Phytogrowth-Inhibitory Lactones Derivatives of Glaucolide B

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The sesquiterpene lactone glaucolide B (1), isolated from Vernonia fruticulosa (Asteraceae), was transformed into six lactones (2–7). The structures of the products were elucidated by spectroscopic analysis. A series of solutions of compounds 1–7, at 200 µm, were tested on the germination and on the root and shoot growth of the dicotyledons Physalis ixocarpa and Trifolium alexandrinum and of the monocotyledons Lolium multiflorum and Amaranthus hypochondriacus. Lactone 5 exhibited clear selectivity towards dicotyledonous species at 200 µm, with an average inhibition of 90% on the germination of P. ixocarpa. Lactones 1, 3 and 4 had a greater effect on root length of monocotyledonous species, inhibiting around 70% at 200 µm in L. multiflorum. It seems that the diol function is required in lactones 4–6 to increase the activity, the polarity in the molecule might be required to reach its target.

Key words: Sesquiterpene Lactones, Herbicidal Activity, Germination and Growth Inhibition

Introduction

Many natural products isolated from plants and microorganisms have been used as pesticides or as lead structures for the preparation of various analogues with highest activity (Arnason et al., 1989). It has been estimated that approx. 100 patents are submitted every year concerning the discovery of new natural compounds with potential use in agriculture for pest control (Pillmoor et al., 1993). Most known phytotoxins are obtained from fungi and bacteria (Duke et al., 1987, 1996; Duke and Lyndon, 1987; Greaves, 1996; Kimura et al., 1997, 1998, 2002; Pillmoor, 1998). Green plants produce hundreds of thousands of compounds that are not involved in primary metabolism, the so-called secondary metabolites. These compounds seem to function as chemical warfare agents against insects, pathogenic organisms, and competing plants. According to their action they are known as allelochemicals and are in fact natural herbicides, and the interactions in which the plant uses them are through the phenomenon of allelophathy. The study of the chemical composition of plants with allelopathic effects leads to the isolation of many compounds, which have a wide diversity of skeletal types, and act as allelochemicals or have phytogrowth inhibitory properties (Cespedes et al., 2000; Dakshini and Einhellig, 1995; Lotina-Hennsen et al., 1998). Among such compounds, many lactones have shown to possess strong phytotoxic activity against several weeds (Macias et al., 1998; Wedge et al., 2000).

In the course of our continuing efforts to discover new natural and synthetic herbicides (Barbosa et al., 1997, 2003; Costa et al., 2000; Demuner et al., 1998; Jimenez et al., 1998; Lima et al., 2003; Rojas et al., 2000), we describe the preparation of new lactones derivatives of glaucolide B, a sesquiterpene isolated from Vernonia fruticulosa (Asteraceae) (Padolina et al., 1974). We also report the phytogrowth effect of a series of aqueous solutions of the lactones at concentrations between 50–200 µm on seed germination and growth of dicotyledonous species (Physalis ixocarpa, Trifolium
*alexandrinum*) and monocotyledonous species (*Lolium multiflorum* and *Amaranthus hypochondriacus*).

**Materials and Methods**

**General procedures**

Melting points were obtained with a MQAPF301 digital apparatus. Infrared spectra were registered on a Perkin Elmer FTIR PARAGON 1000 spectrophotometer, using a potassium bromide disk, scanning from 625 cm⁻¹ to 4000 cm⁻¹. ¹H and ¹³C NMR spectra were recorded on a Bruker ACP 400 instrument (400 MHz and 100 MHz, respectively), using deuterated chloroform as a solvent and tetramethylsilane (TMS) as a reference (δ = 0). The coupling constants are given in Hertz. Chromatographic purifications were carried out using silica gel (63–230 μm). Solvents were purified as described by Perrin and Armarego (1988). Glaucolide B, used as a starting material, was isolated according to the procedure previously described (Costa et al., 2000).

**Syntheses**

**Hydrogenation of glaucolide B.** Glaucolide B (1) (1.1 g, 2.5 mmol) was dissolved in ethyl acetate (10 ml), in a Parr hydrogenation bottle and 10% Pd-C (14 mg) was added as a catalyst. The reaction was carried out under 1.0 × 10⁵ Pa of hydrogen pressure and at room temperature for 6 h. When the hydrogen uptake ceased, the catalyst was filtered off through a Celite pad and the solvent evaporated under reduced pressure. The resultant crude product was purified by silica gel flash chromatography (chloroform/diethyl ether, 5:1 v/v) to afford compound 2 (0.7 g, 1.84 mmol; 74%) and compound 3 (0.24 mg, 0.63 mmol; 25%).

**Data for 2.** White solid, m.p. 145–147 °C. – IR (KBr): ν<sub>max</sub> = 2935, 1770 (C=O, lactone), 1735 (C=O, ester), 1710 (C=O, ketone), 1654 (C=C), 1560, 1457, 1375, 1301, 1244, 1217, 1109 cm⁻¹. – ¹H NMR (CDCl₃, 400 MHz, 333 K): δ = 4.82 (brd, J = 9.5 Hz, H-8), 4.72 (dq, J₁ = 2.0 Hz, J₂ = 9.6 Hz, H-6), 2.88 (m, H-9β), 2.69 (dd, J₁ = 15.2 Hz, J₂ = 4.0 Hz, H-9α), 2.30–2.65 (m, H-2α, H-2β, H-3α, H-3β, H-5), 2.06 (s, COCH₃), 2.02 (s, COCH₃), 1.96 (d, J = 2.0 Hz, 13-CH₃), 1.60 (s, 14-CH₃ and 15-CH₃). – ¹³C NMR (CDCl₃, 100 MHz, 300 K): δ = 206.90 (C=O, ketone), 173.13 (C=O, acetate), 169.97 (C=O, acetae), 169.67 (C=12), 157.30 (C-7), 121.83 (C-11), 85.03 (C-10), 80.93 (C-6), 65.80 (C-8), 64.17 (C-4), 61.01 (C-5), 59.69 (C-13), 40.13 (C-9), 32.83 (C-2), 32.59 (C-3), 20.95 (2 × COCH₃), 20.22 (C-15), 19.00 (C-14), 9.60 (C-13). – C₁₉H₂₄O₈: calcd. C 59.99, H 6.36 and O 33.65%; found C 59.97, H 6.39 and O 33.64%.

**Data for 3.** White solid, m.p. 198–201 °C. – IR (KBr): ν<sub>max</sub> = 2943, 1769 (C=O, lactone), 1738 (C=O, ester), 1720 (C=O, ketone), 1458, 1374, 1341, 1246, 1190, 1107, 1071 cm⁻¹. – ¹H NMR (CDCl₃, 400 MHz, 238 K), Conformer A: δ = 5.60 (t, J = 6.4 Hz, H-8), 4.31 (dd, J₁ = 5.9 Hz, J₂ = 5.1 Hz, H-6), 3.40 (d, J = 5.9 Hz, H-5), 2.80 (dq, J₁ = 5.6 Hz, J₂ = 4.8 Hz, H-11), 2.59 (dd, J₁ = 5.1 Hz, J₂ = 4.8 Hz, H-7), 2.35 (m, H-9α), 2.15 (m, H-9β), 2.02 and 2.09 (2 s, AcO), 1.22 (d, J = 5.4 Hz, CH₃-13), 2.15–2.50 (m, 2H-2, 2H-3), 1.54 and 1.48 (2s, CH₃-15 and CH₃-14). Conformer B: 5.39 (dd, J₁ = 7.0 Hz, J₂ = 3.2 Hz, H-8), 4.38 (dd, J₁ = 5.7 Hz, J₂ = 4.5 Hz, H-6), 2.82 (d, J = 4.5 Hz, H-5), 3.05 (ddd, J₁ = 6.8 Hz, J₂ = 4.9 Hz, J₃ = 3.2 Hz, H-7), 2.75–2.90 (m, H-11), 2.00–2.50 and 3.10–3.20 (m, 2H-2, 2H-3, 2H-9), 2.14 and 2.09 (2 s, AcO), 1.38 (d, J = 5.6 Hz, CH₃-13), 1.62 and 1.75 (2 s, CH₃-14 and CH₃-15). – ¹³C NMR (CDCl₃, 100 MHz, 238 K), for both conformers: δ = 209.14 and 203.69 (C=O), 176.54 and 177.54 (C=O, O-COCH₃), 170.40 and 170.36 (C=O, O-COCH₃), 169.53 and 169.36 (C-12), 83.57 and 83.44 (C-10), 80.42 and 79.00 (C-6), 67.63 and 63.35 (C-8), 60.71 and 60.45 (C-4), 60.01 and 58.85 (C-5), 47.38 and 44.10 (C-11), 40.04 and 38.69 (C-7), 37.53 and 36.82 (C-9), 36.22 and 35.56 (C-3), 34.39 and 32.94 (C-2), 29.71 and 24.58 (O-COCH₃), 21.13 and 21.02 (O-COCH₃), 18.16 and 17.80 (C-15), 16.64 (C-14), 11.90 and 9.64 (CH₃-11). – C₁₉H₂₆O₈: calcd. C 59.68, H 6.85 and O 33.47%; found C 59.65, H 6.85 and O 33.50%.

**Hydrolysis of glaucolide B.** A mixture of glaucolide B (0.3 g, 0.68 mmol), trifluoroacetic acid (5 ml) and water (0.5 ml) was stirred at room temperature for 30 min. After this time, the solvent was removed under reduced pressure in a rotary evaporator to leave a white residue. To this residue diethyl ether (20 ml) and a saturated solution of NaHCO₃ (10 ml) were added. The two phases were separated and the aqueous layer was extracted with diethyl ether (5 × 20 ml). The combined organic extracts were washed with water, followed by drying over MgSO₄. After filtration the solvent was removed under reduced pressure and the yellow oil obtained was purified by silica gel column chromatography (diethyl ether/diethyl-
romethane 10:1 v/v) to afford 65% (0.2 g, 0.44 mmol) of compound 4 as a white solid.

**Data for 4:** M.p. 136–138 °C. – IR (KBr): ν\textsubscript{max} = 3447, 2928, 1749 (C=O, lactone, ester and ketone), 1654 (C=C), 1458, 1374, 1458, 1375, 1259, 1158, 1065 cm\(^{-1}\). – \( ^{1}\)H NMR (CDCl\(_3\), 400 MHz, 300 K): δ = 5.90 (brs, OH), 5.10 (d, J = 9.2 Hz, H-6), 4.91 (d, J = 12.8 Hz, H-13a), 4.75 (d, J = 12.8 Hz, H-13b), 3.56 (d, J = 9.2 Hz, H-5), 3.20–3.40 (br signal, OH), 2.58 (dd, J\(_{1}\) = 15.6 Hz, J\(_{2}\) = 3.6 Hz, H-9\(\alpha\)), 2.10–2.25 (m, H-2α, 2β, 3α, 3β), 1.83 (m, 9β), 2.09 (COCH\(_3\)), 2.09 (COCH\(_3\)), 2.19 (COCH\(_3\)), 1.62 (H-15), 1.50 (H-14). – \( ^{13}\)C NMR (CDCl\(_3\), 100 MHz, 300 K): δ = 171.60 (COCH\(_3\)), 171.06 (COCH\(_3\)), 170.30 (COCH\(_3\)), 169.75 (C-12), 168.54 (C-7), 123.20 (C-11), 86.98 (C-10), 86.59 (C-8), 82.03 (C-6), 76.18 (C-4), 68.49 (C-5), 55.81 (C-13), 41.73 (C-9), 34.70 (C-2), 33.93 (C-3), 22.22 (COCH\(_3\)), 21.97 (COCH\(_3\)), 20.93 (COCH\(_3\)), 20.28 (C-15), 17.56 (C-14). – C\(_{21}\)H\(_{28}\)O\(_{11}\); calcd. C 55.26, H 6.17 and O 38.53%.  

**Hydrolysis of compound 2.** Treatment of compound 2 (0.3 g, 0.78 mmol) under the same conditions as previously described for glaucolide B, afforded lactone 5 in 76% yield (0.23 g, 0.59 mmol).

**Data for 5:** White solid, m.p. 147–149 °C. – IR (KBr): ν\textsubscript{max} = 3447, 2928, 1740 (C=O, lactone, ester and ketone), 1654 (C=C), 1458, 1374, 1244, 1112, 1075 cm\(^{-1}\). – \( ^{1}\)H NMR (CDCl\(_3\), 400 MHz, 300 K): δ = 5.85 (m, H-8), 5.01 (dq, J\(_{1}\) = 2.0 Hz, J\(_{2}\) = 9.6 Hz, H-6), 3.73 (m, H-8), 3.66 (d, J = 9.6 Hz, H-5), 2.62 (dd, J\(_{1}\) = 15.2 Hz, J\(_{2}\) = 4.0 Hz, H-9β), 2.10–2.25 (m, H-2α, 2β, 3α, 3β, 9α, OH), 2.09 (s, COCH\(_3\)), 2.03 (brs, COCH\(_3\)), 1.92 (d, J = 2.0 Hz, H-13), 1.64 (s, H-15), 1.51 (s, H-14). – \( ^{13}\)C NMR (CDCl\(_3\), 100 MHz, 300 K): δ = 173.16 (COCH\(_3\)), 171.50 (COCH\(_3\)), 169.62 (C-12), 162.47 (C-7), 87.15 (C-10), 86.65 (C-8), 83.70 (C-6), 76.20 (C-5), 68.80 (C-4), 34.83 (C-2), 33.90 (C-3), 22.25 (COCH\(_3\)), 21.90 (C-15), 20.46 (C-14), 9.87 (C-13). – C\(_{19}\)H\(_{28}\)O\(_{9}\); calcd. C 56.99, H 7.05 and O 35.96%; found C 57.00, H 7.02 and O 35.98%.

**Deoxgenation of compound 2.** Trimethylsilylchloride (0.20 ml, 1.56 mmol) was added via syringe to a stirred solution of lactone 2 (0.30 g, 0.79 mmol) and sodium iodide (0.23 g, 1.56 mmol) in dry THF (8 ml). The mixture was stirred at room temperature for 3 h under nitrogen atmosphere. After that period of time, the reaction was quenched by addition of Na\(_2\)S\(_2\)O\(_3\) (0.5 m, 7 ml) and the product extracted with diethyl ether (5 × 20 ml). The combined organic extracts were washed with saturated aqueous solution of NaCl (3 × 30 ml) and with water (30 ml). The organic phase was dried (MgSO\(_4\)) and concentrated to give a pale yellow oil. This oil was purified by silica gel flash chromatography (hexane/diethyl ether, 2:1 v/v), to afford compound 7 as a pale yellow oil in 26% yield (0.076 g, 0.21 mmol).

**Data for 7:** IR (NaCl): ν\textsubscript{max} = 3080, 2990, 2920, 1750 (C=O, lactone and ester), 1712 cm\(^{-1}\) (C=O, ketone). – \( ^{1}\)H NMR (CDCl\(_3\), 400 MHz, 300 K): δ = 4.76 (brd, 2H, J = 9.6 Hz, H-5 and H-8), 4.60 (d, J = 8.4 Hz, H-6), 1.70–2.50 (m, 2H-2, 2H-3, 2H-9), 2.08 (s, OC\(_{2}\)O\(_{3}\)), 2.07 (s, OC\(_{2}\)O\(_{3}\)), 1.94 (s, CH\(_{3}\)-13), 1.63 and 1.60 (CH\(_{3}\)-15 and CH\(_{3}\)-14). – C\(_{19}\)H\(_{28}\)O\(_{7}\); calcd. C 62.63, H 6.64 and O 30.73%; found C 62.58, H 6.68 and O 30.74%.

**Seed germination and growth (root and shoot elongation) bioassays**

Seeds of the dicotyledons Physalis ixocarpa and Trifolium alexandrinum and the monocotyledons Lolium multiflorum and Amaranthus hypochon-
driacus were obtained from Central de Abastos, market in Mexico City. All undersized and damaged seeds were discarded and the assay seeds were preselected for uniformity. Bioassays were performed by germinating 40 seeds of each species for 5 d (three for germination and two for root and shoot growth) in 9 cm Petri dishes containing a 10 cm sheet of Whatman n° 1 paper and 10 ml of test or control solution. Seeds were incubated in the dark at 25 °C in a controlled chamber. A stock solution (20 mM) was prepared using DMSO as the initial solubilizing agent. This was then diluted with water to give a final concentration of 200 µM. Control experiments were also conducted with deionized water and with the same DMSO concentration. The seed germination is presented as percentage differences from control in Fig. 2 and 3, after 3 d of incubation. After two more days of incubation, the root and shoot length were measured to the nearest millimeter. All treatments were replicated four times in a completely randomized design. The percentage of root and shoot growth inhibition was calculated in relation to the control. Zero represents the control, positive values represent stimulation of the studied variables and negative values represent inhibition. Tukey’s test with a significance of 0.05 was applied between two sets of experiments.

**Results and Discussion**

Catalytic hydrogenation of glaucolide B, isolated from V. fruticulosa yielded, after 6 h, a mixture of two solid compounds identified as lactones 2 (74%) and 3 (25%). These compounds have been previously prepared (Padolina et al., 1974), but were only partially characterized. Compound 2 presented an EIMS with a molecular ion at m/z 380.1468 and compound 3 at m/z 382.1625, corresponding to the respective molecular formulas C₁₉H₂₄O₈ and C₁₉H₂₆O₈. As in the case of glaucolide B, the ¹H NMR spectra of 2 and 3, at −35 °C and 27 °C, were very complex and not well resolved, and this was attributed to the presence of several conformers. For compound 2, when its spectrum was obtained at the temperature of 60 °C, some of the signals were still broad, but the spectrum was less complex, showing two singlets at δ 2.06 and 2.02 assigned to the acetate groups, and a doublet (J = 2 Hz) at δ 1.96, due to the new methyl group attached to C-11. The homoallylic coupling between H-6 and CH₃-11 was confirmed by the COSY experiment. For this compound, a ¹³C NMR spectrum was obtained at 27 °C, and although some of the signals were broad, it was possible to make all the assignments, as the spectrum was very close to that of glaucolide B.

The ¹H NMR spectra of compound 3 obtained at −35 °C and 27 °C were almost identical, and showed a mixture of two conformers, in an approximate ratio of 55:45. The assignment of the signals for each one was possible by the analysis of the COSY spectrum. Although no attempt was made to propose the structure for each conformer, it was observed that for conformer A, the resonance of H-7 was a double doublet (J₁ = 5.1 and J₂ = 4.8 Hz) at δ 2.59 that was correlated with H-6 and H-11 in the COSY experiment, but not with H-8. So, the triplet observed for H-8 at δ 5.60 reveals that this hydrogen has a vicinal coupling only with the methylene group. In the case of conformer B the signal of H-7 appeared as a double doublet (δ 3.05). In the COSY spectrum, it was observed that this hydrogen is coupled with H-6, H-8 and H-11. From these data it is clear that in this conformation H-8 is coupled only with one of the hydrogens at C-9.

The ¹³C NMR spectrum also revealed duplicated signals, and no effort was made to complete unambiguous assignment for all carbons.

Treatment of compounds 1, 2 and 3 with trifluoroacetic acid yielded respectively the alcohols 4 (65%), 5 (76%) and 6 (68%). For all these three compounds the infrared spectra showed a very strong band around 3400–3500 cm⁻¹, confirming the presence of the hydroxyl groups. Compounds 4 and 5 also showed large and strong absorptions at 1749 cm⁻¹ and 1740 cm⁻¹, respectively, attributed to all carbonyl (lactone, ester and ketone) groups. In case of 6, absorptions at 1754 cm⁻¹ (C=O, ester and lactone) and 1709 cm⁻¹ (C=O, ketone) were observed.

For compound 4 the ¹H NMR spectrum was run at −35 °C and at 27 °C. Although in both cases signals corresponding to only one conformer were observed, small differences were observed. For example, H-9α gave rise to a broad doublet at δ 2.48 (J = 9.9 Hz) at −35 °C, and a double doublet at δ 2.58 (J₉,₃₉α = 15.60 Hz, J₉₆,₈ = 3.60 Hz, H-9α) at 27 °C. These differences suggest that compound 4 exists in different conformations at each temperature. At 27 °C two singlets at δ 1.62 and δ 1.50, attributed to the methyl groups (CH₃-14 and CH₃-15), and also the singlets at δ 2.19 and δ 2.09 (from
the acetates) confirm the existence of only one conformer.

The $^1$H NMR spectrum of 5 at 27 °C was consistent with the required structure and showed signals corresponding to only one conformer. The major diagnostic signals were the four singlets corresponding to the methyl groups, and the doublet ($J = 2.0$ Hz) at $\delta 1.92$ assigned to the methyl group attached to C-11, that showed a homoallylic coupling with H-6. The signals corresponding to the other hydrogens appeared as a broad band, as it was also the case of singlets at $\delta 2.09$ and $\delta 1.64$ that were broadened, as observed for glaucolide B (Costa et al., 2000). The $^{13}$C NMR spectrum showed some very broad signals and the presence of a minor conformer, by the observation of several low intensity peaks, although it was not observed in the $^1$H NMR spectrum.

For compound 6 the $^1$H NMR and $^{13}$C NMR spectra were also run at $-35 \degree C$ and at 27 °C and in both cases signals corresponding to only one conformer were observed. A detailed assignment of the NMR signals was possible by 2D COSY and HETCOR experiments. At $-35 \degree C$ the $^1$H NMR spectrum showed two multiplets at $\delta 5.09$ and $\delta 3.05$ for H-8 and H-7, respectively, and at 27 °C those hydrogens gave rise to two double double doublets at $\delta 5.14$ and $\delta 3.04$ for H-8 and H-7. All the other signals were almost identical at both temperatures.

Treatment of compound 2 with chlorotrimethylsilane and sodium iodide resulted in 7 as a pale yellow oil. The key feature in the $^1$H NMR spectrum, run at 27 °C, is the broad doublet at $\delta 4.75$, that integrated for three hydrogens and assigned to the olefinic protons H-5, H-6, and H-8. At $-35 \degree C$ the resonance of H-6 appeared as a doublet ($J = 8.4$ Hz) at $\delta 4.60$ and the signals of H-5 and H-8 appeared superimposed as a broad doublet at $\delta 4.76$. All the five methyl groups gave rise to sharp singlets in the expected region, and other hydrogen signals appeared as a complex multiplet in the region of $\delta 1.70$–2.50.

The results of the effects produced by the lactones 1–7 (Fig. 1) on the germination and growth (root and shoot development) inhibition of T. alexandrinum, L. multiflorum, P. ixocarpa, and A. hypochondriacus are shown by Fig. 2 and 3.

The most relevant effect observed was a strong inhibition of germination of the P. ixocarpa species by 5, 90% at 200 µm (Fig. 3) over all others species tested. None of the other compounds (1–4 and 6, 7) caused a significant effect on the germination (< 50% inhibition) of A. hypochondriacus, T. alexandrinum and L. multiflorum (Fig. 2 and 3) at 200 µm concentration.

Sensitivity of compound 5 on germination inhibition decreases in L. multiflorum (22%, 200 µm), A. hypochondriacus (12%, 200 µm) and T. alexandrinum (5%, 200 µm) species. Therefore, compound 5 is selective in affecting the germination of the dicotyledon P. ixocarpa. However, compounds 4 and 6 have significant inhibition on ger-
germination of *L. multiflorum*, 4 40% at 200 µm and 6 48% at 200 µm, respectively. All the other compounds had little effect on the germination of these plants (< 30%) (Fig. 2 and 3).

Root length (Fig. 2 and 3) is inhibited for almost all the lactones tested in different degrees except for 2 in *L. multiflorum*. The most significant effects found for the weed *L. multiflorum* species were obtained with 1, 3 and 4 by around 70% at 200 µm, 6 with 58% at 200 µm (Fig. 2) and for the dicotyledon *P. ixocarpa* species it was produced with 5, 59% at 200 µm (Fig. 3). The other species were less inhibited by 5 at 200 µm, *T. alexandrinum* < 40% and *A. hypochondriacus* 14%, and the same plants by 30% and 35% with 7 at 200 µm, respectively. Compound 2 exerts a minor effect on root length development of the other species tested (Fig. 2 and 3).

On the other hand, compound 5 inhibited shoot length of *T. alexandrinum*, *P. ixocarpa* and *A. hypochondriacus* by 70%, 47% and 50%, respectively, at 200 µm (Fig. 3) and compound 6 inhibited shoot length of both *L. multiflorum* and *A. hypochondriacus* by 40 and 42% at 200 µm (Fig. 2). Other compounds had minor effects on shoot length (Fig. 2 and 3). In low concentrations (50–100 µm) the effects of these lactones on the radical and shoot length as well as on germination, were little or not significant (data not shown). A review of the literature on allelopathy reveals that most allelochemicals were thought to be involved in plant-plant interactions are not very phytotoxic when compared to commercial herbicides (Duke and Lyndon, 1987) and most of the compounds partially inhibit the activity. Here we found that compound 5 at 200 µm, caused a stimulatory effect (90%) on the germination of *P. ixocarpa*.

The work reported leads to the following conclusions. These compounds exhibit clear selectivity over germination inhibition of the dicotyledon *P. ixocarpa* (90% at 200 µm) by compound 5, the other species are less affected (< 50%). Compounds 1, 3 and 4 exhibit also a clear selectivity over root length development inhibition by 70%, 72% and 73% at 200 µm of the weeds *L. multiflorum*; the other species were less sensible. Compound 5 shows selectivity in inhibiting shoot length of *T. alexandrinum* by 70% at 200 µm and the other species tested were less affected. The other compounds tested had between moderate and minor effects. Therefore the lactones studied in this work produce an inhibitory effect on germi-
nation and growth of the plant species tested. The above finding suggests that compound 5 had a wide spectrum of action against germination and growth of monocotyledonous and dicotyledonous species, being more active on dycotyledonous (P. ixocarpa). However, some lactones (1, 3, 4) inhibit the weed L. multiflorum.

Among the 500 compounds tested in our laboratory on T. alexandrinum only 5 was active in inhibiting germination and growth. Therefore, further studies on compound 5 are in progress.

Although no clear structure-activity relationships can be obtained from the results presented, it is clear that the diol function (4–6) is required for increasing activity in general. This difference in activity could be due to the polarity of the compounds, or to a change in conformation, as suggested by Macias et al. (1998) for other germacrarnolides. On the basis of these results, such compounds are good candidates as lead templates for the preparation of more active analogues that could result in a new generation of green agrochemicals derivatives.

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