) and 59% δ-lactone (**9**), which were obtained with 18 and 17% yield respectively. The doublet of doublets protons H-8 in γ-lactone (4.24 ppm) and δ-lactone (4.74 ppm) were found in the ^1H NMR spectra and also proved that intermolecular cyclization occurred.

The dehydro-halogenation reaction of δ-halo-γ-lactones (**6**),(**8**) and γ-halo-δ-lactone (**7**),(**9**) with 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) gave new cyclic lactones (**10**),(**11**) in good yields (47–57%) as the only products (Scheme 1). The formation of unsaturated lactone (**10**) as the only product of the dehydrohalogenation of lactones (**6**),(**8**) is a result of E2 elimination. In the IR spectrum of unsaturated lactone (**10**), absorption bands for stretching vibrations for the carbonyl group at 1778 cm^{-1} were observed. The location of a double bond in the side chain of bicyclic lactone (**10**) between C-8 and C-9 was



Scheme 1 Synthesis of lactones from β-damascone. Reagents (i) $LiAlH_4$; (ii) $CH_3C(OEt)_3$, CH_3COOH, 138 °C; (iii) 1. KOH, EtOH, 2. HCl; (iv) NBS/NCS, THF; (v) DBU.

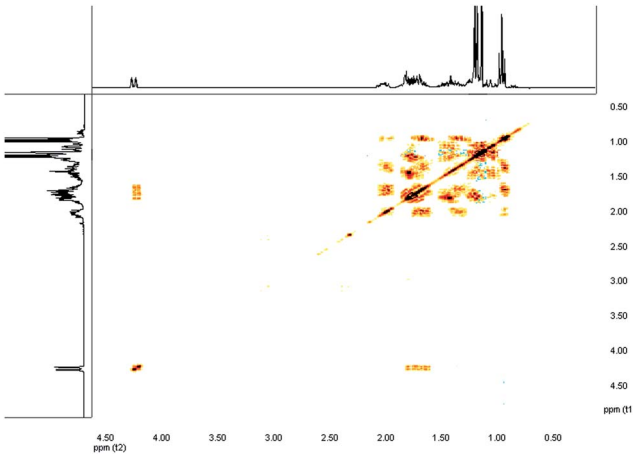


Fig. 1 COSY spectrum of lactone **11**.

confirmed by the signals from olefin protons in the ^{1}H NMR spectrum. The doublets of triplets from H-8 and H-9 protons, at 5.60 ppm and 5.77 ppm, respectively, indicate the presence of double bonds. Moreover, the coupling constant $J = 15.5$ Hz between these protons proved their *trans* orientation. The shift of the absorption band of the C=O band in the lactone moiety from the range of 1716–1733 cm^{-1} for δ-lactones to a higher frequency of 1762 cm^{-1} indicates that in the case of product (**11**), the conversion of the δ-lactone ring into γ-lactone occurred. In the ^{1}H NMR spectrum, the signal from proton H-8 appeared as a doublet of doublets. It was coupled with protons at C-9 at 4.25 ppm with coupling constant $J = 10.9$ and 2.2 Hz. These couplings are clearly observed in the 2D COSY spectrum (Fig. 1). This type of cyclization is the result of E2 elimination. The lack of signal from one of the CH_2-3 protons as well as any signals characteristic for olefinic protons and finally the shape of the signal from the H-8 proton indicated the presence of the cyclopropane ring. Its formation can be explained by expulsion of the proton at C-3 by DBU, leading to carbanion stabilization by the enolate anion. The second step, cyclization, is the result of the attack of the electron pair as a nucleophile on the C-7a carbon atom with simultaneous removal of halogen.[35]

Biological studies

Feeding deterrent activity against *Myzus persicae*

The potency and mechanism of antifeedant activity of the compounds studied varied depending on the compound structure and the duration of exposure of aphids to β-damascone (**1**) and its derivatives. On control leaves, aphids did not leave the substrate (=leaf) during the no-choice 15 minute experiment, and the total probing time was approximately 70% of the time spent on the leaf. The aphids started probing immediately after exposure (the delay was 14 seconds on average), and the average probe was relatively long, *i.e.*, approximately 3.5 minutes (Table 1). In the choice-test, there were no significant differences in aphid preferences to settle on either the untreated or the control (*i.e.*, ethanol-treated) leaf, irrespective of the exposure time (Fig. 2). β-Damascone (**1**) appeared to be a weak attractant during the initial contact with the treated leaves in the no-choice test, as it was observed that although the first probe was delayed in comparison to the control, further probing was rarely interrupted. There were twice as few probes, and the probes were twice as long in comparison to aphids on control leaves (Table 1). However, this effect did not translate into aphid preferences during settling, as no significant differences in the number of aphids on treated

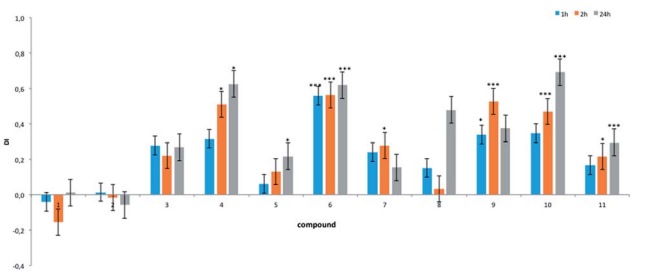


Fig. 2 The effect of β-damascone and its derivatives on settling preferences of *Myzus persicae* in the choice test. The data are expressed as values of indices of deterrence (DI). The standard error is indicated on the bar. *P < 0.05; ***P < 0.001; (Student's t-test).

Table 1 Modification of *Myzus persicae* behaviour by β-damascone and its derivatives in nochoice tests[a]

Compound	Time spent on the leaf (s)	Total probing time (s)	Time to first probe (s)	Number of probes	Mean probing time (s)
Control	900.0 ± 0.0	608.6 ± 51.8	13.7 ± 6.3	5.4 ± 0.9	209.8 ± 69.7
1	892.5 ± 7.5	640.6 ± 44.1	123.1 ± 37.2*	2.4 ± 0.5*	435.7 ± 90.1*
2	844.5 ± 47.9	575.8 ± 79.3	51.5 ± 28.5	6.2 ± 1.3	194.8 ± 65.9
3	809.5 ± 51.3	457.3 ± 68.0	14.8 ± 3.0	7.7 ± 0.9	77.7 ± 22.5*
4	867.2 ± 30.0	467.5 ± 78.7	154.2 ± 50.0*	4.1 ± 0.9	204.2 ± 66.6
5	892.8 ± 7.2	585.8 ± 68.4	12.5 ± 5.0	5.8 ± 1.0	189.6 ± 76.7
6	539.0 ± 117.0*	256.6 ± 80.8*	104.7 ± 64.4	4.5 ± 1.0	43.9 ± 15.8*
7	900.0 ± 0.0	707.5 ± 36.4	25.7 ± 7.8	4.3 ± 0.7	249.3 ± 70.8
8	799.8 ± 68.6	545.6 ± 76.8	27.4 ± 7.2	4.3 ± 0.8	264.5 ± 93.2
9	887.2 ± 9.1	635.4 ± 68.0	22.7 ± 6.1	5.4 ± 1.0	208.9 ± 47.5
10	888.6 ± 10.4	640.1 ± 102.1	26.9 ± 11.6	3.3 ± 0.6	335.8 ± 102.7

[a] Values represent means of $n = 12$ replicates ±SE. An asterisk within a column denotes statistically significant differences in relation to control ($P < 0.05$).

and untreated leaves were found during the 24 hour experiment (Fig. 2).

The compound synthesized in the first step in the β-damascone modification, the dihydro-β-damascone (**2**), did not evoke any changes in aphid behaviour during initial contact with the treated leaves (no-choice test) as well as in the long-term experiment showing aphid preferences during settling (choice-test). Similar results were found after the application of dihydro-β-damascol (**3**), except that the probes were significantly four times shorter in comparison to the control. However, aphids did not restrain from probing – the total probing time was comparable to the control, which finally caused no discrimination between treated and untreated leaves during the 24 hour settling experiment. The β-damascone-derived ethyl 2-(2-butylidene-1,3,3-trimethylcyclohexyl)-acetate (**4**) did not cause significant differences in aphid responses to plants during the initial 15 minutes after exposure, except for considerable delay before the first probe (Table 1).

From the second hour after exposure onwards, the aphids showed a significant preference for untreated leaves. The indices of deterrence reached relatively high values of 0.5 and 0.6 after 2 and 24 hours, respectively (Fig. 2). Further molecular modification by the synthesis of 2-(2-butylidene-1,3,3-trimethylcyclohexyl) acetic acid (**5**) did not cause any change in the biological activity towards *M. persicae* initially, but after 24 hour exposure, aphid settling was significantly hindered (ID = 0.2). In contrast, the incorporation of the lactone moiety and the halogen atoms into the molecule had significant impact on aphid behaviour. However, the four halolactones obtained (**6–9**) differed in activity depending on the size of the lactone ring and the kind and position of the halogen atoms in the molecule. In the behavioural no-choice test, the application of δ-bromo-γ-lactone (**6**) caused a significant decrease in the total time spent on the treated leaves and total and mean probing time, while the γ-bromo-δ-lactone (**7**) did not (Table 1). Consequently, the exposure to δ-bromo-γ-lactone (**6**) resulted in the avoidance of treated leaves by freely moving aphids in the choice-test, from the beginning until the end of the experiment in contrast to γ-bromo-δ-lactone (**7**) (Fig. 2). The replacement of the bromine atom by a chlorine atom to synthesize the respective δ-chloro-γ-lactone (**8**) and γ-chloro-δ-lactone (**9**) did not cause changes in aphid behaviour during initial contact with the studied compounds (Table 1). However, long-term exposure to γ-chloro-δ-lactone (**9**) significantly impeded aphid settling (Fig. 2). Similar effects on aphid settling and behaviour were caused by unsaturated bicyclic γ-lactone (**10**) and bicyclic δ-lactone (**11**): aphid initial responses on treated leaves did not differ from those on control leaves in the no-choice experiment (**Table 1**) but the settling of aphids was significantly impeded after longer exposure times. The settling-deterrent effect of lactones (**10**) and (**11**) increased in potency over the course of time, with indices of deterrence reaching 0.7 and 0.3 after 24 hours for lactones (**10**) and (**11**), respectively (Fig. 2).

In summary, the antifeedant activity of β-damascone-derived compounds manifested as both immediate and delayed effects on aphid behaviour. The immediate effects during initial contacts with the allelochemical were expressed as the reduction of total time spent on the leaf, total probing time (δ-bromo-γ-lactone (**6**)), delayed time to the first probe (β-damascone (**1**) and ester (**4**)), and the reduction of mean probing time (dihydro-β-damascol (**3**) and δ-bromo-γ-lactone (**6**)). Considering average durations of probes (0.7–1.2 minutes), aphid stylets on (**3**)- and (**6**)-treated leaves did not penetrate beyond the epidermis.[36] The delayed effects were expressed after longer times of aphid exposure to the compounds studied. The strongest and the most durable effects on aphid settling were evoked by the application of ethyl 2-(2-butylidene-1,3,3-trimethyl-cyclohexyl)-acetate (**4**), δ-bromo-γ-lactone (**6**), and unsaturated bicyclic γ-lactone (**10**). Moreover, the deterrent effect increased in potency over the course of time. With the exception of δ-bromo-γ-lactone (**6**), the effects of ester (**4**) and lactone (**10**) were most likely only post-ingestional, because the impediment to settling occurred later than two hours after exposure. Partial pre-ingestional and ingestional/post-ingestional deterrent activity was observed with δ-bromo-γ-lactone (**6**), as the aphids were discouraged from probing immediately after they had gained access to plants but the probing was not entirely eliminated, which most likely allowed the consumption of plant sap.

Feeding deterrent activity against *Alphitobius diaperinus*

The compounds studied exhibited varying antifeedant activity, which depended on the structure of the compound and the developmental stage of the lesser mealworm. Starting β-damascone (**1**) was a moderate feeding deterrent for both developmental stages, especially in the no-choice test. The food consumed by the larvae and adults in this test represented 59.26 and 51.5% of the consumption in the control, respectively (Fig. 3). The introduction of a lactone moiety into a β-damascone molecule changed its antifeedant properties.

Unsaturated γ-lactone (**10**) and bicyclic δ-lactone (**11**) were excellent feeding deterrents, but only against adults. In bioassays with larvae, strong activity in the choice test was observed. Unfortunately, in the no-choice tests, larvae intensively ate the treated food, as in the case of unsaturated lactone (**10**) (Table 2).

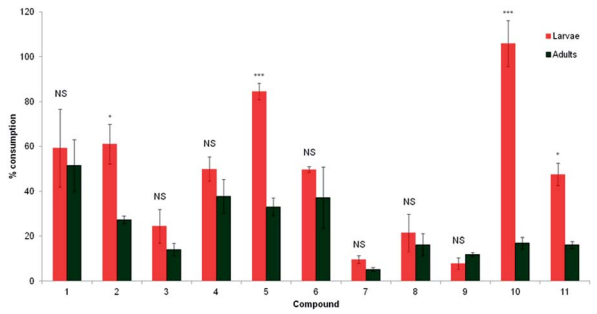


Fig. 3 The effect of β-damascone and its derivatives on the feeding of *Alphitobius diaperinus* in the no-choice test. The data are expressed as percentages of control consumption. The standard error is indicated on the bar. *$P < 0.05$; ***$P < 0.001$; NS: not significant; (Student's *t*-test).

Table 2 Feeding deterrent activity of the studied compounds in choice and no-choice tests against *A. diaperinus*

	Deterrence coefficients ± SE[a]					
	Larvae			Adults		
Compound	*A*	*R*	*T*	*A*	*R*	*T*
Dose	**1%**					
1	30.70 ± 15.94 abc	55.59 ± 3.41 a	86.30 ± 17.37 a	34.74 ± 12.00 a	56.94 ± 4.94 ab	91.68 ± 10.52 ab
2	25.30 ± 6.80 ab	70.69 ± 4.46 b	95.99 ± 9.24 a	57.22 ± 2.05 ab	63.20 ± 5.93 ab	120.42 ± 6.68 abc
3	62.41 ± 9.69 c	76.71 ± 3.60 bc	139.12 ± 6.4 ab	75.47 ± 4.12 b	59.34 ± 9.29 ab	134.81 ± 12.81 bcd
4	33.90 ± 1.20 abc	81.74 ± 4.55 bcde	115.64 ± 3.32 ab	46.53 ± 15.52 a	65.59 ± 11.02 b	112.13 ± 16.07 abc
5	8.47 ± 4.85 ab	90.42 ± 1.88 cdef	98.89 ± 3.84 a	50.74 ± 7.85 a	81.05 ± 17.72 b	131.79 ± 4.59 bcd
6	33.58 ± 2.69 c	77.80 ± 3.11 bcd	111.38 ± 3.67 ab	50.34 ± 1.63 a	23.20 ± 0.30 a	73.54 ± 20.70 a
7	82.45 ± 11.50 d	80.19 ± 1.17 bcde	162.64 ± 1.83 b	90.13 ± 7.63 b	91.05 ± 1.38 b	181.17 ± 1.64 d
8	67.04 ± 4.36 c	97.77 ± 0.53 f	164.81 ± 10.56 b	72.94 ± 1.29 b	89.48 ± 10.36 b	162.42 ± 8.72 cd
9	85.71 ± 5.30 d	98.29 ± 2.96 f	184 ± 4.29 b	78.70 ± 3.82 b	64.69 ± 8.23 b	143.38 ± 10.34 bcd
10	-2.08 ± 4.74 a	91.43 ± 1.07 def	89.35 ± 7.42 a	71.24 ± 2.43 b	77.45 ± 2.60 b	148.69 ± 9.58 cd
11	36.01 ± 2.07 b	93.77 ± 2.77 ef	129.79 ± 5.21 ab	72.24 ± 4.60 b	81.27 ± 4.13 b	153.5 ± 2.87 cd
Dose	**0.5%**					
7	29.78 ± 14.53 a	84.61 ± 4.97 b	114.39 ± 16.06 ab	82.45 ± 8.84 ab	66.95 ± 2.44 a	149.40 ± 11.06 a
8	29.99 ± 6.83 a	84.22 ± 6.03 ab	114.21 ± 8.75 a	85.53 ± 1.02 b	61.61 ± 8.90 a	147.14 ± 9.46 a
9	57.12 ± 2.47 a	65.62 ± 11.96 a	122.74 ± 12.22 b	64.28 ± 3.10 a	63.24 ± 8.86 a	127.53 ± 9.43 a
Dose	**0.1%**					
7	NT	NT	NT	51.91 ± 10.71 a	50.67 ± 14.70 a	102.58 ± 20.2 a
8	NT	NT	NT	65.71 ± 24.34 a	31.99 ± 8.74 a	97.70 ± 27.43 a
9	NT	NT	NT	47.56 ± 5.07 a	62.65 ± 14.87 a	110.2 ± 17.46 a

[a] Values are the means of the four replicates, each set up with ten larvae or adults ($n = 40$). *A*: absolute coefficients; *R*: relative coefficients; *T*: total coefficients. NT: Not tested. Means followed by the same letters within each column and for each concentration are not significantly different one-way ANOVA and Tukey's test ($P < 0.05$).

A significant decrease in the feeding of both developmental stages of *A. diaperinus* in the presence of halolactones was observed. The bromolactone activity was related to the size of the lactone ring. A much stronger antifeedant for both developmental stages of the lesser mealworm was γ-bromo-δ-lactone (**7**) in comparison with δ-bromo-γ-lactone (**6**). The bromolactone with the larger ring was the most potent antifeedant for adults among the studied compounds, with only 5.22% consumption compared with the control. Its activity is comparable with that of the most active known antifeedant, azadirachtin.[17] This compound also strongly reduced the larval feeding in relation to the control, which was 9.69% in the no-choice test (Fig. 3). When a chlorine atom replaces a bromine atom, δ-chloro-γ-lactone (**8**) is obtained, which results in an increase of activity. Compared with δ-bromo-γ-lactone, δ-chloro-γ-lactone with a smaller ring reduced larval feeding by a factor of three and adult feeding by a factor of two (Fig. 3). Generally, chlorolactones (**8**, **9**) strongly reduced insect feeding, but for larvae, the best antifeedant was the chlorolactone with a larger ring. Its activity was similar to the activity of azadirachtin. In the case of adults, this compound (**9**) was somewhat weaker as a feeding deterrent in comparison with δ-chloro-γ-lactone (**8**), but these differences were not significant, especially in the no-choice test (Table 2). Considering the results of the no-choice tests, the strongest antifeedants for both developmental stages were halolactones with a larger ring (Fig. 3). These particularly active compounds, *i.e.*, γ-bromo-δ-lactone (**7**) and both chlorolactones (**8**, **9**), were also very strong antifeedants against adults at the lower dose of 0.5%. For larvae, their activity was poor except for chlorolactone (**9**) in the no-choice test. These compounds used as 0.1% solutions also affected the feeding of adults. According to the classification of Nawrot *et al.*, they can be considered as good antifeedants.[37] Our experiments show that the halogen atoms are responsible for the high antifeedant activity of lactone derivatives of β-damascone (**1**).

A dramatic reduction of consumption of food treated with halolactones obtained from piperitone was also observed. In the presence of the above-mentioned compounds, the food consumed by lesser mealworm adults in the no-choice test represented only 2.16–2.46% of the consumption of the control.[21] Halogen atoms also modify the antifeedant activity of the other monoterpenes. For instance, the number and kind of halogen (Cl or Br) substituents in the cyclohexane ring of cyclic monoterpenes isolated from *Plocamium cartilagineum* significantly affect the activity of the obtained derivatives towards the chrysomelid, *Leptinotarsa decemlineata* L. and two aphid species, *Myzus persicae* Sulz and *Ropalosiphum padi* L.[38] The menthol derivatives with halogen substituents show a much stronger activity against mosquitoes than the starting product.[39] Among intermediate products of synthesis of lactones from β-damascone (**1**), a good antifeedant was dihydro-β-damascol (**3**),

which reduced feeding in the no-choice tests by 75.52% for larvae and 85.83% for adults (Fig. 3). Adult feeding reduction of over 60% was also observed in the presence of the ester (**4**) and acid (**5**). The results showed a significantly higher sensory sensitivity of *A. diaperinus* adults in comparison with larvae. Taking into account the total deterrence coefficients among the 11 compounds, only bromolactone (**6**) was a clearly weaker deterrent for adults (Table 2).

Conclusion

The deterrent activity of individual β-damascone derivatives varied in potency, time of expression, and duration of effect, depending on the spatial structure of the lactone and species/developmental stage of the insect.

The most successive structural modifications of β-damascone in terms of antifeedant activity towards *M. persicae* were the incorporation of a lactone moiety and concomitant presence of bromine in the side chain. δ-Bromo-γ-lactone (**6**) was the most potent antifeedant at pre-, post- and intraingestional levels. Ester (**4**) and unsaturated γ-lactone (**10**) were active at postingestional levels by impeding aphid settling after longer exposure times.

All β-damascone derivatives with a lactone moiety deterred the feeding of adults and larvae of *A. diaperinus*. The potency of the activity was related to the size of the ring and the incorporation of chlorine or bromine into the molecule. Generally, halo-δ-lactones were more active than halo-γ-lactones, and the adults were more sensitive to the compounds studied than larvae. Nevertheless, the strongest antifeedants for both developmental stages of the lesser mealworm were γ-bromo-δ-lactone (**7**), δ-chloro-γ-lactone (**8**), and γ-chloro-δ-lactone (**9**).

Experimental section

Reagents

β-Damascone (90% purity), *N*-bromosuccinimide (99% purity), and *N*-chlorosuccinimide (98% purity) were purchased from Aldrich; triethylorthoacetate was purchased from Fluka.

General procedures

Analytical TLC. Analytical TLC was performed on silica gel (Kieselgel 60 F_{254}, Merck) with a mixture of hexane, acetone, and diethyl ether in various ratios as developing systems. Compounds were detected by spraying the plates with a solution of $Ce(SO_4)_2$ (1 g), $H_3[P(Mo_3O_{10})_4]$ (2 g) in 10% H_2SO_4, followed by heating to 120–200 °C.

Column chromatography. Column chromatography was performed on silica gel (Kiesel gel 60, 230–400 mesh ASTM, Merck) with a mixture of hexane, acetone, and diethyl ether (in various ratios) as eluents.

Gas chromatography. Gas chromatography was performed on an Agilent Technologies 6890N Network GC instrument equipped with autosampler, split injection (20 : 1) and FID detector using an HP-5 column (30 m × 0.32 mm × 0.25 μm) with hydrogen as the carrier gas. The temperature programme was as follows: injector 250 °C, detector (FID) 250 °C, column temperature: 100 °C, 100–300 °C (rate 30° min^{-1}), 300 °C (hold 2 min).

^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC and ^{1}H–^{1}H COSY. ^{1}H NMR, ^{13}C NMR, DEPT 135, HMQC and ^{1}H–^{1}H COSY spectra were recorded in $CDCl_3$ solutions on a Brüker Avance AMX 300 spectrometer.

IR spectra. IR spectra were determined using a Mattson IR 300 Thermo-Nicolet spectrophotometer with KBr pellets or as neat.

Melting points. Melting points (uncorrected) were determined on a Boetius apparatus.

The indexes of refraction. The indexes of refraction were measured on a Carl Zeiss Jena refractometer.

Chemistry

Synthesis and separation of compounds

Synthesis of dihydro-β-damascone (2). A solution of β-damascone **1** (4 g, 20.8 mmol) in anhydrous diethyl ether (10 mL) was added dropwise to the stirred solution of $LiAlH_4$ (0.4 g) in dry diethyl ether (20 mL). After 5 h (GC, TLC) water was added, and the product was extracted with diethyl ether. The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and filtered. The solvent was evaporated *in vacuo*, and pure ketone (**2**) was obtained (3.9 g, 99% yield); the physical and spectral data were consistent with previously published data.[32]

Synthesis of dihydro-β-damascol (3). Using the same reduction reaction for the double bond in the side chain of compound (**1**), alcohol **3** was obtained (3.6 g, 89% yield) from the ketone (**2**), with the physical and spectral data consistent with the literature report.[33]

Synthesis of ethyl 2-(2-butylidene-1,3,3-trimethylcyclohexyl)-acetate (4). A mixture of alcohol (**3**) (3.6 g, 18.4 mmol), triethylorthoacetate (21.6 mL, 115.2 mmol), and a catalytic amount of propionic acid (1 drop) was heated at 138 °C for 10 h under the conditions for distillative removal of ethyl alcohol. When the reaction was completed (GC, TLC), the mixture was chromatographed on silica gel. Elution with hexane/acetone (19 : 1) gave the pure ester (**4**) (4.7 g, 98% yield); (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.89 (t, $J = 7.3$ Hz, 3H, CH_3-10), 1.17 (s, 3H, one of the $(CH_3)_2C<$), 1.20 (s, 3H, one of the $(CH_3)_2C<$), 1.22 (t, $J = 7.4$ Hz, 3H, CH_3-17), 1.27–1.65 (m, 11H, CH_2-9, CH_2-4, CH_2-5, CH_2-6, CH_3-13), 2.14 (m, 2H, CH_2-8), 2.37 and 2.48 (two d, $J = 13.7$ Hz, 2H, CH_2-14), 4.05 (q, $J = 7.4$ Hz, 2H, CH_2-16), 5.20 (t, $J = 7.0$ Hz, 1H, H-7). ^{13}C NMR ($CDCl_3$), δ:13.96 and 14.30 (C-10 and C-17),17.59 (C-5), 23.76 (C-9), 30.51 and 30.64 (C-11 and C-12), 31.73 (C-13), 32.31 (C-8), 35.33 (C-1), 36.33 (C-6), 39.63 (C-3), 41.21 (C-4), 47.70 (C-14), 59.75 (C-16), 126.82 (C-7), 149.07 (C-2), 172.19 (C-15). IR (film, cm^{-1}): 2959 (s), 2932 (s), 2870 (m), 1743 (s), 1193 (w).

Synthesis of 2-(2-butylidene-1,3,3-trimethylcyclohexyl)acetic acid (5). Ester **4** (4 g, 15 mmol) was heated under reflux for 3 h in 10% ethanol solution of KOH (40 mL). After evaporation of ethanol *in vacuo*, the residue was diluted with water, and organic impurities were removed by extraction with diethylether (3 × 40 mL). The water layer was acidified with 0.1 M HCl, and

the product was extracted with diethyl ether (3 × 40 mL). The ethereal fraction was washed with brine and dried over anhydrous $MgSO_4$. Evaporation of solvent *in vacuo* afforded pure acid **5** (3 g, 85% yield); (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.87 (t, $J = 6.3$ Hz, 3H, CH_3-10), 1.19 (s, 6H, $(CH_3)_2C<$), 1.23 (s, 3H, CH_3-13), 1.29–1.62 (m, 9H, CH_2-9, CH_2-4, CH_2-5, CH_2-6), 2.11–2.21 (m, 2H, CH_2-8), 2.41 and 2.55 (two d, $J = 13.8$ Hz, 2H, CH_2-14), 5.26 (t, $J = 7.0$ Hz, 1H, H-7), 9.1 (s, 1H, –COOH). ^{13}C NMR ($CDCl_3$), δ: 13.88 (C-10), 17.55 (C-5), 23.63 (C-9), 30.52 and 30.58 (C-11 and C-12), 31.62 (C-13), 32.23 (C-8), 35.29 (C-1), 36.36 (C-6), 39.43 (C-3), 41.29 (C-4), 47.54 (C-14), 127.12 (C-7), 148.88 (C-2), 178.32 (C-15). IR (film, cm^{-1}): 2958 (s), 1706 (s), 1463 (m).

*Preparation of bromolactones (**6**), (**7**).* A solution of acid (1.86 g, 7.8 mmol) and *N*-bromosuccinimide (7.8 mmol) in THF (30 mL) and a drop of acetic acid were stirred at room temperature for 24 h. The mixture was diluted with diethyl ether and then washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo*. The mixture of products was subjected to column chromatography (hexane/acetone, 3 : 1). The data for isolated lactones are given below.

*7a-(1-Bromobutyl)-3a,7,7-trimethylhexahydrobenzofuran-2-one (**6**).* (0.42 g, 17% yield); (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.94 (t, $J = 7.3$ Hz, 3H, CH_3-11), 1.10 (s, 3H, CH_3-14), 1.34 and 1.40 (2s, 6H, $(CH_3)_2C<$), 1.50–2.33 (m, 10H, CH_2-4, CH_2-5, CH_2-6, CH_2-9, CH_2-10), 2.50 and 2.73 (two d, $J = 18.1$ Hz, 2H, CH_2-3), 4.28 (dd, $J = 10.3$ and 1.2 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$), δ:13.36 (C-11), 17.27 (C-10), 22.62 (C-5), 27.03 and 28.11 (C-12 and C-13), 30.11 (C-14), 36.29 (C-9), 38.75 (C-4), 38.97 (C-6), 40.88 (C-3a), 44.30 (C-7), 45.27 (C-3), 59.13 (C-8), 91.91 (C-7a), 175.90 (C-2). IR (film, cm^{-1}): 2958 (m), 2874 (m), 1776 (s), 1463 (w), 1226 (w).

*7a-Bromo-3a,7,7-trimethyl-8-propyloctahydroisochromen-3-one (**7**).* (0.67 g, 27% yield); (colourless crystal); mp: 95–104 °C; ^{1}H NMR ($CDCl_3$), δ:0.98 (t, $J = 7.0$ Hz, 3H, CH_3-11), 1.16–1.27 (m, 2H, CH_2-10), 1.32 (s, 3H, CH_3-3a), 1.40 and 1.45 (two s, 6H, $(CH_3)_2C<$), 1.52–1.97 (m, 8H, CH_2-6, CH_2-5, CH_2-4, CH_2-9), 2.44 and 3.15 (two d, $J = 19.6$ Hz, 2H, CH_2-3), 4.93 (d, $J = 10.6$ Hz, 1H, H-8).[13] CNMR ($CDCl_3$), δ:13.69 (C-11), 18.24 (C-10), 21.22 (C-5), 24.08 (C-14), 28.48 (C-3a), 33.62 and 35.67 (C-12 and C-13), 35.24 (C-9), 35.67 (C-4), 40.70 (C-6), 41.05 (C-3), 84.89 (C-7a), 84.97 (C-8), 170.90 (C-2). IR (KBr, cm^{-1}): 3007 (s), 2918 (s), 1716 (s), 1460 (s).

*Preparation of chlorolactones (**8**),(**9**).* A solution of acid (1.87 g, 7.8 mmol) and *N*-chlorosuccinimide (7.8 mmol) in THF (30 mL) and a drop of acetic acid were stirred at room temperature for 24 h. The mixture was diluted with diethyl ether and then washed with saturated $NaHCO_3$ solution and brine. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was evaporated *in vacuo*. The mixture of products was subjected to column chromatography (hexane/diethyl ether, 3 : 1). The data for isolated lactones are given below:

*7a-(1-Chlorobutyl)-3a,7,7-trimethylhexahydrobenzofuran-2-one (**8**).* (0.39 g, 18% yield); (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.94 (t, $J = 7.3$ Hz, 3H, CH_3-11), 1.11 (s, 3H, CH_3-3a), 1.33 and 1.41 (two s, 6H, $(CH_3)_2C$ < 7), 1.50–2.24 (m, 10H, CH_2-4, CH_2-5, CH_2-6, CH_2-9, CH_2-10), 2.52 and 2.62 (two d, $J = 18$ Hz, 2H, CH_2-3), 4.24 (dd, $J = 10.6$ and 1.6 Hz, 1H, H-8). ^{13}C NMR ($CDCl_3$), δ: 13.43 (C-11), 17.18 (C-10), 21.90 (C-5), 26.61 and 28.27 (C-12 and C-13), 29.60 (C-14), 36.84 (C-9), 37.91 (C-6), 38.82 (C-4), 40.10 (C-3a), 43.96 (C-7), 45.50 (C-3), 66.74 (C-8), 92.63 (C-7a), 175.95 (C-2). IR (film, cm^{-1}): 1464 (s), 1775 (s), 2875 (s), 2959 (s).

*7a-Chloro-3a,7,7-trimethyl-8-propyloctahydroizochromen-2-one (**9**).* (0.36 g, 17% yield); (colourless crystal); mp: 81–96 °C; ^{1}H NMR ($CDCl_3$), δ: 0.97 (t, $J = 7.3$ Hz, 3H, CH_3-11), 1.27, 1.31 and 1.35 (three s, 9H, $(CH_3)_2C<$, CH_3-3a), 1.51–2.16 (m, 10H, CH_2-4, CH_2-5, CH_2-6, CH_2-9, CH_2-10), 2.38 and 3.11 (two d, $J = 19.5$ Hz, 2H, CH_2-3), 4.74 (dd, $J = 10.3$ and 1.6 Hz, 1H, H-8).[13] C NMR ($CDCl_3$), δ:13.72 (C-11), 18.15 (C-10), 21.11 (C-5), 24.63 (C-14), 31.10 and 33.02 (C-12 and C-13), 34.31 (C-9), 35.07 (C-4), 39.95 (C-6), 40.02 (C-7), 40.22 (C-3a), 41.58 (C-3), 80.64 (C-7a), 84.57 (C-8), 175.95 (C-2). IR (KBr, cm^{-1}): 2968 (w), 2931 (w), 2869 (w), 1733 (s), 1463 (s).

Dehydrohalogenation procedure. 1,8-Diazabicyclo[5.4.0]undec-7-ene (6 mmol) was added to a solution of the corresponding halolactone (3 mmol) in dry methylene chloride (20 mL) at room temperature. When the reaction was completed (GC, TLC), the mixture was concentrated *in vacuo* to remove methylene chloride. The residue was diluted with diethyl ether. The ethereal solution was washed with brine, dried over anhydrous $MgSO_4$, and evaporated *in vacuo*. The residue was chromatographed on silica gel. Elution with hexane/acetone (6 : 1) gave lactones (**10**) and (**11**) (47–57% yield) with the following data:

*7a-((E)-But-1-enyl)-3a,7,7-trimethylhexahydrobenzofuran-2-one (**10**).* (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.92 (s, 3H, one of the $(CH_3)_2C<$), 1.01 (t, $J = 7.5$ Hz, 3H, CH_3-11), 1.03 (s, 3H, one of the $(CH_3)_2C<$), 1.25 (s, 3H, CH_3-3a), 1.40–1.62 (m, 6H, CH_2-4, CH_2-5, CH_2-6), 1.97 and 2.42 (two d, $J = 16.3$ Hz, 2H, CH_2-3), 2.11 (dqd, $J = 7.5$, 6.3 and 1.3 Hz, 2H, CH_2-10), 5.60 (dt, $J = 15.5$ and 1.3 Hz, 1H, H-8), 5.77 (dt, $J = 15.5$ and 6.3 Hz, 1H, H-9). ^{13}C NMR ($CDCl_3$), δ: 13.80 (C-11), 17.75 (C-5), 21.66 and 24.99 (C-12 and C-13), 25.66 (C-10), 26.47 (C-14), 36.25 (C-3a), 36.49 (C-4), 36.57 (C-6), 41.75 (C-7), 45.91 (C-3), 91.98 (C-7a), 125.01 (C-9), 134.33 (C-8), 176.73 (C-2). IR (film, cm^{-1}): 2964 (s), 2932 (s), 1778 (m), 1462 (m), 1289 (m), 1125 (m), 971 (m).

*3a,7,7-Trimethyl-8-propylhexahydro,cyclopropa[1,2]benzofuran-2(3H)-one (**11**).* (oily liquid); ^{1}H NMR ($CDCl_3$), δ: 0.96 (t, 3H, $J = 7.3$ Hz, CH_3-11), 1.14 and 1.18 (two s, 6H, $(CH_3)_2C<$), 1.21 (s, 3H, CH_3-3a), 1.29–2.11 (m, 11H, CH_2-6, CH_2-5, CH_2-4, CH_2-9, CH_2-10 and H-3), 4.25 (dd, $J = 10.9$ and 2.2 Hz, 1H, H-8).[13]C NMR ($CDCl_3$), δ: 13.92 (C-11), 17.73 (C-10), 17.86 (C-14), 20.45 (C-5), 26.13 (C-12), 28.04 (C-3a), 29.50 (C-13), 30.52 (C-7), 33.11 (C-9), 34.12 (C-3), 37.27 (C-6), 41.51 (C-4), 45.59 (C-7a), 82.73 (C-8), 175.13 (C-2). IR (film, cm^{-1}): 2936 (s), 2873 (s), 1762 (s), 1466 (m), 1327 (m), 1188 (s), 976 (s).

Bioassays

Myzus persicae

Cultures of aphids and their settling (choice-test) have been previously described by Grudniewska *et al.*[18]

Aphid initial behavioural responses (no-choice test)

Aphid behaviour during the initial contact with the studied compound was measured by direct monitoring of the freely moving aphids on a treated leaf, using a video recording. This

bioassay allows studying the preingestional (activating olfactory receptors) and ingestional (activating gustatory receptors) effects of a chemical, and the results may show the mode of action of the compound – whether it is active at the plant surface or within plant tissues. The experiment was carried out for 15 min with 12 aphids/compound. The following parameters were derived from data obtained in this experiment: total time of aphid presence on the leaf, total time of probing, number of probes, and mean duration of a probe. The time spent on the leaf and the duration of probing were recorded based on the relationship between antennal and body movements and penetration of the stylets as described by Hardie *et al.*, who associated styled penetration with the position of antennae parallel to the abdomen and the cessation of body movements.[40]

Statistical analysis

The data derived from the choice-test (aphid settling) were analysed using Student's *t*-test. If aphids showed clear preference for the leaf treated with the tested compound ($P < 0.05$), the compound was described as having attractant properties. If aphids settled mainly on the control leaf ($P < 0.05$), the compound studied in the respective choice-test was classified as a deterrent. From the data thus obtained, the relative index of deterrence (DI) was calculated using the equation:

$$\mathrm{DI} = (C - T)/(C + T)$$

where C and T are the numbers of aphids settled on control and treated leaves, respectively. The value of DI ranged between plus 1 (ideal deterrent) and minus 1 (ideal attractant).

The data derived from the no-choice-test (aphid behavioural responses) were analyzed by means of one-way analysis of variance (ANOVA) followed by comparison of deterrence coefficients using Tukey's test ($P < 0.05$).

Alphitobius diaperinus

The insect culture of *Alphitobius diaperinus* culture has been previously described by Szczepanik *et al.*[21]

To investigate the antifeedant activity of compounds studied, choice and no-choice tests were used according to a procedure previously described.[17] The choice test is very sensitive, but insects could easily avoid treated food. Feeding-deterrent activity of chemical compounds observed in a choice test thus needs to be confirmed with a no-choice test. The application of these two types of tests also allowed us to evaluate the mode of action of the compounds studied: did they act on the taste organs only (according to the definition of antifeedants) or were they toxic to the insects?

For the feeding assays, 1 mL of acetone solution of the test compounds at a concentration of 1% was applied to 1 g of oat flakes (Melvit SA Warsaw, Poland) using a micropipette. After evaporation of acetone, weighed flakes were placed in Petri dishes and offered to 10 larvae or 10 unsexed adults during the following three-day period. All trials were kept in an incubator at 29 ± 1 °C in the dark. The amount of food eaten was the basis for calculating the deterrence coefficients (relative R and absolute A) according to the formulae:[36,41]

$$R = (C - E)/(C + E) \times 100 \text{ (choice test)}$$

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

where C, CC and E, EE are the weights of the control and the treated food consumed by the insects in the choice and no-choice tests, respectively.

The total deterrence coefficient, which ranged from −200 to +200, served as an activity index. A compound for which both indices reach a value of 100 and the sum of 200 is called an ideal deterrent. Activity of the tested compounds was evaluated according to the classification of Nawrot *et al.* and Koul.[16,42]

The compounds with the highest activity (**7**, **8**, **9**) were used for further studies as 0.5 and 0.1% solutions. Because 0.5% solution moderately decreased larval feeding, the lowest concentration of these halolactones was used only against adults. The research methodology was the same as for the case of 1% solutions. Four replicates of both tests for each compound were conducted on each insect life stage.

From a practical point of view, it is important to evaluate the level of insect feeding in a 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:

$$(\mathrm{EE}/\mathrm{CC}) \times 100$$

where EE and CC are the weights of the treated and control food consumed in no-choice tests, respectively.

Statistical analysis

Antifeedant activity was analyzed by means of one-way analysis of variance (ANOVA) followed by comparison of deterrence coefficients with Tukey's test ($P < 0.05$) using PAST (Paleontological Statistics) software.[26] Student's *t*-test (Microsoft Office 2010, Excel) was used to compare the mean values of consumption by the larvae and adults in the no-choice test.

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