

Sesquiterpene Lactones with Potential Use as Natural Herbicide Models (I): *trans,trans*-Germacranolides[†]

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A structure–activity study to evaluate the effect of the *trans,trans*-germacranolide sesquiterpene lactones costunolide, parthenolide, and their 1,10-epoxy and 11,13-dihydro derivatives (in a range of 100–0.001 μM) on the growth and germination of several mono and dicotyledon target species is accomplished. Results are compared with those obtained in the same bioassay with an internal standard, the commercial herbicide Logran, to validate the results with a known active formulation and to compare the results with a commercial product to test their potential use as natural herbicide models. These compounds appear to have a more selective effects on the radicle growth of monocotyledons. Certain factors such as the presence of nucleophile-acceptor groups and their accessibility enhance the inhibitory activity. The levels of radicle inhibition obtained with some compounds on wheat are totally comparable to those of Logran and allow to propose them as lead compounds.

Keywords: *trans,trans*-Germacranolide; costunolide; parthenolide; Logran; phytotoxicity; allelopathy; herbicide; *Lactuca sativa* L.; *Lycopersicon esculentum* L.; *Lepidium sativum* L.; *Allium cepa* L.; *Triticum aestivum* L.

INTRODUCTION

Modern agricultural techniques heavily rely on the use of pesticides and herbicides with the subsequent environmental risks. Otherwise, the development of resistant species and cross-resistance phenomena are challenging the use of herbicides for certain weeds (Maxwell and Mortimer, 1994). Allelopathy offers a new approach for the discovery of new lead compounds and their use as herbicides and pesticides from plants, fungi, and microorganisms (Duke et al., 1996). Several classes of compounds have been named as common allelopathic agents: phenolic compounds, monoterpenes, sesquiterpenes, and alkaloids among them (Waller, 1987).

Sesquiterpene lactones have been reported as allelopathic agents (Spring et al., 1981; Macías et al., 1993) in many plants belonging to the family Compositae. Despite the high number of sesquiterpene lactones described (more than 7500 (Fraga, 1998)) and their biological activities (Marles et al., 1995; Francois et al., 1996; Beekman et al., 1997), little has been done to determine which of them are really phytotoxic, the necessary structural requirements to show this kind of bioactivity (Ando et al., 1992; Baruah et al., 1994), or their possible modes of action in plants (Lotina-Hennsen et al., 1992; Galindo et al., 1999). Going on with our systematic study of sesquiterpene lactones as natural herbicide models (Macías et al., 1992; Macías et al., 1999a), the results of bioassays with seven different *trans,trans* germacrane-type sesquiterpene lactones are presented. Costunolide (1), parthenolide (2), 1,10-

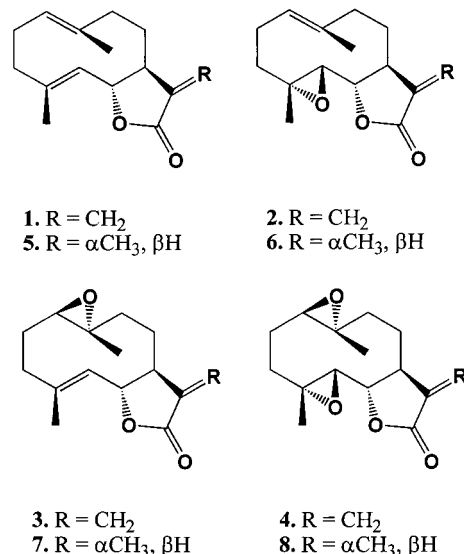


Figure 1. *trans,trans*-Germacranolides tested.

epoxycostunolide (3), 1,10-epoxyparthenolide (4), and their 11,13-dihydro derivatives (5–7) (Figure 1) have been tested for germination and growth activity on dicotyledonous and monocotyledonous standard target species. Dicots assayed are lettuce (*Lactuca sativa* L. cv. Roman), tomato (*Lycopersicon esculentum* L. cv. Tres Cantos), and cress (*Lepidium sativum* L. cv. Común). Monocots assayed are onion (*Allium cepa* L. cv. Valenciana) and wheat (*Triticum aestivum* L. cv. Cortex). The study has been centered on the three main macroscopic parameters: germination and root and shoot elongation. These parameters are accepted as indirect measures of other different physiological processes affected by the assayed chemicals. A commercial herbicide is introduced within the same bioassay to serve as internal standard.

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[†] Natural Products as Allelochemicals. 11. For part 10, see: Macías et al., 1999a.

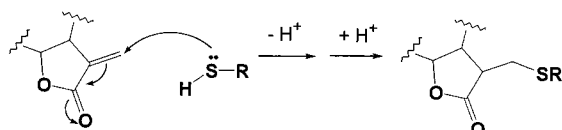


Figure 2. Michael nucleophilic attack of a nucleophile to the α -methylene moiety.

Thus, the comparison of the commercial herbicide effects in the same conditions as the sesquiterpene lactones tested allow to validate the results with a well-known active formulation and to establish the relative levels of activity of these natural products respect to the herbicides actually in the market.

EXPERIMENTAL PROCEDURES

Costunolide (**1**) was obtained from costus resin oil (*Saussurea lappa*) by previous column chromatography (CC) separation and then purified by crystallization from hexane/ethyl acetate mixtures. Parthenolide (**2**) was easily available from magnolia leaf extract (*Magnolia grandiflora* L.) by CC and purification by crystallization from hexane/ethyl acetate. Epoxide derivatives **3** and **4** were prepared by treatment with *m*-chloroperbenzoic acid from **1** and **2**, respectively, as previously described (Rodríguez et al., 1978). Dihydro derivatives **5–7** were prepared respectively from **1–3** by reduction with NaBH_4 in a 0 °C methanolic solution during 1 h. All the structures were confirmed by comparison of their spectroscopical data (IR, EIMS, and ^1H and ^{13}C NMR) with those in the literature. Logran (terbutryn 59.4% + triasulfuron 0.6%) was supplied by Novartis.

Epoxidation of Costunolide (1) and Parthenolide (2). Fifty milligrams of **1** was dissolved in 10 mL of dried methylene chloride (DCM), and then 50 mg of sodium acetate was added. Afterward, 50 mg of *m*-chloroperbenzoic acid (*m*-CPBA) dissolved in dried DCM was added dropwise with stirring. After 1 h, the reaction was stopped and the workup was carried out as follows: the reaction mixture was extracted with NaOH (aq) 5% (2 \times) to separate the rest of the *m*-CPBA and *m*-chlorobenzoic acid; then, the pH of the organic phase was adjusted to nearly 7.0 by washing with distilled water (2 \times).

All of the aqueous phases were extracted with DCM, and the combined organic phases were dried on anhydrous sodium sulfate. After separation by CC, compound **3** was obtained in a crystalline form (77%). Epoxidation of **2** as described for **1** yield the crystalline product **4** (60%).

Reduction of 1–3. A 2 mL methanolic solution with 15 mg of each compound was kept in a Dewar glass at 0 °C. While the solution was stirring, NaBH_4 (1:1.4 molar relationship) was added during the first 5 min of reaction. After 1 h, the reaction was stopped by addition of 2 mL of distilled water. Extraction with AcOEt and HPLC purification (hexane/ethyl acetate mixt) yielded the dihydro derivatives **5–7** (average 90%).

Molecular Modeling. Minimum energy conformations and molecular properties were obtained by using MMX calculations (PCModel ver. 6.0, Serena Software, Bloomington, IN).

Germination and Seedling Growth Bioassays. Seeds of *L. sativa* L. cv. Roman, *L. esculentum* L. cv. Tres Cantos, *L. sativum* L. cv. Común, *A. cepa* L. cv. Valenciana, and *T. aestivum* L. cv. Cortex were obtained from FITÓ, S. L. (Barcelona, Spain). All undersized or damaged seeds were discarded, and the assay seeds were selected for uniformity. Bioassays were carried out in 9 cm \varnothing plastic Petri dishes, using Whatman No. 1 filter paper as support.

Bioassay Methodology. The general procedure was as follows: *L. sativum*, 25 seeds per dish, 5 mL of test soln, 4 days in the dark, 25 °C, and four replicates of each conc; *L. sativa* and *L. esculentum*, 25 seeds per dish, 5 mL of test soln, 5 days in the dark, 25 °C, and four replicates of each conc; *A. cepa*, 25 seeds per dish, 5 mL of test soln, 7 days in the dark, 25 °C, and four replicates of each conc; *T. aestivum*, 10 seeds per dish, 5 mL of test soln, 5 days in the dark, 25 °C, and 10 replicates of each conc (Macías et al., 1999b).

Test solutions (10^{-4} or 10^{-5} M) were prepared using MES (2-[*N*-morpholino]ethanesulfonic acid) 10 mM, and the rest were obtained by dilution. Parallel controls were performed. All pH values were adjusted to 6.0 before bioassay with MES. All products were purified prior to the bioassay using HPLC equipped with a refractive index detector. Minimum degree of purity was of 99% as extracted from the chromatograms.

Data are presented as percentage differences from control in graphics (Figures 4–6). Thus, zero represents the control; positive values represent stimulation of the studied parameter and negative values represent inhibition.

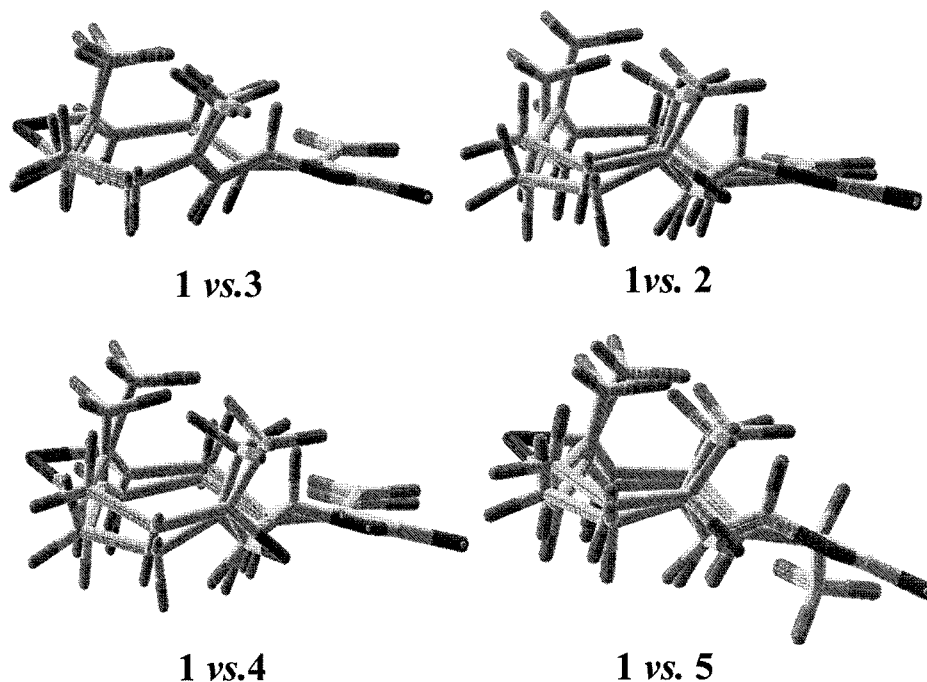


Figure 3. Selected comparison of minimum energy conformers obtained by using MMX calculations. Each figure represents the superimposed structures obtained by fixing those atoms of the γ -lactone ring taking as the reference structure the corresponding of costunolide (**1**).

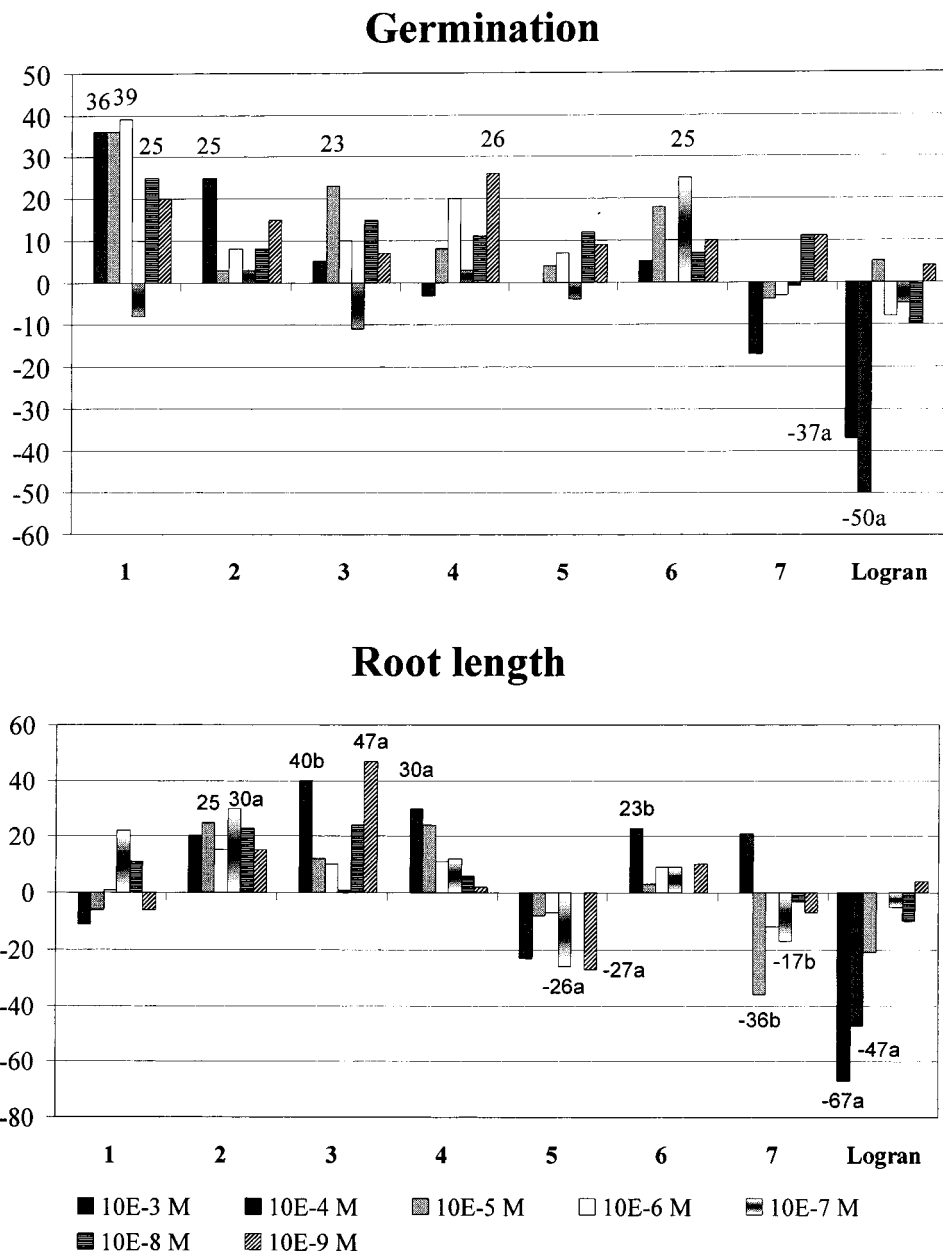


Figure 4. Germination and root length effects of compounds 1–7 on lettuce. Values are expressed as percentage differences from the control: (a) $P \leq 0.01$; (b) $0.01 < P \leq 0.05$; rest of the values, $P > 0.05$.

Statistical Treatment. Germination and root and shoot length values were tested by Welch's test (Zar, 1984), the differences between test solutions and controls being significant with $P \leq 0.01$.

RESULTS

Bioassay results are presented in Figures 4–6. Table 1 presents a resume of the main trends showed by every test compound on each target species.

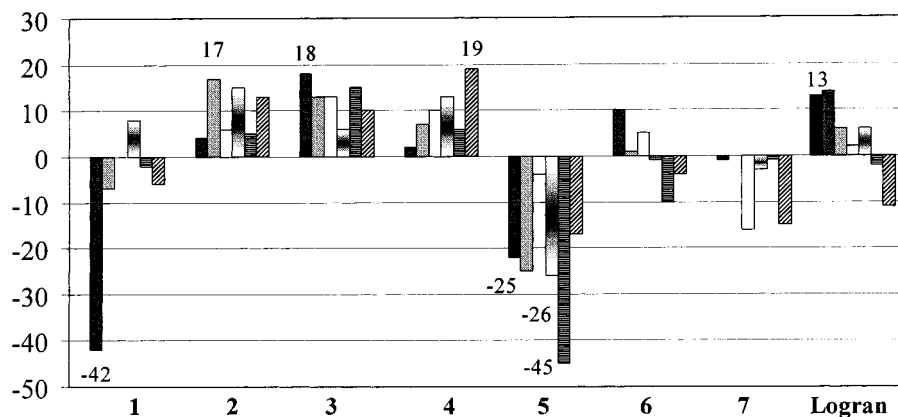
***L. sativa* L. (Figure 4).** Test compounds tend to stimulate the germination, with costunolide (1) being the most active. This effect reaches the maximum at 0.1–0.001 μM , and then it is lowered with the concentration. The introduction of an epoxide moiety (compounds 2–4, 6, 7) or an 11,13-dihydro functionalitation (5–7) appears to have a decreasing effect on the activity. No big differences of activity appear between parthenolide (2) and its 11,13-dihydro derivative. The internal standard (Logran) presents a clear inhibitory activity at 1 and 0.1 μM .

Root length is the other sensitive parameter. The activity of costunolide (1) at 10 μM over root growth of *Phaseolus vulgaris* (Kalsi et al., 1983) has been reported. Nevertheless, it has no important root-promoting effects on lettuce. Otherwise, the presence of an epoxide moiety in C4–C5 (2), C1–C10 (3), or both of them (4) seems to have an enhancing effect on the root growth. On the other hand, the reduction of the α -methylene group of parthenolide (2) to yield 6 results in a decrease of the activity. Reduction of 1 and 3 to yield 5 and 7, respectively, turns the activity on inhibitory.

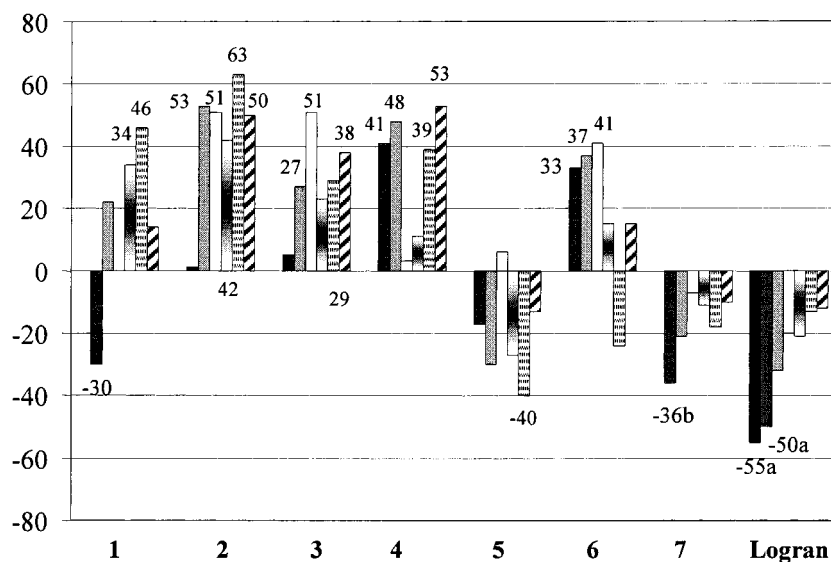
There is no activity on shoot length (data not shown) except that showed by compound 5, which inhibits shoot growth. All of these data are in good accordance with previous results obtained for the root-promoting activity of some guaianolides and some other sesquiterpenes (Kalsi et al., 1981, 1983).

***L. sativum* L. (Figure 5).** Costunolide (1) strongly inhibits cress germination at 100 μM , decreasing the activity with the dilution in a typical dose–response

Germination



Root length



■ 10E-3 M ■ 10E-4 M ■ 10E-5 M □ 10E-6 M ■ 10E-7 M ■ 10E-8 M ▨ 10E-9 M

Figure 5. Germination and root length effects of compounds 1–7 on cress. Values are expressed as percentage differences from the control: (a) $P \leq 0.01$; (b) $0.01 < P \leq 0.05$; rest of the values, $P > 0.05$.

format. Compounds 2–4, bearing an epoxide ring, generally tend to stimulate the germination, although the values are low. Reduction of costunolide (1) results in an enhancing of the inhibition at the lower concentrations, while reduction of compounds 2 and 3 results on a loss of the activity. Logran presents no phytotoxic effects in cress germination.

Root length and shoot length parameters present a similar behavior: both are stimulated by all the α -methylene-containing compounds, root length being more stimulated in cress than in lettuce. Otherwise, 100 μ M costunolide (1) shows a higher activity (inhibitory at high concentrations and stimulatory at low concentrations) in cress than in lettuce. This effect is more pronounced when an epoxide function is introduced (compounds 2–4). Comparison of the activities of compounds 1–4 with their dihydroderivatives 5–7 results again in a decrease of the stimulatory activity, changing some of them to inhibitors of the root growth (5, 7). Logran presents a pronounced root length inhibitory activity only at 1000 and 100 μ M and a weaker activity in shoot length.

A. cepa L. Tested compounds have no effects in germination and little effects on root or shoot growth (data not shown). Though the effects measured are low, all of them show a general trend to inhibit either root or shoot, the roots being a little more affected. This represents behavior opposite of that shown over dicotyledonous species. There are no big differences between compounds with or without epoxide and/or α -methylene moiety. Only compound 4, with two epoxide functionalizations, seems to maintain the activity at the lower concentrations.

T. aestivum L. (Figure 6). There is a general trend to enhance germination, but only the 11,13-dihydro derivative of costunolide (5) presents important values. Except for this case, little difference can be found on the activity related with the presence or absence of epoxides and/or α -methylene groups. Logran inhibitory effect is low and only significant for the highest concentration.

Root and shoot length are both generally inhibited by almost all compounds, except for the 11,13-dihydro derivative 7. The higher levels of root inhibition are

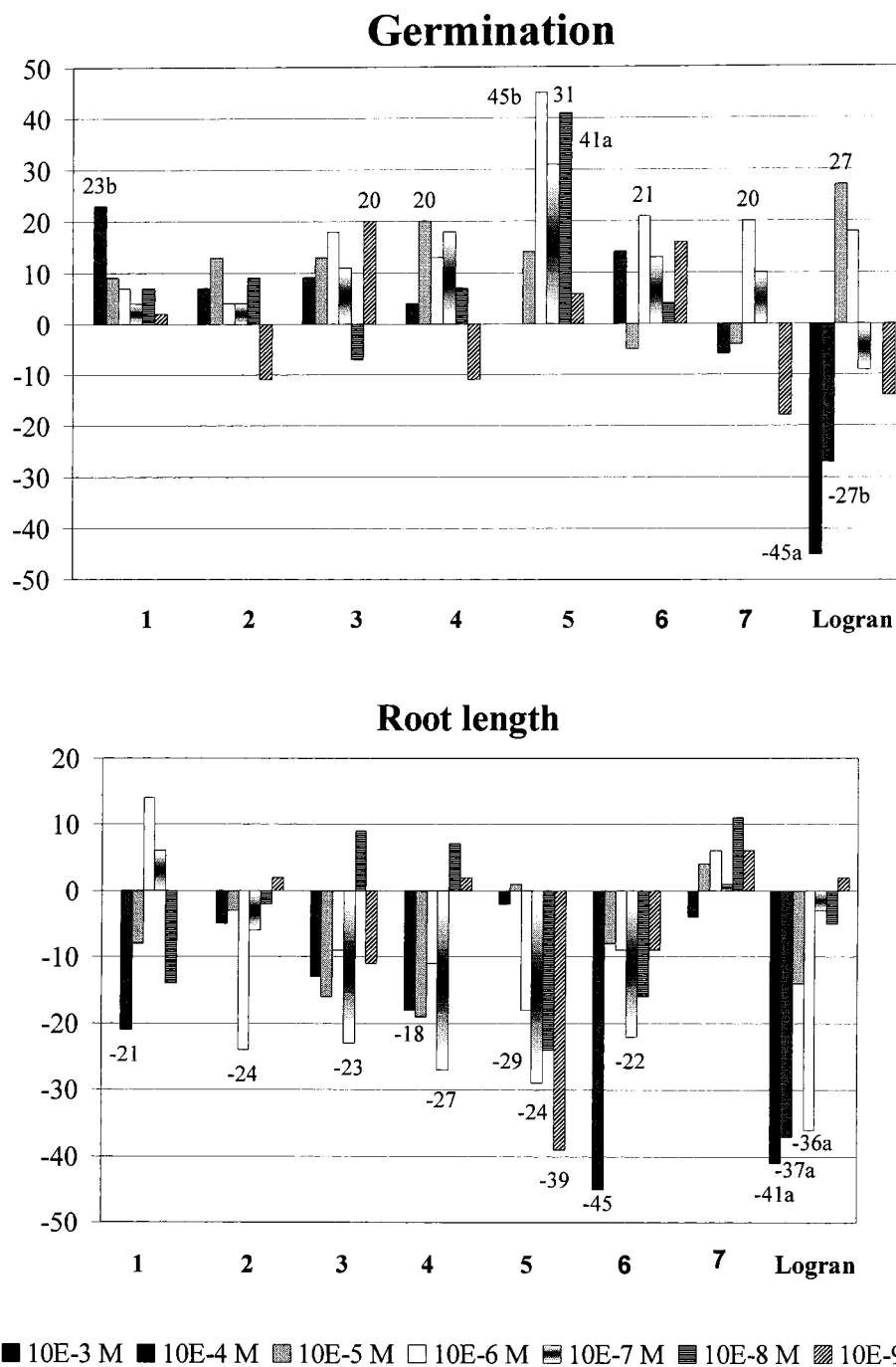


Figure 6. Germination and growth effects of compounds 1–7 on wheat. Values are expressed as percentage differences from the control: (a) $P \leq 0.01$; (b) $0.01 < P \leq 0.05$; rest of the values, $P > 0.05$.

those from compounds 5 and 6, both without the α -methylene group. At any rate, no distinctions can be made on the basis of the presence of an epoxide moiety or an 11,13-exomethylene group. This behavior is also illustrated in the shoot length parameter, where compound 5 is again the most active one.

DISCUSSION

From the results shown in Table 1 and Figures 4–6, three main observations can be made. First, the different mode of action shown by these compounds depends on if the target species belongs to a monocotyledons or dicotyledons, or even among the same family. While monocots are generally inhibited, dicots are stimulated.

Second, no activity is observed in the three parameters studied in tomato, which appears to be the less sensitive species. This result has been previously observed for other sesquiterpene lactones (Macías et al., 1999b).

Third, comparison of the values and profiles of activity shown by these lactones and those of the herbicide reveals that they are less effective than the commercial product on dicotyledons but show similar values of activity on monocotyledons.

The response of many test compounds with the concentrations may depend on their ability to act as inhibitors or as stimulants (phytohormone-like activity). In the case when they act as inhibitors, and if the range of test concentrations is correctly chosen, compounds

Table 1. Resume of the Bioactivity Data of Compounds 1–7^a

		lettuce	tomato	cross	onion	wheat
logran	germination	0	0	0	0	-
	root length	-	=	-	=	(-)
1	shoot length	=	-	-	=	(-)
	germination	++	0	-	0	+
2	root length	0	0	(-)(+)	(-)	-
	shoot length	0	0	(-)+	(-)	(-)
3	germination	+	0	(+)	0	(+)
	root length	+	0	++	(-)	(-)
4	shoot length	0	0	+	(-)	(-)
	germination	(+)	0	(+)	0	(+)
5	root length	++	0	+	0	(-)
	shoot length	0	0	+	(-)	-
6	germination	(+)	0	(+)	0	(+)
	root length	(+)	0	+	(-)	-
7	shoot length	0	0	+	(-)	(-)
	germination	0	0	0	(+)	0
	root length	0	0	(-)	(-)	0
	shoot length	0	(+)	(-)	(-)	0

^a 0: not active; (+), (-): stimulatory or inhibitory values below 20%; +', '-': Stimulatory or inhibitory values between 20% and 40%; ++, ==: stimulatory or inhibitory values higher than 40%.

usually exhibit a dose–response format activity profile: at the highest concentrations they tend to act as inhibitors and the activity decreases as the concentration lowers. In many cases, the activity can turn stimulatory as the test solution is diluted. This behavior can be found in the following cases: lettuce, compound 7, germination; cross, compound 1, germination and root growth, and compound 7, root growth; wheat, compound 6, root growth.

When the test compound is not a powerful inhibitor or the range of concentrations chosen falls out of the “inhibitory” zone, a different scenario can be drawn: there is low or no activity at the higher concentrations (no inhibition) and as the test solutions are more diluted the activity turns on stimulatory. Once the maximum of stimulatory activity has been reached, the activity decreases again toward to zero. This behavior can be observed in the following cases: cross, compounds 2–4, germination and root length; wheat, compound 1, germination and root length and compounds 3–6, germination; and lettuce, compounds 1–4, germination and root length.

Since these compounds seem to have enhancing and inhibiting activities depending on the concentration and they seem to be related with defensive roles in nature (mainly antifeeding and allelopathic), it does not look like they have a phytohormone-like activity, and it does not matter if they have a stimulatory activity at low concentrations.

Previously, the inhibitory effect of the *trans,trans*-germacranolides argophyllins A and B (*Helianthus tuberosus*) on the IAA-induced elongation of *Azukia angularis* hypocotyl sections (Watanabe et al., 1982), the inhibitory effect on the root growth of chinese cabbage by tatrudin B and (11*R*)-11,13-dihydrotatrudin B (Sashida et al., 1983), or the stimulation of witchweed germination by several germacranolide-type lactones (Fischer et al., 1990) have been reported.

Costunolide (1) is one of the two major constituents of the roots of *Saussurea lappa* (Kalsi et al., 1977), and

it has been reported to enhance rooting in stem cuttings of *Phaseolus aureus* (Kalsi et al., 1983). Its activity has been related to the presence of an α -methylene moiety in the γ -lactone ring and is enhanced up to four times when replaced by a cyclopropyl ring, very tensioned and susceptible to undergo ring-opening reactions by different reagents attacking in the same way as α -methylenes do with nucleophiles (Figure 2).

Recent reports show also costunolide (1) and the 1,10-epoxy (3) and 11,13-dihydro derivatives (5, 7) to elicitate the germination of the parasitic weed witchweed (*Striga asiatica* L., Kuntze) (Fischer et al., 1990). No significant differences were found among them, no matter the functionalization they possess. These compounds, and some other eudesmanolides with the same “double crown” conformation in the Decalin part of the molecule, are very active elicitors, while other guaianolides present low or even no activity. Thus, it was hypothesized that activity is given by a conjunction between the lactone moiety, also present in the natural witchweed germination promoter strigol, and the conformation of the medium ring, also close to that of strigol. No other reports about the effects of these kind of compounds on growth or germination have been published so far.

In a previous study (Macías et al., 1992), it has been proposed that the conformation of the molecule determines the accessibility of the lactone ring and thus, the activity of the molecule. Dramatic changes in the medium backbone ring cause important changes in the orientation of the lactone moiety and similar changes in the bioactivity should be expected. Some important theoretical molecular properties of the tested compounds, such as selected dihedral angles in the lactonic ring and van der Waals and water solvation surfaces, are shown in Table 2. These parameters have been selected since they are related to the orientation of the lactonic ring, the total volume of the molecule, and their solvation ability, which could be related with the activity.

Main changes in the orientation of the lactonic ring are observed when an epoxy group is introduced at positions C4–C5 and (C1–C10, C4–C5) (see Table 2, Figure 3, selected dihedral angles entry; comparison of compound 1 with compounds 2, 4, 6, and 8). Nevertheless, the most important differences can be observed in the distances between the two methyl groups at C-14 and C-15 where the closest position is obtained when the two epoxy groups at C1–C10, C4–C5 are presents in the molecule. These structural changes can be related with the different activity behavior of the tested compounds but not exclusively.

From the functional point of view, it should be expected that the introduction of a new electrophilic moiety will cause an enhancement of the inhibition, if a nucleophilic attack of the biomolecule to the inhibitor is postulated. The introduction of an oxirane ring at 4,5 causes a depletion in the inhibitory activity of 1. However, the introduction of an oxirane ring at 1,10 presents just the opposite effect (see effects on wheat root growth, Figure 6), which can be observed also in the 1,10–4,5 derivative 4. This behavior can be explained by the different accessibility of the oxirane ring in each side. The lactonic β -face is very hindered, and the effect of the introduction of this moiety is the change in the orientation of the lactonic ring (Table 2, Figure 3). The presence of an epoxide at 1,10 does not change

Table 2. Theoretical Molecular Properties Obtained Using MMX Calculations^a

	1	2	3	4	5	6	7	8
Selected Dihedral Angles (deg)								
H3 α ,C14	-151.05	-162.72	-149.01	-161.16	-151.21	-163.56	-148.84	-161.68
H5,H6	161.99	147.96	157.81	143.64	163.18	150.81	160.49	148.06
H6,H7	-167.77	-159.68	-168.21	-160.54	-166.64	-161.62	-168.15	-163.73
H7,C13	95.44	87.01	95.00	87.17	37.79	34.14	38.60	35.59
H6,C=O	-89.21	-93.76	-87.72	-92.17	-89.62	-92.05	-87.95	-89.92
H6,C12	-91.65	-96.03	-90.63	-94.89	-91.84	-93.92	-90.42	-92.10
van der Waals Area (Å ²)								
NPSSA	184.2	184.4	176.2	181.9	210.3	210.2	202.7	203.8
NPSUA	24.3	18.6	21.9	11.7	13.1	4.7	6.6	0.0
PSA	31.9	49.0	49.8	62.3	35.9	51.1	49.8	61.1
TSA	240.	252.	247.	255.	259.	265.	259.	265.
Water Solvation Shell (Å ²)								
NPSSA	314.5	309.2	310.0	304.0	349.2	342.9	341.7	336.9
NPSUA	30.5	18.9	21.1	14.6	10.6	2.6	6.0	0.0
PSA	71.0	89.1	90.3	106.8	67.4	85.6	86.9	103.9
TSA	416.	417	421.	425.	427.	430.	434.	440.
Accessible Water Solvation Shell (Å ²)								
NPSSA	296.4	291.4	292.6	286.9	328.6	323.1	322.1	317.6
NPSUA	21.8	13.5	15.0	10.4	7.5	1.8	4.3	0.0
PSA	56.7	71.1	72.1	85.4	53.9	68.5	69.6	83.3
TSA	374.	376.	379.	382.	390.	393.	396	400.

^a Key: NPSSA, nonpolar saturated surface area; NPUSA, nonpolar unsaturated surface area; PSA, polar surface area; TSA, total surface area.

so much the orientation of the lactone moiety (Figure 3), but introduces a more accessible and nucleophilic group, as can be observed in compound **4**. Thus, the observed increment of the inhibition could be explained through the introduction of the new electrophilic and accessible epoxy group.

Another point to compare is the effect on the activity of the reduction of the double bond conjugated with the lactonic carbonyl group. An enhancement of the growth inhibitory activity or a depletion of the stimulatory activity is observed on lettuce, cress, and wheat for the pairs (**1**, **5**), (**2**, **6**), and (**3**, **7**) (Figures 4–6). Thus, the reactivity cannot be related with the presence of an α,β -unsaturated carbonyl system. On the other hand, the polar surface area (PSA) in both the van der Waals and the solvation shells are smaller in the dihydroderivatives **5–7** than in the unsaturated compounds **1–4**. Thus, it should be possible that the hydrophobic interactions could be more important in this case for the activity. Another possibility is to consider the total van der Waals surface, which can be related with the total volume of the molecule. This is higher in the reduced compounds and could induce a greater change once they are bounded to the target site due to their higher steric requirements.

Some conclusions can be obtained from these results:

(a) The lactone group is necessary for the activity, but it does not seem to be necessarily unsaturated. Moreover, the α,β -unsaturated lactone system in *trans,trans*-germacranolides seems to be less effective than the saturated group.

(b) The introduction of an easily accessible nucleophilic group such as oxirane ring enhances the activity.

(c) The reduction of the α,β -unsaturated system produces molecules with a higher volume and higher steric requirements that turn into higher growth inhibitory activities.

(d) The second effect of the reductions carried out is smaller polar areas and bigger hydrophobic areas. There is not any satisfactory explanation to how this effect could affect the activity.

(e) The levels of activity of some of these compounds are similar to those of Logran on monocotyledons.

(f) Compounds such as costunolide (**1**) and its 11,13-dihydro derivative **5** can be proposed as lead compounds for the development of new models of herbicides.

ABBREVIATIONS USED

DCM, methylene chloride; mixt, mixture; conc, concentration; soln, solution.

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