



Allelochemicals from sunflower leaves cv. Peredovick[☆]

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Received 28 October 1998; received in revised form 5 February 1999; accepted 1 March 1999

Abstract

Isolation, structural elucidation and allelopathic bioassays of 13 compounds, isolated from sunflower cultivar cv. Peredovick are described. Two new sesquiterpene lactones, helivypolide D and helivypolide E and the bisnorsesquiterpene, annuionone D, which is reported at the first time as natural product, are described. The effects of a series of aqueous solutions at 10^{-4} – 10^{-9} M of these compounds were studied on the root and shoot lengths of *Lactuca sativa*, *Lepidium sativum* seedlings (dicotyledons) and *Hordeum vulgare* and *Allium cepa* (monocotyledons). © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Helianthus annuus*; Sesquiterpene lactones; Helivypolides D and E; Bisnorsesquiterpene; Annuionone D; Allelopathy; *Lactuca sativa*; *Lepidium sativum*; *Hordeum vulgare*; *Allium cepa*

1. Introduction

Modern crop production technology depends on synthetic insecticides, herbicides and other chemicals. The increasing dependence on chemicals for pest control poses a serious threat to our health and environment, because after the field application of pesticides, some of their degradation products are adsorbed in the soil and persist for a long period. Besides, another problem faced in chemical weed control is the development of resistant weed biotypes (De Prado, Jorrín, & García-Torres, 1997). Allelopathy, an emerging branch of applied sciences which studies biochemical plant-plant and plant-microorganisms interactions, may help in overcoming such problems through development of crop varieties which have a greater ability to smother

weeds, by using natural phytotoxins from plants or microbes as herbicides (Macías, 1995; Macías, Varela, Torres, & Molinillo, 1996; Macías, Varela, Torres, & Molinillo, 1997).

In continuation of our systematic study of the allelopathic activity of different cultivar of *Helianthus annuus* L. (Macías, Varela, Torres, Molinillo, & Fronczek, 1993; Macías, Varela, Torres, & Molinillo, 1993; Macías, Molinillo, Varela, Torres & Fronczek, 1994), we report the isolation, structural elucidation and allelopathic bioassay of 13 compounds isolated from sunflower cultivar cv. Peredovick. Two new sesquiterpene lactones, helivypolide D and helivypolide E and the bisnorsesquiterpene, annuionone D, which is reported at the first time as natural product, are described.

In order to evaluate the potential allelopathic activity of the new compounds, we have studied the effect of a series of aqueous solutions between 10^{-4} – 10^{-9} M on the root and shoot lengths of *Lactuca sativa*, *Lepidium sativum* seedlings (dicotyledons), *Hordeum vulgare* and *Allium cepa* (monocotyledons).

[☆] Part 14 in the Series 'Allelopathic Studies in cultivar Species'; for Part 13 see: Macías, F. A.; Varela, R. M.; Torres, A. & Molinillo, J. M. G. (1999). Heliannuol E. A Novel Bioactive sesquiterpene of the heliannane family. *Tetrahedron Letters*, 40, 0000, 4725.

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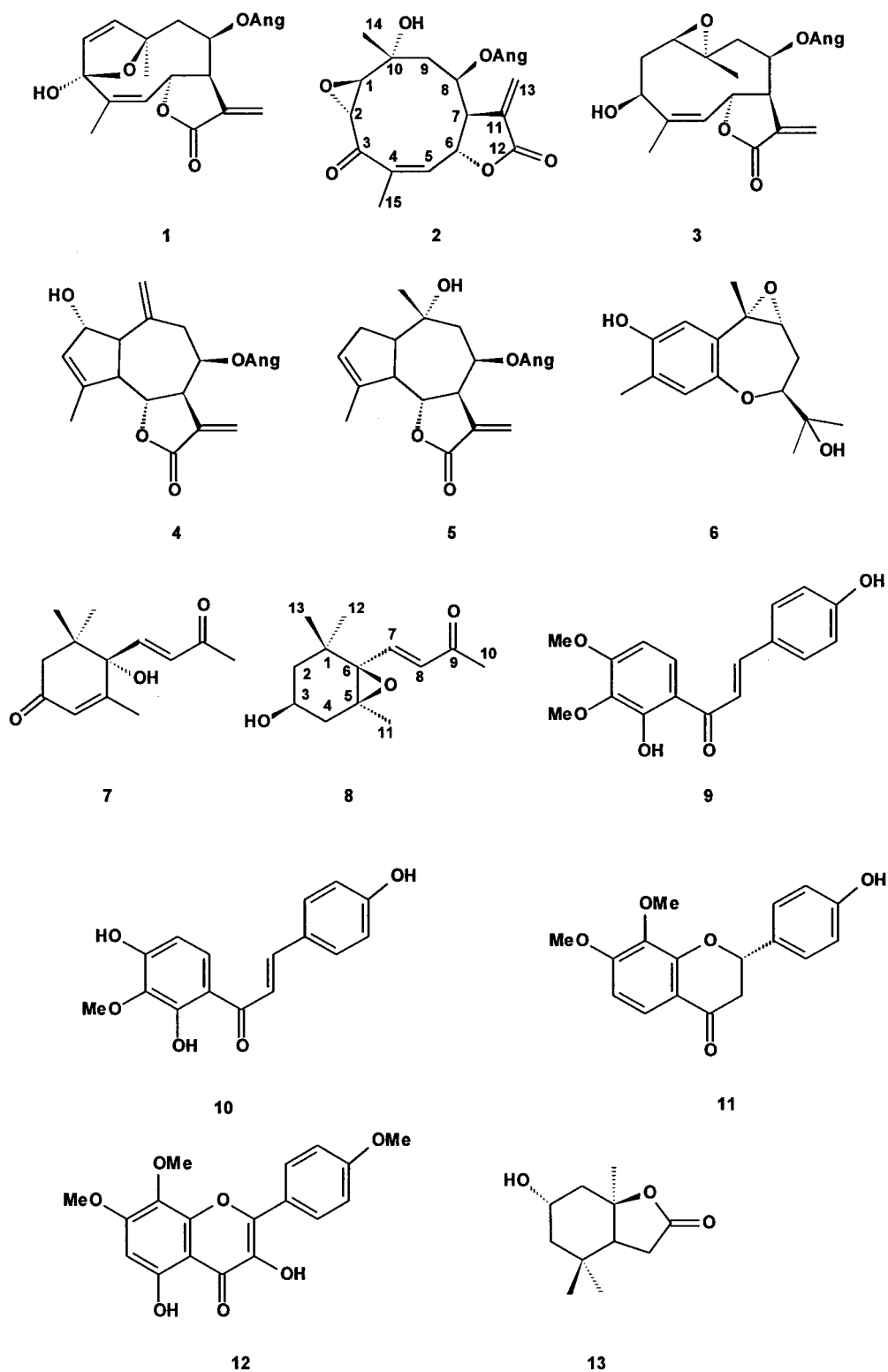


Fig. 1. Allelochemicals from sunflower leaves cv. Peredovick.

2. Results and discussion

The extraction of dry leaf of *H. annuus* L. cv. Peredovick with dichloromethane gave a crude extract which was chromatographed on a silica gel column (Flash chromatography) eluted with hexane-acetone mixtures of increasing polarity. Medium polar fractions yielded 13 compounds (Fig. 1): 5 sesquiterpene lactones, two heliangolides, helivypolide D (**1**) and leptocarpin (**3**), one *cis,cis*-germacranolide, helivypolide E (**2**), two guaianolides, annuolide F (**4**), 8 β -angeloiloxicumambranolide (**5**); one heliannuol, heliannuol J (**6**); two bisnorsesquiterpenes, (+)-dehydrovomifoliol (**7**), annuonone D (**8**); 4 flavonoids, heliannone A (**9**), kukulkanine B (**10**), heliannone B (**11**) tambuline (**12**) and one degradation compound from carotenoids, (+)-loliolide (**13**). The compounds **1** and **2** are new ones in the literature, and the bisnorsesquiterpene **8** is described at first time as natural product. Spectroscopic data of **3** (Martínez, Ayamonte, Núñez-Alarcón, & Romo de Vivar, 1979), **4** (Macías, Torres, Molinillo, Varela, & Castellano, 1996), **5** (Gershenzon & Mabry, 1984), **6** (Macías, Varela, Torres, & Molinillo, 1999), **7** (Takasugi, Anetai, Katsui, & Masumune, 1973) and **9** (Macías, Molinillo, Torres, Varela, & Castellano, 1997), **10** (Domínguez et al., 1989), **11** (Bose & Bose, 1939), **12** and **13** (Isoe, Katsumura, Hyeon, & Sakan, 1971) were identical to those previously reported.

Helivypolide D (**1**) was isolated as a gum, $[M]^+$ at m/z 360, which together with the $^1\text{H-NMR}$ data (Table 1) was in agreement with the molecular formula $\text{C}_{20}\text{H}_{24}\text{O}_6$.

The $^1\text{H-NMR}$ spectrum of **1** (Table 1) exhibited typical signals of a sesquiterpene lactone with an exocyclic α -methylene- γ -lactone at δ 6.30 and 5.70 assigned to H-13b and H-13a, and an angelate ester [δ 6.22 (*qq*, H-3'), 1.96 (*dg*, 3H, H-4'), 1.82 (*dq*, 3H, H-5')]. Compound **1** exhibited also two doublets at δ 5.79 and 6.33 coupled to each other, characteristic of a furane ring. The chemical shift and the multiplicity of the signal assigned to H-14 (δ 1.40, *s*) was in agreement with an oxygen atom attached to C-10. The β -orientation for the furane ring oxygen was assigned by comparison with the spectroscopic data of other furanoheliangolides (Herz & Sharma, 1975; Chowdhury, Sharma, Thyagarajan, Herz, & Govindan, 1980; Zdero, Bohlmann, & Scott, 1987; Pereira, Días, Vichnewsi, Nassi, & Herz, 1997).

$^1\text{H-NMR}$ 2D COSY spectrum of **1** showed the following series coupling protons: H-13a (*d*, δ 5.70) and H-13b (*d*, δ 6.30) with H-7 (*dddd*, δ 3.44); H-7 with H-6 (*dd*, δ 5.94); H-6 with H-5 (*dq*, δ 5.68). The chemical shift of H-5 suggested the presence of a double bond at position 4–5. The geometry of this double bond (Δ^{4-5}) as well as the orientation of H-8 was established

Table 1

$^1\text{H-NMR}$ of compounds **1**, **2** and **8** (399.952 MHz, CDCl_3 , signal of residual CHCl_3 centred at δ 7.25 ppm)^a

H	1	2	8
1	5.8 <i>d</i>	3.2	—
2 α	6.3 <i>d</i>	—	1.3 <i>dd</i>
2 β	—	3.6	1.6 <i>dd</i>
3 α	—	—	3.9 <i>ddd</i>
4 α	—	—	1.6 <i>dd</i>
4 β	—	—	2.4 <i>dd</i>
5	5.7 <i>dq</i>	6.4	—
6 β	5.9 <i>dd</i>	5.6	—
7	3.4 <i>dddd</i>	3.3	6.3 <i>d</i>
8	5.1 <i>dd</i>	5.4 <i>ddd</i>	7.0 <i>d</i>
9 α	2.4 <i>d</i>	2.2 <i>dd</i>	—
9 β	2.4 <i>d</i>	2.0 <i>dd</i>	—
10	—	—	2.3 <i>s</i>
11	—	—	1.6 <i>s</i>
12	—	—	1.0 <i>s</i>
0.04	5.7 <i>d</i>	5.9	1.2 <i>s</i>
13b	6.3 <i>d</i>	6.4	—
14	1.4 <i>s</i>	1.3	—
15	1.9 <i>dd</i>	1.9	—
3'	6.2 <i>qq</i>	6.1	—
4'	2.0 <i>dq</i>	2.0	—
5'	1.8 <i>dq</i>	1.8	—

^a Multiplicities are indicated when the coupling constant can be measured and they are not repeated if identical with those in the preceding column. $J(\text{Hz})$: **1**: 1,2=5.7; 5,6=6.6; 5,15=1.4; 6,7~3; 6,15=1.4; 7,8~1.5; 7, 13a=2.3; 7,13b=2.6; 8,9=3.6; 3',4'=7.2; 3',5'=1.5; 4',5'=1.5. **2**: 1,2=2.5; 5,6=5.5; 5,15=1.4; 6,7~2; 6,15=1.4; 7,13a=1.7; 7,13b=1.8; 7,8=3.0; 8 α ,9 α =4.3; 8 α ,9 β =9.4; 9 α ,9 β =15.2; 3',4'=7.2; 3',5'=1.5; 4',5'=1.5. **8**: 2,2'=14.5; 2,3=1.9; 2',3=4.9; 3,4=5.1; 3,4'=8.8; 4,4'=14.3; 7,8=15.0.

by NOE difference experiments (Fig. 2). The observed NOE effects between H-5 and H-15, was in agreement with a *Z*-double bond between C-4 and C-5. A NOE effect between H-7 and H-8, and the small coupling constant value ($J_{7,8\alpha}=1.5$ Hz) required an α -orientation of H-8.

The absence of the signal of C-3, and the molecular formula led to the structure shown in Fig. 1 with an α -oriented hydroxyl group at C-3.

Helivypolide E (**2**) exhibits typical signals of an α -methylene- γ -lactone moiety at δ 6.36 (*d*, H-13b) and 5.86 (*d*, H-13a) and an angelate ester [δ 6.14 (*qq*, H-3'), 1.97 (*dq*, 3H, H-4') and 1.82 (*dq*, 3H, H-5')].

The molecular ion $[M]^+$ at m/z 376 ($\text{C}_{20}\text{H}_{24}\text{O}_7$) was not observed but a peak at m/z 358 $[M-\text{H}_2\text{O}]^+$ indicate the presence of an hydroxyl group, the following peaks confirmed the presence of the angelate ester: m/z 259 $[M-\text{H}_2\text{O}-\text{C}_5\text{H}_7\text{O}_2]^+$, m/z 83 $[\text{C}_5\text{H}_7\text{O}]^+$, 55 $[\text{C}_4\text{H}_7]^+$.

The $^1\text{H-NMR}$ 2D COSY spectrum of **2** showed the following series of coupling protons: H-13a (*d*, δ 6.58) and H-13b (*d*, δ 6.36) with H-7 (*dddd*, δ 3.30), H-7 with H-6 (*dd*, δ 5.62), H-6 with H-5 (*dq*, δ 6.37). H-6 and H-5 are coupled with H-15 (*dd*, δ 1.94). H-7 was also coupled H-8 (*ddd*, δ 5.44) and H-8 with H-9 α (*dd*,

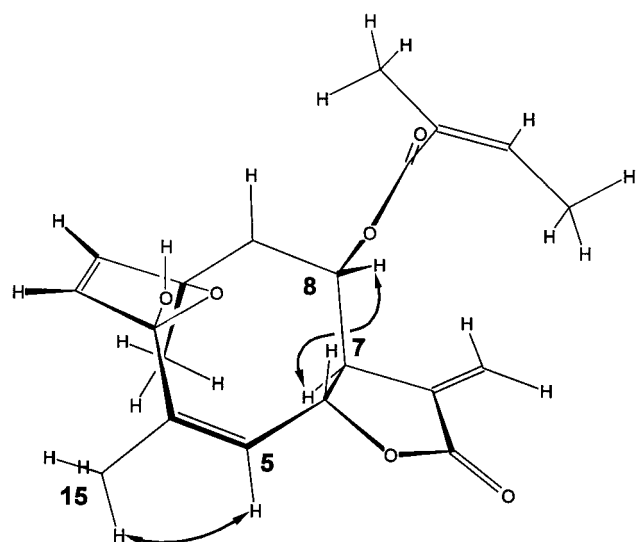
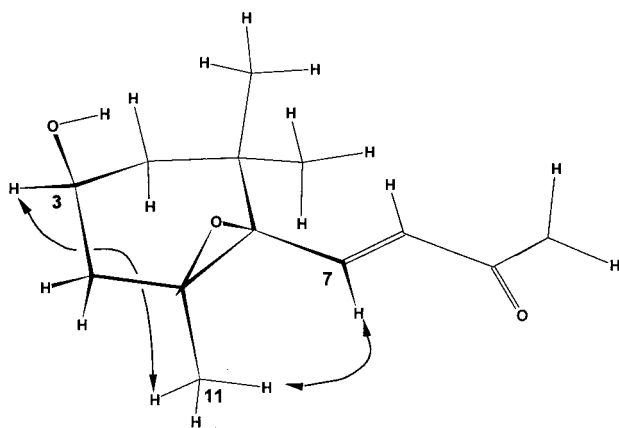
**Helivypolide D****Annuionone D**

Fig. 2. Observed NOEs for the most stable conformers of **1** and **8** using PM3 calculations.

$\delta 2.20$) and H-9 β (*dd*, $\delta 2.00$). Finally, H-1 (*d*, $\delta 3.23$) was coupled with H-2 (*d*, $\delta 3.67$). H-1 and H-2 have the characteristic chemical shifts of protons attached to an oxirane ring.

The absence of H-3 and the molecular formula led us to propose a carbonyl group at C-3. This is in agreement with the deshielding of the signal assigned to H-5 ($\delta 6.37$), corresponding to a vinylic proton of a double bond conjugated with a carbonyl group.

The proposed structure is shown in Fig. 2, with an α -oriented hydroxyl group at C-10, that was confirmed by the chemical shift observed for H-14 ($\delta 1.26$).

The stereochemistry was established by comparison with the spectroscopy data of 1 β -2 α -epoxytaginine C, a

related compound with an isobutyrate ester attached to C-8 (Zdero et al., 1987).

Annuionone D (**8**) was isolated a colourless oil. EIMS showed a molecular ion at m/z 224, which together with the ^{13}C NMR data were in good agreement with the molecular formula of a bisnorsesquiterpene $\text{C}_{13}\text{H}_{20}\text{O}_3$.

^1H NMR 2D COSY spectrum analysis showed the correlation of H-3 α ($\delta 3.90$, *dddd*) with H-2 α ($\delta 2.38$; *dd*), H-2 β ($\delta 1.64$; *dd*), H-4 α ($\delta = 1.62$; *dd*) and with H-4 β ($\delta 1.27$; *dd*). Other observed correlations were: H-2 α with H-2 β ($J_{2\alpha,2\beta} = 14.5$ Hz) and H-4 α with H-4 β ($J_{4\alpha,4\beta} = 14.3$ Hz). The coupling constant observed for H-7 led us to propose an *E* geometry for the double bond 7–8.

These correlations, together with the presence of three methyl groups at $\delta 2.27$ (H-10), $\delta 1.24$ (H-11), $\delta 0.96$ and 1.18 (H-12 and H-13), suggested an ionane skeleton with a carbonyl group at C-9.

The chemical shifts of H-3 ($\delta 3.90$) and C-3 ($\delta 64.0$) in ^1H NMR (Table 1) and ^{13}C NMR spectra, together with the observed absorption at 3407 cm^{-1} in the IR spectrum, indicated the presence of an hydroxyl group at C-3.

The absence of signals assigned to H-5 and H-6, as well as the multiplicity of H-7 ($\delta 5.6$, *d*) and H-11 ($\delta 1.24$, *s*), and the molecular formula, indicated the presence of an oxirane ring between C-5 and C-6. The NOE effects between H-11 and H-7 (Fig. 2) confirmed a *cis* stereochemistry for this epoxide. Otherwise, the NOE effect between H-3 and H-11 indicate the same relative orientation for the hydroxyl group and the epoxide.

Absolute configuration was determined by comparison of the optical rotation data of this compound and the previous synthetic intermediate (Broom, Ede, & Wilkins, 1992). This is the first time that compound **8** is reported as a natural product.

2.1. Bioassay data discussion

In order to evaluate their potential allelopathic activity, we have studied the effect of a series of aqueous solutions ranged between 10^{-4} – 10^{-9} M of isolated compounds on germination, root and shoot length of *Lactuca sativa*, *Lepidium sativum*, *Allium cepa* and *Horedum vulgare*, as standard target species (STS).

Compounds **1** and **2** showed an inhibitory profile for the three tested parameters (germination, root and shoot length) on *Lactuca sativa* cv. Roman (Fig. 3) with inhibition values of 40%. The highest activity is shown by **1** at 10^{-6} M with a value of -46% on germination. Anyway, the activities produced by compound **2** solutions are, in general, higher than those produced by **1**, specially on root (**2**: 10^{-5} M, 41%; 10^{-6} M, 22%; 10^{-7} M, 33%; 10^{-8} M, 40%; 10^{-9} M,

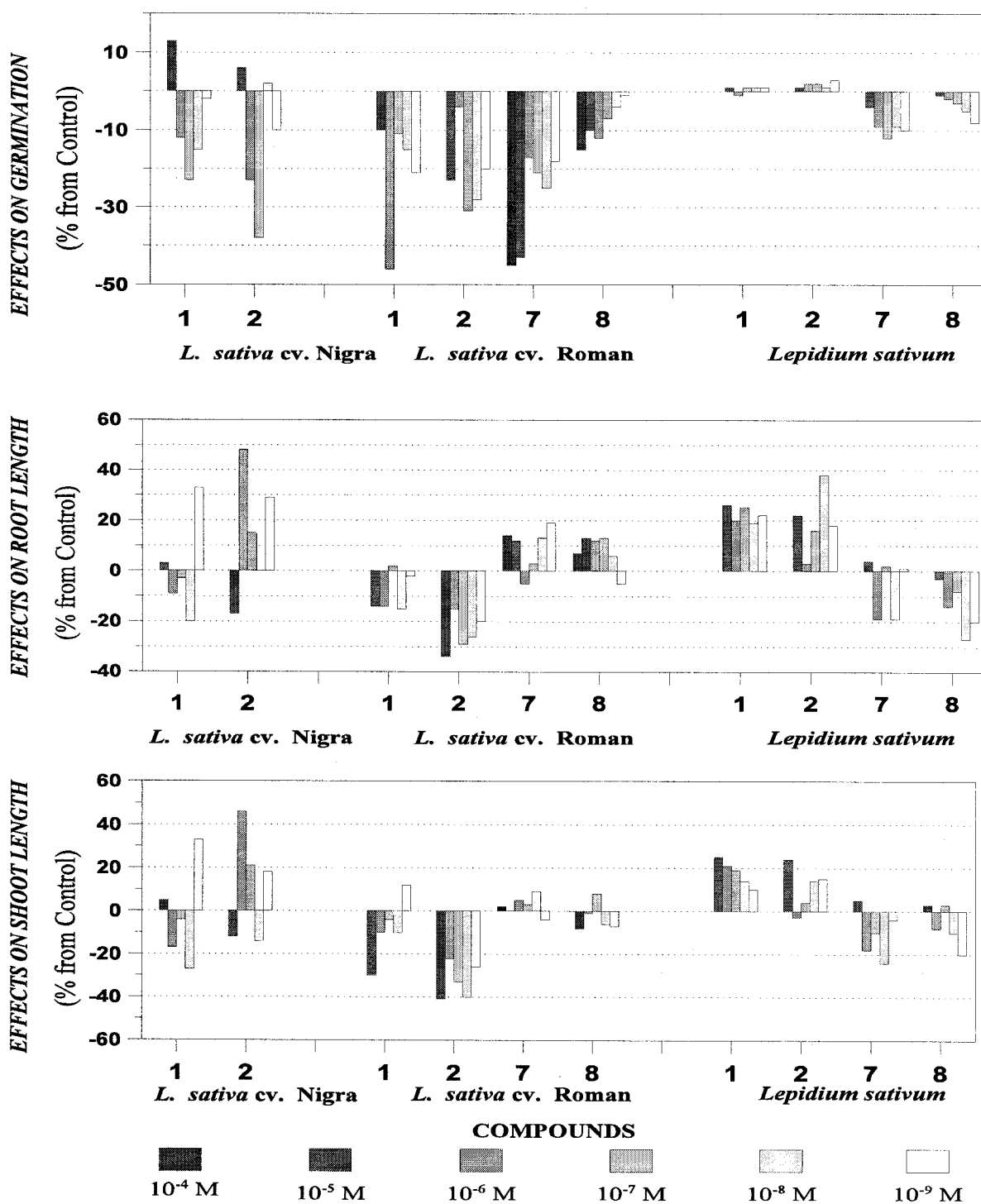


Fig. 3. Effects of lactones **1**, **2** and norsesquiterpenes **7**, **8** on the germination, radicle and shoot length of dicotyledon standard target species (STS) *L. sativa* L. cv. Nigra (left), *L. sativa* L. cv. Roman (centre) and *L. sativum* L. (right).

26%). This can be due to a major flexibility of molecule **2**. It has been reported that the different spatial arrangements that the molecule can adopt play an important role in the activity, as well as the presence of an α -methylene- γ -lactone moiety, and other functionalizations (Macías, Galindo, & Massanet, 1992).

The compounds generally had no effect on germination of *Lepidium sativum*, but they had a moderate stimulatory profile on root and shoot growth. We can point out that the effect of **1** on root growth was maintained with the dilution, with significance $P < 0.05$, (Fig. 3).

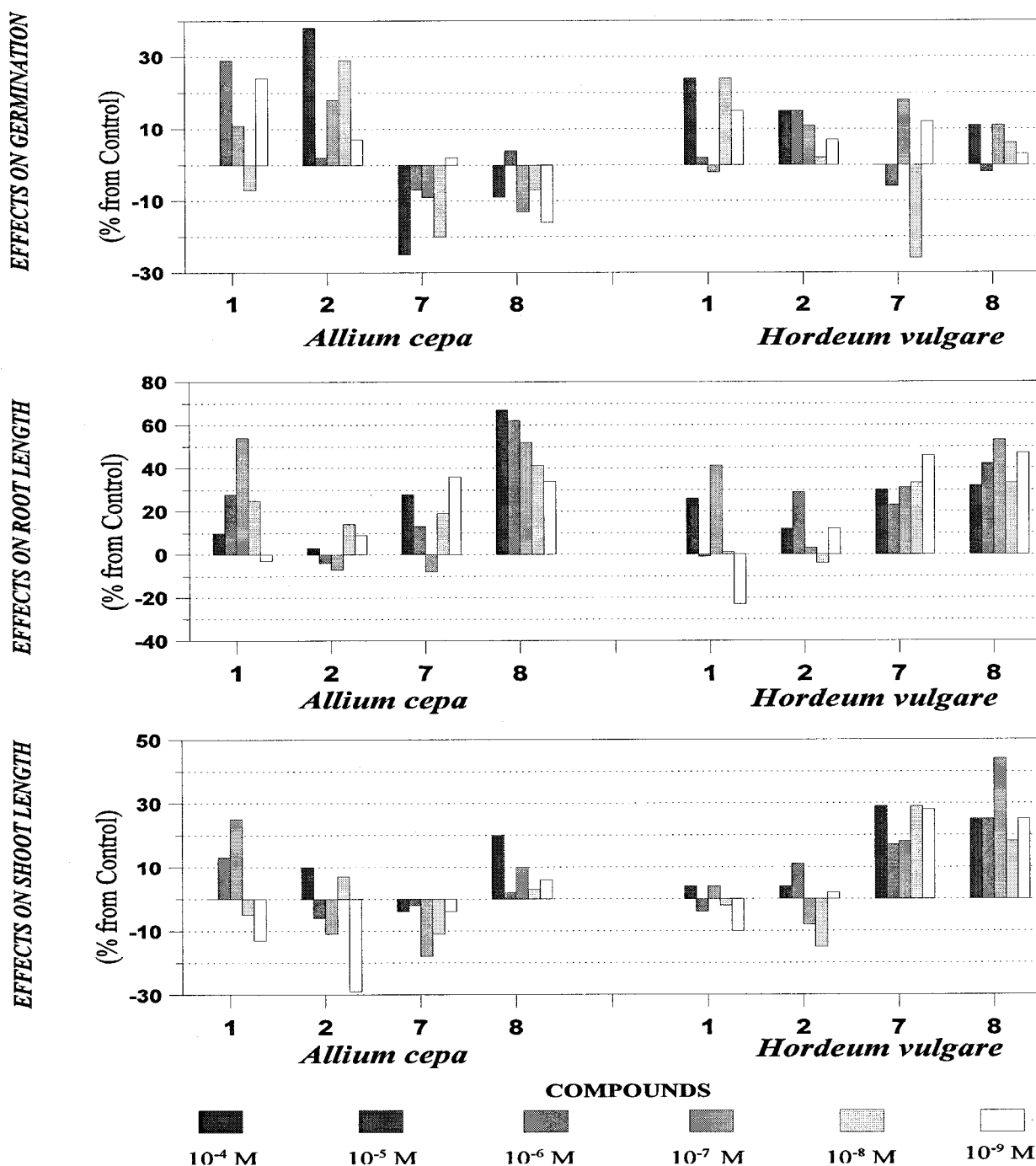


Fig. 4. Effects of lactones 1, 2 and norsesquiterpenes 7, 8 on the germination, radicle and shoot length of monocotyledon standard target species (STS) *A. cepa* L. (left) and *H. vulgare* L. (right).

The observed effect of 1 and 2 over *Allium cepa* and *Hordeum vulgare* are of small significance.

The most significant effects found for the dicotyledon species (*L. sativa* and *L. sativum*) were obtained with 7, which showed a strong inhibitory effect on the germination of *L. sativa* cv. Roman in a range of concentrations between 10⁻⁴–10⁻⁶ M with an average of -45% (Fig. 3).

Compounds 7 and 8 showed the most relevant observed effects over monocotyledons species (*Allium cepa* and *Hordeum vulgare*) (Fig. 4). Compound 8 stimulated root growth of *Allium cepa* in all tested concentrations (10⁻⁵–10⁻⁹ M), with values that decrease with the dilution (10⁻⁵ M, 67%; 10⁻⁶ M, 62%; 10⁻⁷ M, 52%; 10⁻⁸ M, 41%; 10⁻⁹ M, 34%). Compounds 7 and 8 also showed stimulatory effects

on root and shoot growth of *Hordeum vulgare* (average 35%). These stimulatory effects over *Hordeum vulgare* (especially on root) are in good agreement with our previous observations for other series of bisnorsesquiterpenes (Macías, Torres, Varela, Oliva, & Molinillo, 1998).

3. Experimental

3.1. Plant material

Leaves of *H. annuus* L. cv. Peredovick[®] were collected in September 1990 during the third plant development stage (Macías, 1995) (plants 1.2 m tall with flowers, 1 month before harvest) and were provided by Rancho de la Merced, Agricultural Research Station (CIFA), Junta de Andalucía, Jerez, Spain.

3.2. Extraction and isolation

Dry leaves (1.7 kg) were soaked in CH₂Cl₂ (fresh plant:solvent 1:3) for 24 h at 25°C in the dark. The CH₂Cl₂ extract was separated by 'Dry Flash' chromatography on silica gel using *n*-hexane-acetone mixts of increasing polarity yielding 123 × 50 ml frs which were reduced to 7 frs after comparison by TLC: A (0–2%), B (5%), C (10–20%), D (40%), E (60–100%), F (100% AcOEt) and G (100% MeOH).

By following the bioactivity exhibited by the fractions of *L. sativa*, *Lepidium sativum*, *Allium cepa* and *Hordeum vulgare*, fr. C was chromatographed using silica gel and eluted with *n*-hexane-acetone mixts of increasing polarity. After separations using HPLC with a Hibar Si60 (MERCK) column we obtained **1** (1 mg), **2** (0.5 mg), **3** (2.5 mg), **4** (0.5 mg), **5** (1 mg), **6** (1 mg), **7** (1 mg), **8** (1 mg), **9** (2 mg), **10** (1 mg), **11** (0.5 mg), **12** (4 mg) and **13** (1.5 mg).

3.3. Helivypolide D (**1**)

C₂₀H₂₄O₆, colourless oil, IR $\nu_{\text{max}}^{\text{KBr, neat}}$ cm⁻¹: 3488 (OH), 1759 (α,β -unsaturated- γ -lactone), 1715 (α,β -unsaturated ester). EI-MS (70 eV) *m/z* (rel. int): 360 [M]⁺ (8); 260 [M-C₅H₈O₂]⁺ (4), 83 [C₅H₇O]⁺ (100), 55 [C₄H₇]⁺ (81); ¹H NMR: see Table 1. ¹³C NMR (100.23 MHz, CDCl₃): δ 169.5 (*s*, C-12), 164.2 (*s*, C-1'), 140.7 (*d*, C-3'), 139.5 (*d*, C-2), 139.4 (*s*, C-4), 139.2 (*s*, C-11), 131.1 (*d*, C-1), 130.5 (*d*, C-2'), 127.6 (*d*, C-5), 124.1 (*t*, C-13), 104.1 (*s*, C-3), 82.9 (*s*, C-10), 76.4 (*d*, C-8), 74.7 (*d*, C-6), 47.9 (*d*, C-7), 43.7 (*t*, C-9), 31.6 (*q*, C-14), 20.6 (*q*, C-15), 20.5 (*q*, C-5'), 15.7 (*q*, C-4'). HREIMS calcd for C₂₀H₂₄O₆ 360.1573, found 360.1579.

3.4. Helivypolide E (**2**)

C₂₀H₂₄O₇, colourless oil, IR $\nu_{\text{max}}^{\text{KBr, neat}}$ cm⁻¹: 3418 (OH), 1767 (α,β -unsaturated- γ -lactone), 1717 (α,β -unsaturated ester). EI-MS (70 eV) *m/z* (rel. int): 358 [M-H₂O]⁺ (4), 259 [M-H₂O-C₅H₇O₂]⁺ (16), 83 [C₅H₇O]⁺ (100), 55 [C₄H₇]⁺ (94); ¹H NMR: see Table 1. HREIMS calcd for C₂₀H₂₂O₆ 358.1416 [M-H₂O]⁺, found 358.1413.

3.5. Annuionone D (**8**)

C₁₃H₂₀O₃, oil, IR $\nu_{\text{max}}^{\text{KBr, neat}}$ cm⁻¹: 3408 (OH), 1671 (α,β -unsaturated C=O). EI-MS (70 eV) *m/z* (rel. int): 224 [M]⁺ (2.7), 123 [M-C₆H₁₃O]⁺ (100), 43 [CH₃CO]⁺ (75); ¹H NMR: Table 1. ¹³C NMR (100.23 MHz, CDCl₃): δ 197.5 (*s*, C-9), 142.4 (*d*, C-7), 132.6 (*d*, C-8), 77.2 (*s*, C-6), 69.3 (*s*, C-5), 64.0 (*d*, C-3), 46.6 (*t*, C-4), 40.5 (*t*, C-2), 35.1 (*s*, C-1), 29.3 (*q*, C-10), 28.3 (*q*, C-11), 24.9 (*q*, C-13), 19.8 (*q*, C-12).

3.6. Bioassays

Seeds of *Lactuca sativa* L. cv. Nigra, and cv. Roman, *Allium cepa* L., and *Hordeum vulgare* L. were obtained from FITÓ, SL. (Barcelona, Spain). All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity. Germination and growth bioassays for tested species were as follows: *Lactuca sativa*, *L. sativum* and *A. cepa*, 25 seeds per dish, 5 ml test soln, 5 days dark, 25°, and four replicates of each conc; *H. vulgare*, 10 seeds per dish, 5 ml test soln, 5 days dark, 25°, and 10 replicates of each conc (Macías, Castellano, & Molinillo, 1999).

Test solns (10⁻⁴M or 10⁻⁵ M) were prepd using H₂O-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 1M NaOH_(aq). Osmotic pressure values were measured on a vapour pressure osmometer (WESCOR 5500) and ranged between 30 and 38 mM/kg.

Data are presented as percentage differences from control in graphics and tables (Table 2 and Figs 3, 4). Thus, zero represents the control; positive values represent stimulation of the studied parameter, and negative values represent inhibition.

3.7. Statistical treatment

Germination root, and shoot length values were tested by the Welch test; differences between experimental and controls were significant (*P* = 0.01) (Table 2).

Table 2
Germination and growth activity of compounds **1**, **2**, **7** and **8** over STS of dicotyledons and monocotyledons^a

Compound	Germination (% difference from the control)					Root length (% difference from the control)					Shoot length (% difference from the control)							
	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M	10 ⁻⁴ M	10 ⁻⁵ M	10 ⁻⁶ M	10 ⁻⁷ M	10 ⁻⁸ M	10 ⁻⁹ M
<i>Lactuca sativa</i> L. cv. Nigra																		
1		13	-12	-23	-15	-2		3	-9	-3	-20	33		5	-17	-4	-27	33
2		6	-23	-38	2	-10		-17	48	15	0	29		-12	46	21	-14	18
<i>Lactuca sativa</i> L. cv. Roman																		
1		-10	-46	-11	-15	-21		-14	-14	2	-15	-2		-30	-10	-4	-10	12
2		-23a	-4	-31b	-28a	-20		-34a	-15	-29b	-26a	-20		-41b	-22	-33b	-40b	-26
7	-45	-43	-17	-21	-25	-18b	14	12	-5	3	13	19	2	0	5	3	9	-4
8	-15	-10	-12	-7a	-4	-1	7	13	12	13	6	-5	0	-8	-1	8	-6	-7
<i>Lepidium sativum</i> L.																		
1		1a	-1b	1a	1b	1a		26a	20b	25a	19b	22a		25a	21a	19a	14b	10
2		1b	2	2b	1b	3		22b	3	16b	38b	18		24a	-3	4	14b	15b
7		-4	-9	-12	-9a	-10		4	-19	2	-19a	1		5	-18a	-10	-24	-4
8		-1	-2	-3	-5	-8		-3	-14	-8	-27a	-20a		3	-8	3	-10	-20a
<i>Hordeum vulgare</i> L.																		
1		24	2	-2	24	15		26	-1	41	1	-23		4	-4	4	-2	-10
2		15	15	11	-2	0		12	29	3	-4	12		4	11	-8	-15	2
7		0	-6	18	-26	12		30	23	31	33	46		29	17	18	29	28
8		11	-2	11	6	3		32	42	53	33	47		25	25	44	18	25
<i>Allium cepa</i> L.																		
1		0	29	11	-7	24		10	28	54	25	-3		0	13	25	-5	-13
2		38	2	18	29	7		3	-4	-7	14	9		10	-6	-11	7	-29
7		-25	-7	-9	-20	2		28	13	-8	19	36		-4	-2	-18b	-11	-4
8		-9	4b	-13	-7a	-16		67	62b	52	41a	34a		20	2	10	3	6

^a Values presented as percentage differences from the control (e.g. 16% means 116% compared with the control). $P > 0.05$ for the Welch's test; a: $P < 0.01$; b: $0.01 < P < 0.05$.

Acknowledgements

This research has been supported by the Dirección General de Investigación Científica y Técnica, Spain (DGICYT, Project No. PB95-1231). We thank Dr Alberto García de Luján Gil de Bernabé and Mr Miguel Lara, Rancho de la Merced, Agricultural Research Station (CIFA), Junta de Andalucía, Jerez, Spain, for providing plant material. We also thank FITÓ SL. for providing monocot and dicot seeds for bioassays.

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