Synthesis and Insecticidal Activity of Lactones Derived from Furan-2(5H)-one

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Ten 4,7-methanoisobenzofuran-1(3H)-ones were synthesized and their insecticidal activities against the insect pest Diaphania hyalinata were evaluated. The most active substances have been selected from the initial screening to find the dose to kill 50% (LD10) and 90% (LD90) of the melonworm. Time-mortality curves of the three selected formulations at the LD90 concentration have been made against D. hyalinata. From the time-mortality curves we found that the formulation (3aR,4R,5S,6S,7S,7aS)- and (3aS,4S,5R,6R,7R,7aR)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3H)-one + (3aR,4R,5R,6R,7S,7aS)- and (3aS,4S,5S,6S,7R,7aR)-5,6-dichlorohexahydro-4,7-methanoisobenzofuran-1(3H)-one has killed 50% of the melonworm after 2 h, presenting the best knockdown effect. Bioassays against Solenopsis saevissima and Tetragonisca angustula were carried out for the lactones selected in the initial screening against D. hyalinata. The formulation (3aS,4R,5S,6S,7S,7aR)- and (3aR,4S,5R,6R,7R,7aS)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3H)-one + (3aS,4S,5R,6R,7S,7aS)- and (3aR,4S,5S,6S,7R,7aR)-5,6-dibromohexahydro-4,7-methanoisobenzofuran-1(3H)-one has killed 31.25 and 68.30% of the pest natural enemy and the pollinator bee, respectively. At the same concentration this formulation killed 90% of D. hyalinata. The selectivity in favor of the non-target organisms has rendered this formulation a position as a promising agrochemical.

Keywords: Diaphania hyalinata, Tetragonisca angustula, Solenopsis saevissima, insecticide

Introduction

The melonworm Diaphania hyalinata (Lepidoptera: Crambidae) is an important pest of plants of the family Cucurbitaceae and occurs in the Americas.1-3 The leaves are first attacked by these insects, limiting plant photosynthesis, therefore, growth and development. After this the stems and shoots are damaged leading to the plant death. When attacked by the caterpillar, fruits become unfit for human consumption.3

Solenopsis saevissima, an ant widely distributed in South America, is among the natural enemies of D. hyalinata. Worker individuals of this species are aggressive and have a painful sting.4 Predation by S. saevissima contributes significantly to the biological control of agricultural crops.5 Species of the genus Solenopsis have been considered important predators contributing to insect pest reduction in the tropics and subtropics.6

Bees are another important non-target group of organisms that contribute to the biodiversity of the ecosystems. However, an alarming fact is that during the last decades there were losses in the number of colonies in locations around the world.7 Multiple environmental factors can potentially contribute to these losses, including biotic ones like pathogens, parasites, availability of resources due to habitat fragmentation and loss; and abiotic ones like climate change and pollutants.8-10 Although the causes of the loss of pollinators are still under study, which currently go through a screening process, extensive use of chemical pesticides against insect pests for crop protection may have contributed to the decline of pollinators.11 One hypothesis for the disappearance of bees relates to the use of neonicotinoid insecticides.12-17 Concern for bees is justified by the fact that they play a vital role in agriculture.18 Nearly 35% of the world’s food crops depend on pollination mostly by insects,19 especially bees and butterflies. The pollinator insects can increase genetic biodiversity in the cultivated species by the cross pollination leading to better fruits, seeds and plants.20

In recent years, several species are rapidly going into extinction because the insecticides have been planned to be very effective against pests in general, but with great
potential to affect beneficial organisms. This concept has fueled concerns about the ecological consequences of biodiversity loss, leading to a flurry of studies examining how changes in diversity affect the ecosystems, particularly those that provide goods and services that humans depend upon.

Pest control should be based on equilibrium between the use of chemical agents and survival of the natural enemies. Therefore, to avoid the possible interruption of the natural control of pests it is important to evaluate the effects of pesticides against the beneficial organisms.

The idea that beneficial organisms and insecticides were incompatible has been modified in recent years. The outdated broad-spectrum insecticides have been superseded by more modern agrochemicals like spinosads and novel pyrethroids that present selectivity in favor of the beneficial organisms and a desirable ecotoxicology. The novel pyrethroids have been evaluated for their toxicological effect against target insects and adverse activity on natural enemies.

The conservation of beneficial organisms is an essential component in integrated pest management. The most frequent source of mortality for phytophagous arthropods in ecosystems is attack by natural enemies.

The synthesis of new lactones has been inspired by small molecules from natural products that have been the mainstay of research in organic chemistry since its initial development. Within this context, phthalides or isobenzofuranones, natural volatile compounds present in apiaceous plants have attracted the attention of many research groups. This class of compound has presented itself in the NMR (nuclear magnetic resonance) spectrum as doublet of doublets at 6.18 and 6.23, respectively. The double bond is confirmed by the multiplet at 6.25-6.33 ppm in the 1H NMR spectrum. These distinct signals of adduct have also been observed in the 13C NMR spectrum. The olefinic protons H5 and H6 of 3 are displayed as doublet of doublets at δ 6.18 and 6.23, respectively. Long distance detection experiments in the NMR (nuclear Overhauser effect (NOE)) have enabled us to differentiate these isomers. Irradiation of the signal of H8 of adduct 2 has resulted in 1.32 and 1.87% increase in the signals of

\[ \text{exo-3} : \text{endo-2} = 3.2 \]
H7a and H3a, respectively. However, irradiation of H8 of adduct 3 has resulted in 0.85% NOE in the signal of H3′ which is close in space to H8.

In the next stage, the double bond of lactones 2 and 3 was oxidized with meta-chloroperoxybenzoic acid to give only epoxides 4 (98% yield) and 5 (83% yield) with stereospecific control (Scheme 2). In the 1H NMR of 4 and 5, H5 and H6 (δ ca. 3.2 ppm) were chemically shielded compared to H5 and H6 (δ ca. 6 ppm) at the double bond in compounds 2 and 3, which is a strong evidence of the epoxide formation. The oxygen at the exo position in epoxides 4 and 5 was established by NOE experiments. Irradiation of the signal of H5/H6 of epoxide 4 has resulted in 1.17% increase in the signal of H3’. However, irradiation of the signal of H5 of epoxide 5 has led to 0.9% increase of H3a. To reinforce the exo position of the oxygen in the epoxide 5 we have irradiated the signal of H6 resulting in 2.03% increase of H7a.

Following the idea of preparing novel isobenzofuranone derivatives we have also performed hydrogenation, chlorination, and bromination reactions of these adducts (Scheme 2). Bromination and chlorination of unsaturated lactone 3 have proceeded stereoselectively to give only the trans-1,2-dihalogenated products, as expected. The structures of all compounds have been established by using the spectrometric methods, including in these methods the bidimensional NMR experiments such as heteronuclear single quantum coherence (HSQC), correlation spectroscopy (COSY), and nuclear Overhauser effect spectroscopy (NOESY).

Insecticidal activity

In this study, we have assessed the efficacy of compounds 1-7, 9a, and 9b against second-instar larvae of D. hyalinata. Furthermore, the insecticidal activities of the mixtures of 8a/8b (2:1 ratio), and 9a/9b (1:1 ratio) have also been evaluated. Compounds 8a and 8b have not been evaluated separately because only a small quantity of 8b was isolated and purified (57.5 mg).

There was a significant difference in the mortality of larvae of D. hyalinata as a function of the treatments (analysis of variance (ANOVA), F14.75 = 48.24, p < 0.001). The mortality of D. hyalinata as a result of the treatments is presented in Figure 1 as a histogram with standard deviation bars and assignment letters according to the Scott-Knott grouping analysis test at p < 0.05. The compounds 1, 9a, 9a + 9b, 9b, and 8a + 8b have presented significant insecticidal activities killing more than the negative control. The lactones 9a + 9b, 9b, and 8a + 8b at the concentration of 45.2 µmol g⁻¹ insect were the most potent, killing 84.8, 91.3 and 96.3% of D. hyalinata, respectively. These compounds were more active than the commercial piperine, which has presented 64.5% mortality. Compounds 1 and 9a have shown moderate activities of 23.1 and 40.0%, respectively.

Scheme 2. Reagents: (a) meta-chloroperoxybenzoic acid (MCPBA), CH₂Cl₂; (b) H₂, Pd/C (10%), EtOH; (c) Cl₂, CH₂Cl₂; (d) Br₂, CH₂Cl₂.
Piperine was used as the positive control because the insecticidal activity of this substance has been extensively described in the literature. Furthermore, extracts of black pepper *Piper nigrum* L. (Piperaceae), whose main constituent is piperine are toxic to Lepidoptera insects, including *Ascia monuste orseis* Latreille (Lepidoptera: Pieridae) and *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae).

The isolated isomers 9a and 9b, in the concentration of 45.2 µmol g⁻¹ insect and a period of 48 h, have caused 40.0 and 91.3% mortalities of the insects, respectively. According to Ahern and Whitney, many secondary metabolite classes are responsible for the plant resistance against herbivore. Therefore, totally different compounds could play an important defense mechanism for the plants. To the best of our knowledge we have not found many references describing the ecological effects played by modifying the stereochemistry of the active ingredients.

In some cases, individual isomers have identical bioactivities, but in other cases different isomers can drastically differ in bioactivity.

A significant difference in insecticide activity has been observed for stereoisomers 9a and 9b. The isomer 9b is the most active killing 91.3% of the insect pest. However the formulation 9a + 9b at the ratio of 1:1 has killed 84.8% of the pest, which does not differ significantly from that obtained for the pure isomer 9a. Therefore, the laborious separation of the chemicals 9a and 9b is not necessary considering only the difference in insecticidal activity.

The most active lactones 8a + 8b, 9a + 9b, and 9b were selected for a more thorough investigation after the general insecticidal bioassay. This selection is in line with the Brazilian Health Surveillance Agency (ANVISA) for tests of efficacy on pest control products, which recommends that only values between 90 ± 10% mortality should be considered satisfactory.

The dose-mortality results from the lactones application in larvae of *D. hyalinata* have shown low chi-square test ($\chi^2$) and high $p$ values ($< 2.39$ and $> 0.05$, respectively) indicating the suitability of the probit model for fitting the dose-response curves. Curves with acceptance probability of the null hypothesis by $\chi^2$ greater than 0.05 were accepted.

Table 1. Toxicity of the chemicals 8a + 8b, 9a + 9b and 9b against *D. hyalinata* caterpillars. 48 h after application

<table>
<thead>
<tr>
<th>Lactones</th>
<th>LD$_{50}$ / (µmol g⁻¹ insect)</th>
<th>LD$_{90}$ / (µmol g⁻¹ insect)</th>
<th>$y$</th>
<th>$\chi^2$</th>
<th>df</th>
<th>$p$</th>
</tr>
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<tbody>
<tr>
<td>8a + 8b</td>
<td>20.58 (19.37-21.86)</td>
<td>32.03 (29.57-35.39)</td>
<td>-0.75 + 4.38x</td>
<td>2.39</td>
<td>4</td>
<td>0.67</td>
</tr>
<tr>
<td>9a + 9b</td>
<td>21.49 (19.95-23.08)</td>
<td>33.90 (31.10-37.67)</td>
<td>-0.66 + 4.25x</td>
<td>1.88</td>
<td>3</td>
<td>0.60</td>
</tr>
<tr>
<td>9b</td>
<td>21.40 (19.97-22.86)</td>
<td>36.73 (33.55-41.21)</td>
<td>0.24 + 3.58x</td>
<td>0.72</td>
<td>5</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*The numbers in parentheses are the confidence intervals. LD$_{50}$ and LD$_{90}$: lethal dose to kill 50 and 90% of the insects, respectively; y: curve equation; $\chi^2$: chi-square test; df: degree of freedom; p: probability.*
The chemicals $8a + 8b$, $9a + 9b$ and $9b$ have killed 50% of the population of the pest in intervals of 2, 4 and 3 h, respectively. The rate of activity of the insecticide is very important to control the outbreak of pests in the agriculture. The fast population growth of pests should be controlled to avoid great damages to the crops. The formulation $8a + 8b$ has presented the best time mortality result, which is adequate to control the outbreak of pests because it has killed 50% of the population of $D. hyalinata$ in the shortest period (2 h).

![Figure 3](image.png)

**Figure 3.** Survival curves of $D. hyalinata$ subjected to the application of the chemicals $8a + 8b$, $9a + 9b$, and $9b$ at the LD$_{10}$ concentration.

The insecticides have been planned to be very effective against pests in general, but with great potential to affect beneficial organisms. Thus we have evaluated the toxicity of eleven chemical formulations against the pest $D. hyalinata$, and the three most promising formulations have been tested against the beneficial bee pollinator and the natural enemy fire ant.

The formulations $8a + 8b$, $9a + 9b$, and $9b$ have been evaluated against the beneficial organisms $S. saevissima$ and $T. angustula$ (Table 2). The results from this study showed that lactones $9b$ ($t_{10} = 6.35$, $p < 0.001$) and $9a + 9b$ ($t_{10} = 4.59$, $p < 0.001$) were selective in favor of $S. saevissima$ and $T. angustula$ (more toxic to the pest than to the natural enemy).

Besides the efficiency against insect pests, novel agrochemical agents should preferably provide selectivity to non-target species, especially predators and pollinators. This is important because natural enemies can aid in the control of the population of pests in agricultural crops.$^{62}$ Moreover, the conservation and enhancement of natural enemy activity in agroecosystems is one of the key elements of sustainable agricultural production.$^{63-67}$

However, the natural enemies alone are not able to prevent any damage caused by the pest.$^{24}$ Because of this, finding a balance between the use of chemical insecticides and the survival of natural enemies is a priority of integrated pest management.

Furthermore, pollination is essential to ensure the reproduction of most plants.$^{68}$ Thus pollinator should be retained, so that the biodiversity of the ecosystem is maintained.$^{69,70}$

The International Organization for Biological Control (IOBC) uses a standardized classification for the impact of pesticides on natural enemies for experiments in laboratory which consists of four categories: harmless (< 30% effect), slightly harmful (30-79% effect), moderately harmful (80-99% effect), and harmful (> 99% effect).$^{71}$ The formulation $9a + 9b$ has killed 31.25 and 68.30% of $S. saevissima$ and $T. angustula$, respectively. However, the isolated compound $9b$ has killed 46.99 and 65.33% of $S. saevissima$ and $T. angustula$, respectively. The selectivity of these lactones in favor of $S. saevissima$ and $T. angustula$ surpasses the negative impact of the outdated broad-spectrum insecticides which kill the insects without selectivity. The selectivity provided by lactones ($9a + 9b$ and $9b$) to $S. saevissima$ and $T. angustula$ suggests that the use of these compounds to control $D. hyalinata$ presents a low risk to these non-target insects according to the IOBC classification.

To sum up we have found that a number of structural features have influenced the insecticidal activity, particularly the presence of halogens and the $cis$-relationship between the lactone ring and the methylene bridge. The most active compounds were the $exo$ adducts and the ones containing halogens which is in agreement with literature reports where various compounds with biological activities such as fungicide, herbicide, antibiotic, and insecticide have been described.$^{72-74}$

**Table 2.** Mortalities of the pest natural enemy $S. saevissima$ and the pollinator $T. angustula$ 48 h after application of the formulations $8a + 8b$, $9a + 9b$, and $9b$ at 40.37, 43.03, and 48.74 mmol g$^{-1}$ of insect, respectively

<table>
<thead>
<tr>
<th>Lactones</th>
<th>Average mortality ± standard error / %</th>
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<tbody>
<tr>
<td></td>
<td>$S. saevissima$</td>
</tr>
<tr>
<td>$8a + 8b$</td>
<td>$91.37 ± 4.24$</td>
</tr>
<tr>
<td>$9a + 9b$</td>
<td>$31.25 ± 12.81$</td>
</tr>
<tr>
<td>$9b$</td>
<td>$46.99 ± 6.79$</td>
</tr>
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*Significantly different from the mortality caused by these chemicals to $D. hyalinata$ by t-test at $p < 0.05$.

**Conclusions**

In summary, we have synthesized and determined the insecticidal activity of ten lactones. The agrochemicals
Reagents and solvents were purified, when necessary, according to the procedures described by Perrin and Armarego. The reactions were followed by visualizing the thin layer chromatography (TLC) plates coated with silica-gel in an ultraviolet chamber at 254 nm. The furan-2(5H)-one was obtained as previously described. The cyclopentadiene was obtained by distillation of dicyclopentadiene commercially available (Sigma-Aldrich) before being used in the Diels-Alder reaction. The 1H, 13C NMR, COSY, HSQC, heteronuclear correlation (HETCOR), heteronuclear multiple-bond correlation (HMBC), NOE difference (NOEDIFF), and NOESY spectra were recorded on a Varian Mercury 300 instrument (300 MHz), using deuterated chloroform as solvent. Infrared (IR) spectra were recorded on a Varian 660-IR, equipped with GladiATR scanning from 4000 to 500 cm⁻¹. Mass spectra (MS) were recorded on a Shimadzu GCMS-QP5050A instrument under electron impact (70 eV) conditions. Melting points are uncorrected and were obtained using MQAPF-301 melting point apparatus (Microquímica). Column chromatography was performed over silica gel (60-230 mesh).

Synthesis of lactones

(3aR,4R,5R,6S,7S,7aR) and (3aS,4S,5S,6S,7R,7aS)-5,6-epoxyhexahydro-4,7-methanoisobenzofuran-1(3H)-one (4) meta-Chloroperbenzoic acid (MCPBA) (2.6366 g of MCPBA 70%, 15.2 mmol) was added portionwise to a round-bottom flask containing phthalide 2 (1.1386 g, 7.59 mmol) and dichloromethane (100 mL). The reaction mixture was stirred for 15 h and quenched with aqueous Na2SO3 solution (20%, 100 mL). The mixture was extracted with diethyl ether (3 × 100 mL), and the combined organic layers were washed with aqueous NaHCO3 solution (10%, 100 mL) and brine until neutral. The organic layer was dried with anhydrous MgSO4 and filtered. After evaporation of solvent, the product mixture was purified by column chromatography with silica-gel using hexane:ethyl acetate 1:1 as eluent to give 1.238 g of 4 (98% yield): white solid; TLC Rf = 0.36 (hexane:ethyl acetate 1:1 v/v); m.p. 120-121°C; IR (film) νmax/cm⁻¹ 3065, 2973, 2924, 1761, 1481, 1381, 1345, 1185, 1001; 1H NMR (300 MHz, CDCl3) δ 1.46 (d, 1H, J 8.6 Hz, H8), 1.64 (d, 1H, J 8.6 Hz, H8’), 3.04-3.15 (m, 2H, H3a and H4), 3.25 (dd, 1H, J 9.2, 4.6 Hz, H7a), 3.30-3.36 (m, 1H, H7), 3.79 (dd, 1H, J 9.7, 3.1 Hz, H3’), 4.28 (dd, 1H, J 9.7, 8.3 Hz, H3’), 6.25-6.33 (m, 2H, H5 and H6); 13C NMR (75 MHz, CDCl3) δ 40.1 (C3a), 45.6 (C7), 46.0 (C4), 47.4 (C7a), 51.7 (C8), 70.2 (C3), 134.3 (C5), 136.7 (C6), 178.0 (C1); MS m/z (%) 91 (7), 85 (11), 66 (100).

Data for compound 2

White solid; TLC Rf = 0.44 (hexane:ethyl acetate 2:1 v/v); m.p. 119.8-120.6°C; IR (film) νmax/cm⁻¹ 3065, 2097, 2873, 1765, 1481, 1381, 1345, 1185, 1001; 1H NMR (300 MHz, CDCl3) δ 1.46 (d, 1H, J 8.6 Hz, H8), 1.64 (d, 1H, J 8.6 Hz, H8’), 3.04-3.15 (m, 2H, H3a and H4), 3.25 (dd, 1H, J 9.2, 4.6 Hz, H7a), 3.30-3.36 (m, 1H, H7), 3.79 (dd, 1H, J 9.7, 3.1 Hz, H3’), 4.28 (dd, 1H, J 9.7, 8.3 Hz, H3’), 6.25-6.33 (m, 2H, H5 and H6); 13C NMR (75 MHz, CDCl3) δ 40.1 (C3a), 45.6 (C7), 46.0 (C4), 47.4 (C7a), 51.7 (C8), 70.2 (C3), 134.3 (C5), 136.7 (C6), 178.0 (C1); MS m/z (%) 91 (7), 85 (11), 66 (100).

Data for compound 3

Colorless oil; TLC Rf = 0.56 (hexane:ethyl acetate 2:1 v/v); IR (film) νmax/cm⁻¹ 3061, 2974, 2909, 1761, 1381, 1385, 1005; 1H NMR (300 MHz, CDCl3) δ 1.47 (dt, 1H, J 9.8, 1.7 Hz, H8), 1.55 (duquat, 1H, J 9.8, 1.7 Hz, H8’), 2.51-2.60 (m, 1H, H3a), 2.65 (dt, 1H, J 8.5, 1.7 Hz, H7a), 2.90 (sl, 1H, H4), 3.27 (sl, 1H, H7), 3.96 (dd, 1H, J 8.6, 3.5 Hz, H3’), 4.48 (dd, 1H, J 9.8, 8.7 Hz, H3), 6.18 (dd, 1H, J 5.7, 3.0 Hz, H5) 6.23 (dd, 1H, J 5.7, 3.0 Hz, H6); 13C NMR (75 MHz, CDCl3) δ 41.7 (C3a), 43.1 (C8), 46.2 (C7), 47.8 (C7a), 48.0 (C4), 71.7 (C3), 137.3 (C6), 137.6 (C5), 177.6 (C1); MS m/z (%) 150 ([M]+, 1), 91 (7), 85 (10), 66 (100).

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(20), 92 (17), 91 (25), 82 (42), 81 (100), 80 (11), 79 (49), 78 (19), 77 (45), 70 (27), 69 (57), 66 (17), 65 (14), 55 (17), 54 (27), 53 (29), 51 (21), 41 (22), 40 (12). *These assignments could be reversed.

Data for compound 8a

Colorless oil; TLC Rf = 0.37 (hexane:ethyl ether 1:1 v/v); IR (film) νmax / cm⁻¹ 2969, 1769, 1385, 1001, 853; 13C NMR (300 MHz, CDCl₃) δ 67.7 (C4), 69.3 (C5, C6), 71.7 (C3), 177.0 (C1); MS m/z (%) 224 (M+4⁺), 222 ([M+2⁺], 1.21), 220 ([M⁺], 1.70), 141 (12), 105 (29), 91 (25), 80 (100), 79 (52), 78 (11), 77 (23), 66 (38), 65 (42), 63 (19), 53 (17), 52 (15), 51 (44), 50 (15), 49 (10), 41 (15), 40 (13).

Data for compound 8b

Colorless oil; TLC Rf = 0.31 (hexane:ethyl ether 1:1 v/v); IR (film) νmax / cm⁻¹ 2975, 2911, 1769, 1485, 1385, 1189,
1048, 1009, 821, 785, 653, 493; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 1.57-1.66\) (m, 1H, H8\(^i\)), 2.03 (dquint, 1H, J 12.1, 1.7 Hz, H8\(^j\)), 2.49 (sl, 1H, H4), 2.61 (dd, 1H, J 9.0, 3.7, 1.7 Hz, H3a), 2.93-2.97 (m, 1H, H7\(^i\)), 3.27 (dt, 1H, J 9.0, 1.7 Hz, H7\(^a\)), 3.70 (t, 1H, J 2.7 Hz, H5), 3.97 (dd, 1H, J 9.9, 3.7 Hz, H3\(^a\)), 4.30 (dd, 1H, J 4.1, 2.7 Hz, H6), 4.54 (dd, 1H, J 9.9, 9.2 Hz, H3); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 29.77, 29.11, 176.1, 147.8, 138.0, 1180, 1053, 1009, 757, 613, 485; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 1.60-1.66\) (m, 1H, H8\(^i\)), 2.13 (dquint, 1H, J 12.1, 1.8 Hz, H8\(^j\)), 2.55 (sl, 1H, H4), 2.63 (d, 1H, J 8.7, 3.6, 1.8 Hz, H3a), 2.92-2.96 (m, 1H, H7\(^i\)), 3.31 (ddd, 1H, J 8.7, 1.8, 1.8 Hz, H7\(^a\)), 3.85 (t, 1H, J 2.9 Hz, H5), 3.97 (dd, 1H, J 10.0, 3.6 Hz, H3\(^a\)), 4.47-4.56 (m, 2H, H3 and H6); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 30.8\) (C8), 40.0 (C3a), 43.0 (C7a), 48.3 (C7), 52.6 (C4), 55.9 (C5), 57.6 (C6), 71.6 (C3), 178.6 (C1); MS m/z (%) 231 (33), 229 (35), 106 (34), 105 (31), 75 (12), 66 (66), 65 (65), 63 (31), 53 (25), 52 (22), 51 (74), 50 (27), 49 (20), 41 (23), 40 (24).

Data for compound 9a

Colorless oil; TLC \(R_f = 0.31\) (hexane:diethyl ether 1:1 v/v); IR (film) \(\nu_{max} / \text{cm}^{-1}\) 2976, 2917, 1761, 1475, 1457, 1371, 1180, 1052, 996, 754, 677, 605, 543, 466; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 1.56-1.64\) (m, 1H, H8\(^i\)), 2.08 (d, 1H, J 12.1 Hz, H8\(^j\)), 2.55 (dd, 1H, J 9.0, 3.7 Hz, H4), 2.72 (d, 1H, J 8.9 Hz, H7a), 2.92 (s, 1H, H7\(^i\)), 3.22 (tdd, 1H, J 8.9, 9.0, 3.7 Hz, H3a), 3.88 (t, 1H, J 3.0 Hz, H6), 3.96 (dd, 1H, J 9.9, 3.6 Hz, H3\(^a\)), 4.33 (t, 1H, J 3.7 Hz, H5), 4.56 (t, 1H, J 9.6 Hz, H3); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 30.6\) (C8), 35.7 (C3a), 46.2 (C7a), 50.0 (C4/C7), 56.0 (C6), 58.0 (C5), 71.7 (C3), 177.0 (C1); MS m/z (%) 231 (33), 229 (35), 106 (31), 105 (100), 92 (11), 91 (60), 80 (19), 79 (48), 78 (22), 77 (40), 66 (86), 65 (85), 63 (36), 53 (28), 52 (32), 51 (78), 50 (34), 41 (26), 40 (33).

Data for compound 9b

Colorless oil; TLC \(R_f = 0.29\) (hexane:diethyl ether 1:1 v/v); IR (film) \(\nu_{max} / \text{cm}^{-1}\) 2977, 2911, 1761, 1478, 1380, 1180, 1053, 1009, 757, 613, 485; \(^1H\) NMR (300 MHz, CDCl\(_3\)) \(\delta 1.60-1.66\) (m, 1H, H8\(^i\)), 2.13 (dquint, 1H, J 12.1, 1.8 Hz, H8\(^j\)), 2.55 (sl, 1H, H4), 2.63 (d, 1H, J 8.7, 3.6, 1.8 Hz, H3a), 2.92-2.96 (m, 1H, H7\(^i\)), 3.31 (ddd, 1H, J 8.7, 1.8, 1.8 Hz, H7\(^a\)), 3.85 (t, 1H, J 2.9 Hz, H5), 3.97 (dd, 1H, J 10.0, 3.6 Hz, H3\(^a\)), 4.47-4.56 (m, 2H, H3 and H6); \(^13\)C NMR (75 MHz, CDCl\(_3\)) \(\delta 30.8\) (C8), 40.0 (C3a), 43.0 (C7a), 48.3 (C7), 52.6 (C4), 55.9 (C5), 57.6 (C6), 71.6 (C3), 178.6 (C1); MS m/z (%) 231 (40), 229 (43), 106 (29), 105 (100), 91 (60), 80 (16), 79 (45), 78 (21), 77 (34), 66 (79), 65 (73), 63 (31), 55 (14), 53 (23), 52 (24), 51 (68), 50 (28), 41 (26), 40 (30).

Biological assays

Biological assays were conducted with second-instar larvae of D. hyalinata and adults of S. saevissima and T. angustula. Larvae of D. hyalinata were obtained from a laboratory rearing and adults of S. saevissima and T. angustula were collected from nests located at the University campus.

Screening bioassay for D. hyalinata

The experimental design was completely randomized with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm \(\times\) 2.0 cm) containing ten insects. The average weights of the insects were obtained by measuring, on an analytical balance, the mass of ten insects and taking the average. Bioassays were conducted by topical application. A 10 \(\mu\)L Hamilton micro syringe was employed to apply, on the thoracic tergite of each individual insect, 0.5 \(\mu\)L of a solution of the test compound, dissolved in acetone, corresponding to a concentration of 45.2 pmol g\(^{-1}\) insect. In a negative control experiment, carried out under the same conditions, 0.5 \(\mu\)L of acetone was applied on each insect. After the application, the insects were kept in individual petri dishes containing discs of chayote leaf as food. The petri dishes were placed in an incubator at 25 \(\pm\) 0.5 \(^\circ\)C and 75 \(\pm\) 5\% relative humidity with a photoperiod of 12 h. The mortality counts were made after 6, 12, 24 and 48 h of treatment. Mortalities included dead individuals as well as those without movements. The insecticidal activity of piperine (Sigma-Aldrich) was taken as positive control.

Dose-mortality bioassays for D. hyalinata

The most active lactones against D. hyalinata were subjected to experiments to obtain dose-mortality curves. The experimental design was completely randomized with six replications. Each experimental unit consisted of a glass petri dish (9.5 cm \(\times\) 2.0 cm) containing ten insects. Initially, four doses of each compound were tested to identify the range of concentrations that would provide mortalities greater than zero and less than 100\%. Once the ranges of
concentrations were defined, other doses were tested for each compound. The number of doses used to obtain the dose-mortality curves varied from five to seven. Bioassays were conducted by topical application using the same procedure described above.

**Time-mortality bioassays for D. hyalinata**

The most active lactones were subjected to experiments to obtain curves of survival. The experimental design was completely randomized with twelve replicates. Each experimental unit consisted of ten insects kept on a glass Petri dish (9 cm diameter × 2 cm height) covered with organza. Bioassays were conducted by the same procedure as that described above. The dose used was equivalent to LD$_{50}$ obtained for the three most active lactones. Insect mortality was observed every 30 min during the initial 12 h exposure followed by 5 h intervals of observation until the death of approximately 90% of insects’ populations.

**Risk assessment to non-target insects**

To determine the magnitude of selectivity of the compounds, LD$_{50}$ for the most active lactones against *D. hyalinata* were applied to beneficial insects. The experimental design was completely randomized with six replications. Bioassays were conducted using the same procedure to that described above. *T. angustula* was subjected to a photoperiod of 12 h while *S. saevissima* was kept in the dark during the experiment. After application, the insects were kept in individual Petri dishes, containing a mixture of sugar (85%), honey (15%) and water as food. The mortality counts have been made after 6, 12, 24 and 48 h after treatment.

**Data analysis**

Mortality data were subjected to analysis of variance, and the averages were compared by the Scott-Knott grouping analysis test ($p < 0.05$). Dose-mortality data of active compounds were corrected by Abbott’s method and then subjected to probit analysis using PROC PROBIT procedure of Statistical Analysis System (SAS) program to estimate dose-mortality curves. The curves that presented probabilities greater than 0.05 by the $\chi^2$ test were accepted. These curves were used to estimate the lethal dose (LD) that causes 50 and 90% mortalities. Time-mortality data were subjected to survival analysis ($p < 0.05$) with non-parametric Kaplan-Meier estimator using LIFETEST procedure. The survival curves constructed were compared by log-rank test ($p < 0.05$) and the median survival times (LT$_{50}$) of the larvae were estimated. To evaluate the selectivity, mortality of non-target species were compared with pest mortality by $t$-test for independent samples ($p < 0.05$).

**Supplementary Information**

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

**Acknowledgments**

We are grateful to FAPEMIG, CNPq, and CAPES for financial support.

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Submitted: July 10, 2015
Published online: August 28, 2015