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POTENTIAL ALLELOPATHIC ACTIVITY OF SEVERAL SESQUITERPENE LACTONE MODELS*

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Key Word Index—Sesquiterpene lactones; melampolides; *cis,cis*-germacranolides; guaianolides, eudesmanolides; allelopathy; *Lactuca sativa*.

Abstract—A collection of 12 natural and synthetic sesquiterpene lactones with eudesmanolide, melampolide, *cis,cis*-germacranolide, and guaianolide skeletons have been prepared and tested as allelochemicals. The effect of a series of aqueous solutions at 10^{-4}–10^{-9} M of this collection is evaluated. The specific structural requirements related to their activity is discussed. The natural sesquiterpene lactones soulangianolide A, melampomagnolide A and B, zaluzanin C and isozaluzanin C have been synthesized from costunolide, parthenolide and dehydrocostuslactone using SeO_2 and *tert*-butylhydroperoxide. The structures of the synthetic compounds were established by NMR spectroscopy.

INTRODUCTION

In spite of the large number and wide variety of naturally occurring sesquiterpene lactones (over 3,500 [1, 2]) that have been chemically characterized, little work has been done on their biological activity [3, 4] and ecological significance (antibacterial, antifungal [5], cytotoxic [6], allergenic [7], allelopathic [8–10] and deterrent activity [11], and toxicity and antifeedant activity [12]).

Several studies have suggested that sesquiterpene lactones may act as plant growth regulators. Some sesquiterpene lactones have been reported to be responsible for the allelopathic properties of some plants by affecting the germination and growth of other species [13].

As part of our research on bioactive natural products (coumarins, sesquiterpene lactones, phenolics, etc.), we are attempting to perform a systematic study of their potential as allelochemicals. Thus we are evaluating the regulator effects of suitable test compounds on *Lactuca sativa*, a plant which is widely used in the laboratory bioassay of allelopathic activity [14]. A positive effect (inhibition or promotion) at the concentrations used in this work should allow us to establish a correlation between tested compounds and their potential as allelochemicals. Also, as has been proposed [13], it is reasonable to hypothesize that the low concentrations used in this study might be reached in localized concentrations in the soil, as a result of root exudates or litter decomposition.

In order to obtain information about the specific structural requirements needed for their biological activity, we have studied the effect of a series of aqueous solutions at 10^{-4}–10^{-9} M of 12 natural and synthetic eudesmanolides, melampolides, *cis,cis*-germacranolides and guaianolides on root and shoot lengths of *Lactuca sativa* var. *nigra* seedlings (Fig. 1).

*Part 1 in the series 'Natural Product Models as Allelochemicals'.

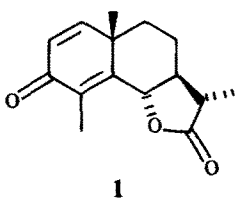
RESULTS AND DISCUSSION

A large part of the influence of substances known for their toxic, allergenic, etc. effects, is postulated to be related to the presence of strong electrophilic or nucleophilic systems. The attack by these systems on specific positions of proteins or enzymes would change their configurations and destroy their activity. One of these systems, very common in many natural products, is the α,β-unsaturated carbonyl group. This electrophilic system can undergo 1,4-Michael additions with electron donors such as the sulphur atom in the sulphydryl moiety, a group which is very abundant in proteins and nucleic acids. The α-methylene-γ-lactone group is present in many of the isolated natural sesquiterpene lactones, and has been proposed as one of the factors which can determine their allelopathic activity [9] in particular, as well as their biological activity in general [15] (Fig. 2).

In the literature, the potential allelopathic activity of sesquiterpene lactones is related to two factors: one is the presence of certain functionalizations (where the α-methylene-γ-lactone moiety plays an important role), and the other is the different spatial arrangements that a molecule can adopt [9, 10, 13]. Searching for a better understanding of the mode of action, we have prepared and tested molecules selected to provide information about the following: (a) the influence of a non-lactonic α,β-unsaturated carbonyl system; (b) the influence of the conformation in decalinic systems with an α-methylene-γ-lactone moiety and (c) the influence of a higher functionalization of α-methylene-γ-lactone-possessing molecules.

Attending to requirement (a), the eudesmanolides **1–3**, where the α-methylene-γ-lactone group is not present, were tested. A striking difference of activity profile between these lactones and the rest of the sesquiterpene lactones tested was observed (Table 1 and Fig. 3). The absence of the α-methylene group in the γ-lactone ring may be responsible for the lack of a significant activity on the seedling. At the same time, eudesmanolides **1–3** showed a strong stimulatory effect on radical and shoot length

Eudesmanolides



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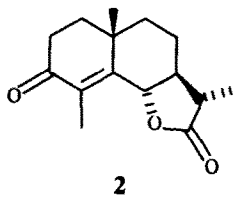

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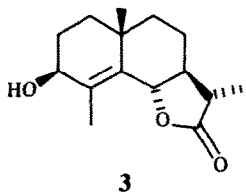

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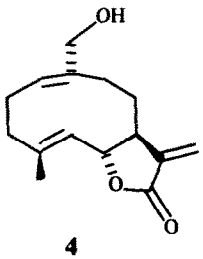
Melampolides



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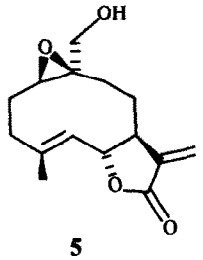

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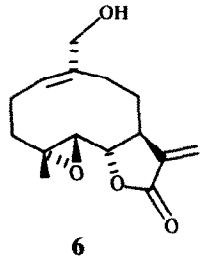

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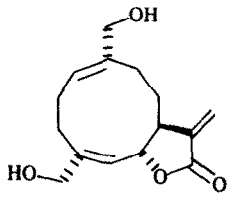
cis, *cis*-Germacranolides



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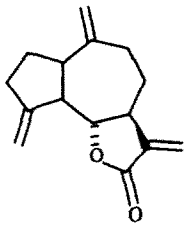
Guaianolides



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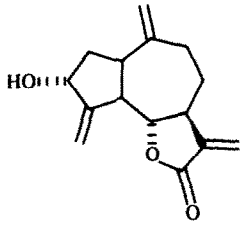

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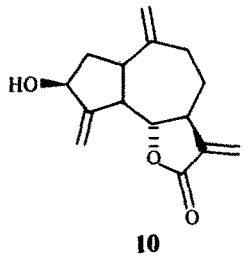

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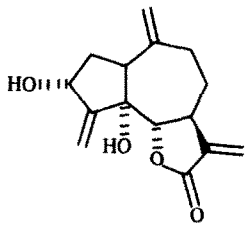

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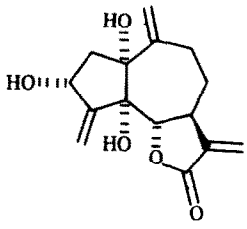


12

Fig. 1. Sesquiterpene lactones tested.

(Figs 4 and 5). So, it may be more reasonable to relate the presence of the α-methylene-γ-lactone functionalization with the germination responses than with shoot and root growth responses, at least in conformationally less flexible systems, which is in agreement with the previous results reported for dihydro and dehydroambrosanolides [10].

The germination response brought about by **1** in comparison to **2** serves to establish a relative α,β-unsaturated carbonyl system accessible-activity rela-

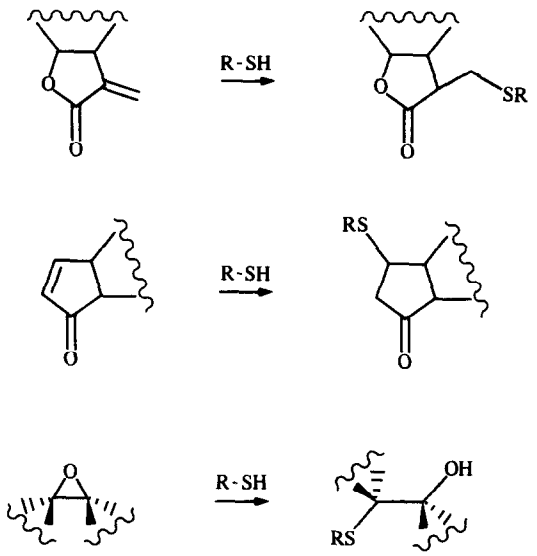


Fig. 2. Alkylating properties of sesquiterpene lactones.

tionship. Thus, the shoot and root length results could be a clear indication that the major requirement is the conformation these compounds can adopt, rather than the functionalization they may possess. So, compounds **1–3** have a similar activity pattern (Table 1, Figs 4 and 5): the radical length effect (%) for the 10^{-4} M series is +72 for **1**, +68 for **2** and +61 for **3**, and for 10^{-7} M series is +45, +36 and +36 for **1**, **2** and **3**, respectively.

As has been shown for costunolide on witchweed germination [10], the structural crown conformation is related to its strong activity. The observation of a stimulatory effect around 50% on *Lactuca sativa* cv. Grand Rapids germination (a minor or no effect has been shown for the other less sensitive species *L. sativa* light insensitive and *L. sativa* light sensitive) [13], of 10^{-4} to 10^{-6} M dihydroparthenolide (crown conformation) provided the means to establish a modified crown conformation-activity relationship.

In order to obtain a deeper insight about the effect on the activity that the conformation and flexibility of a molecule produce [requirement (b)], melampolides **4–6** and the *cis,cis*-germacranolide **7** were synthesized and tested. The synthesis was performed using SeO_2 and *tert*-butylhydroperoxide with costunolide and parthenolide as starting materials. The conditions used bring about a stereospecific modification of the double bonds between C-1 and C-10 (compounds **4–6**) and C-4 and C-15 (compound **7**) by ene reaction [16]. The spectroscopic data are presented in Tables 2 and 3.

Although lactones **4** and **5** show a strong stimulatory effect on germination, lactones **6** and **7**, which also contain an α-methylene-γ-lactone moiety, have less effect (Table 1 and Fig. 3). These results can be related to the changes in the conformation of these conformationally flexible molecules (**4–7**), where significant modifications with respect to the crown system are progressively introduced by changing the configuration of the corresponding double bonds, as we can observe from dihedral angles obtained using molecular mechanics calculations (MMX) [17] and experimental coupling constants (Fig. 6, Table 4). This behaviour has lead us to propose that in

Table 1. Statistical results of allelopathic bioassays (using *L. sativa*) of compounds **1–12**

	(%) Germination						(%) Radical length					
	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
Eudesmanolides												
α-Santonin (**1**)	+10[b]	+18[a]	+2[b]	+15[b]	−3[b]	+10[b]	+51	+21[b]	+30[a]	+30	−44	+15
1 2-Dihydro-α-santonin (**2**)	+8[b]	+2[b]	−5[b]	+8[b]	−11[b]	−4[b]	+64	+57	+33[a]	+22[a]	−23[b]	−8[b]
3β,OH-1,2-Dihydro-α-santonin (**3**)	+5[b]	0	+3[b]	0	+8[b]	+6[b]	+58	+66	+33[a]	+22[a]	+5[b]	+8[b]
Melampolides												
Soulangianolide A (**4**)	+87	+62[a]	+33[a]	+47[a]	+97	+2[b]	+34	+5[b]	−14[a]	−6[b]	+14[a]	−5[b]
Melampomagnolide A (**5**)	+40[a]	+34[a]	+6[b]	+2[b]	+37[b]	+46[a]	+4[b]	−18[a]	−11[a]	−18	−21	11[b]
Melampomagnolide B (**6**)	+11[b]	+20[b]	+2[b]	+15[b]	0	+7[b]	−11[b]	+11[a]	−7[b]	0	+4[b]	−8[b]
cis,cis-Germacranolides												
14,15-diOH-*cis,cis*-Germacranolide (**7**)	+20[b]	+16[b]	−8[b]	+3[b]	+3[b]	+5[b]	+9[b]	+6[b]	−11[b]	−13[a]	+9[b]	+2[b]
Guaianolides												
Dehydrocostuslactone (**8**)	−17[b]	+5[b]	+21[b]	+6[b]	−10[b]	−2[b]	−2[b]	+10[b]	+30[a]	+30	−9[b]	+12[b]
Isozaluzanin C (**9**)	+80[a]	+32	−42	−13[b]	0	+20	+10[b]	+13[a]	+24	+17	+20	−3[b]
Zaluzanin C (**10**)	+77[a]	+32[b]	+2[b]	+26[b]	−16[b]	−5[b]	+3[b]	+31	−2[b]	+20	−30	7[b]
5α-Hydroxy-isozaluzanin C (**11**)	0	+32	−6[b]	+10[b]	+56[a]	+23[b]	+12[b]	+2[b]	+18	−9[b]	+10[b]	−67
1α,5α-Dihydroxyisozaluzanin C (**12**)	+14[b]	+32[b]	+19[b]	+18[b]	−10[b]	−2[b]	−18	−7[b]	0	0	0	+4[b]

Table 1. (*Continued*)

	(%) Shoot length (%)					
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M
Eudesmanolides						
α-Santonin (**1**)	+72	+23[a]	+17[b]	+45	+16[b]	+23
1,2-Dihydro-α-santonin (**2**)	+68	+39[a]	+34[a]	+36	+2[b]	0
3β,OH-1,2-Dihydro-α-santonin (**3**)	+61	+48	+29[a]	+36	+18[b]	+8[b]
Melampolides						
Soulangianolide A (**4**)	−8[b]	−13[a]	−26	−23	−12[a]	−3[b]
Melampomagnolide A (**5**)	−20	+22	+4[b]	−31	−38	−9[b]
Melampomagnolide B (**6**)	−12[a]	0	0	+9[b]	0	+8[b]
cis,cis-Germacranolides						
14,15-diOH-*cis,cis*-Germacranolide (**7**)	−6[b]	−17[a]	+8[b]	+8[b]	−8[b]	+19
Guaianolides						
Dehydrocostuslactone (**8**)	−2[b]	−9[b]	+10[b]	+5[b]	−9[b]	+23
Isozaluzanin C (**9**)	−30	−19	−6[b]	−14[a]	−9[b]	−13[b]
Zaluzanin C (**10**)	−16	+7[b]	−14[b]	+8[b]	−29[b]	−10[b]
5α-Hydroxy-isozaluzanin C (**11**)	−9[b]	−28	−8[b]	−33	−29	−72
1α,5α-Dihydroxyisozaluzanin C (**12**)	−19	+13	−7[b]	+9[b]	−14[a]	+15[a]

$P<0.01$; a: $0.01<P<0.05$; b: $0.05<P$.

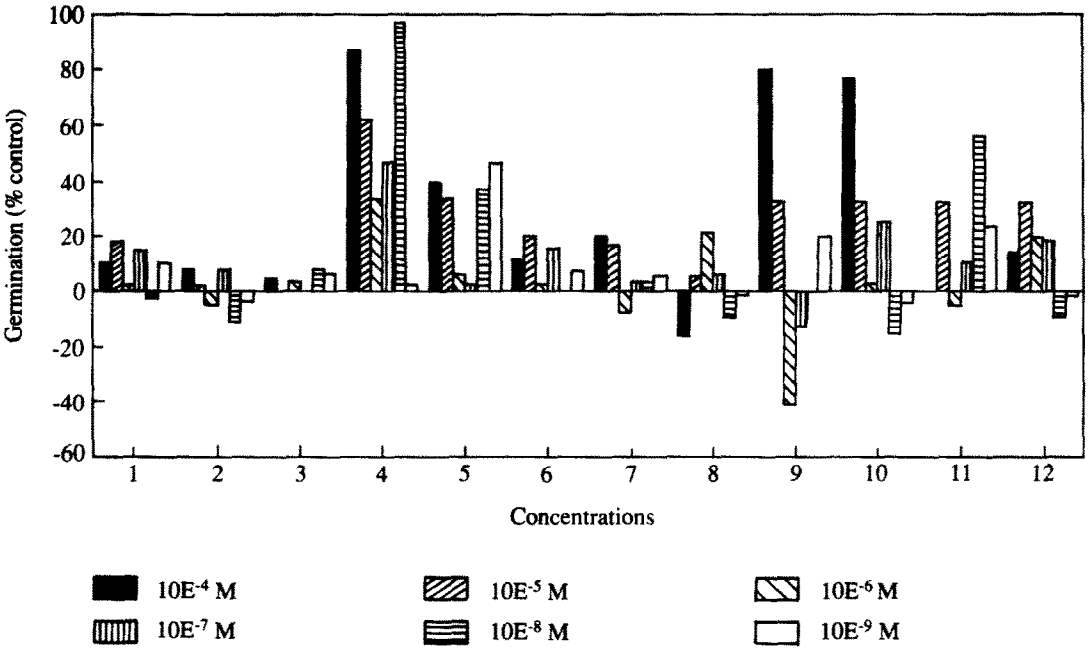


Fig. 3. Effect of lactones **1–12** on the germination of *L. sativa*.

"non-rigid" systems, the germination effect depends both on the presence of alkylable groups (such as α-methylene-γ-lactone) and the conformation of the molecule, which can determine the accessibility for alkylation.

The shoot and root length effects shown by **4–7** (Table 1, and Figs 4 and 5) are in agreement with the conformation–activity relationship indicated above for eudesmanolides.

In order to evaluate the effect of requirement (c) in 'pseudo-rigid' systems, guaianolides **8–12** were prepared and tested. In this case, the reaction with SeO_2 and *tert*-butylhydroperoxide was used to introduce regiospecifically an increasing number of hydroxyl groups (Fig. 1, and Tables 2 and 3).

The presence of a hydroxyl group in the molecule (**9–12**) changes dramatically the activity compared with **8** (Table 1 and Figs 3–5), which could be related with the increment in the number of possible linkage centres. Nevertheless, the different profile of activity shown by **9–12** could be a clear indication that the changes that an

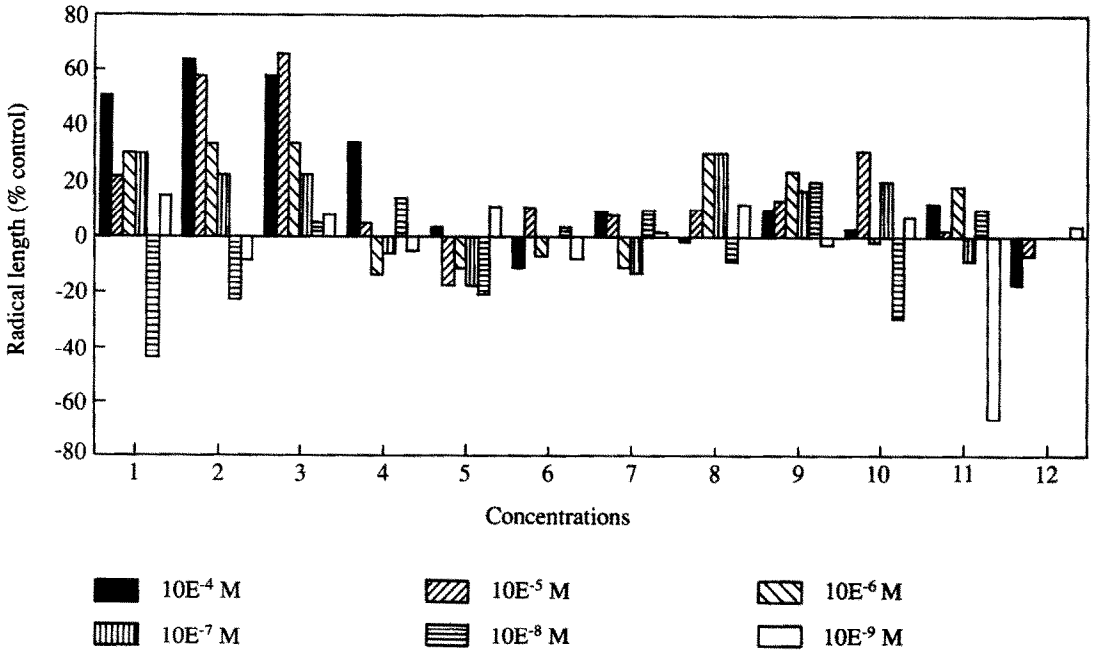


Fig. 4. Effect of lactones **1–12** on radical length of *L. sativa*.

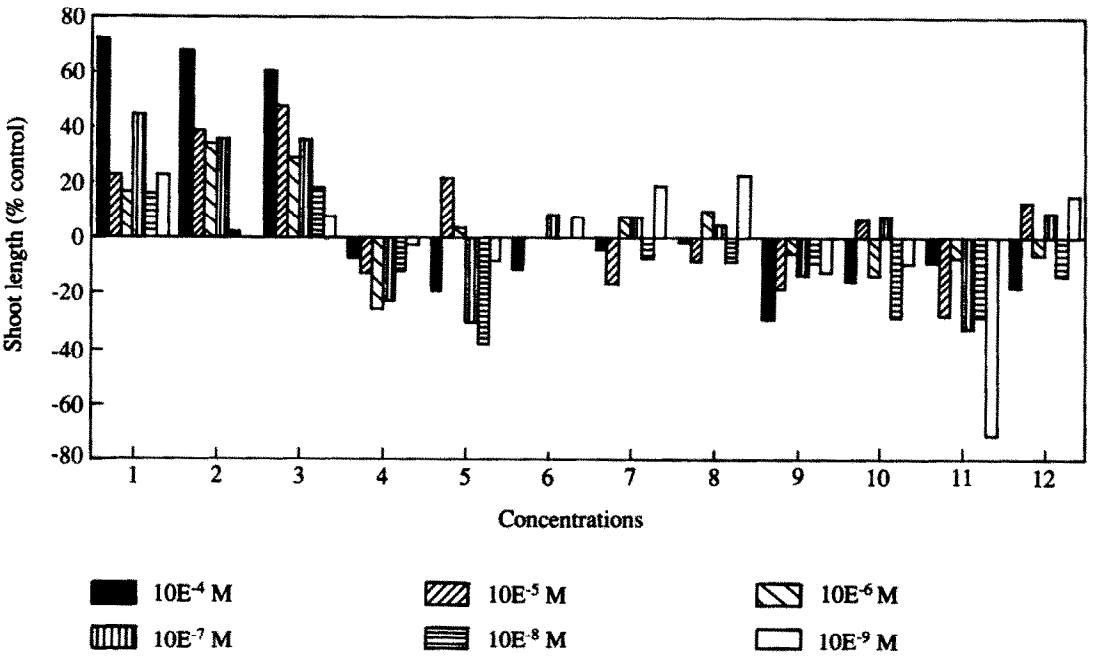


Fig. 5. Effect of lactones **1–12** on shoot length of *L. sativa*.

increasing number of hydroxyl groups produce on the seven-membered ring conformation also need to be considered (Fig. 7). In fact, the low activity showed by **12** indicates that the determining factor is the conformation over the solubility.

The molecular mechanics calculations (MMX) [17] for the two extreme possibilities, twist-chair and twist-boat for the seven-membered ring, show a preferred conformation of twist-boat for **11** and different twist-chair conformations for **8–12** [e.g. Φ (C_1–R_1, C_5–R_2), $J_{exp(6,7)}$, Table 5 and Fig. 7].

The phenomenon of a 'double maximum' of stimulatory activity at high and low values of concentration found in the melampolides has been reported previously [10]. The biochemical explanation of this behaviour needs to evaluate the possibility that the same compound may act on more than one protein or enzyme, with different values of best activity concentrations for each

Table 2. ^{1}H NMR data of compounds **4–7**, **9**, **11** and **12** (201.2 MHz, $CDCl_3$, $CHCl_3$ signal centred at δ7.24 ppm)*

H	4	5	6	7	9	11	12†
1	5.45 *br dd*	3.03 *dd*	5.63	5.48 *m*	3.08 *dd*	2.83	
2α	1.94 *m*[a]	1.21	2.10	2.55[a]	2.16	2.18	2.47 *dd*
2β	2.13 *m*[a]	2.31	2.27	2.22[a]	1.85 *ddd*	2.10	1.81 *dd*
3α	1.88 *m*[b]	2.24	1.07	2.27[b]			
3β	2.18 *m*[b]	2.12	2.10	1.72[b]	4.62 *ddd*	4.69 *dddd*	4.41
5	5.02 *br dd*	5.23 *dd*	2.83 *d*	5.48 *m*	3.08 *dd*		
6	4.57 *dd*	4.60	3.83	5.19	3.89	4.09 *d*	3.96 *d*
7	2.51 *ddddd*	2.76	2.83 *m*	2.71	2.84 *dddd*	3.26	3.30
8α	1.51 *m*	1.60 *dddd*	1.63	2.06 *m*	2.08	1.38	1.32 *dddd*
8β	2.34 *m*	2.28	2.38	2.55	2.21	2.04 *dddd*	2.06 *m*
9α	2.24 *m*	2.04 *ddd*	2.26 *m*	2.27[c]	2.51 *ddd*	2.52	2.62
9β	2.00 *m*	1.38 *ddd*	2.26 *m*	1.72[c]	1.37	2.23	2.06
13a	5.42 *d*	5.46	5.52	5.58	5.30	5.66	5.41
13b	6.12 *d*	6.19	6.20	6.27	6.21	6.22	6.07
14	4.11 *d*	3.95	4.14	4.03	4.92 s	4.92	4.98
14′	3.99 *d*	3.50	4.04		4.77 s	4.74	4.98
15	1.85 *d*	1.85	1.52	4.03	5.49	5.61	5.65
15′					5.34 *d*	5.39	5.48

*Multiplicities are not repeated if identical with those in the preceding column. Assignments were confirmed by COSY experiments.

†Spectrum in $CDCl_3$–MeOD (1:1).

[a–c]Paired values in the same column may be interchanged.

J(Hz): **4**: 1,2α = 1,2β = 7.2; 5,15 = 1.2; 5,6 = 10.2; 6,7 = 9.7; 7,13a = 3.2; 7,13b = 3.4; 14,14′ = 12.5; **5**: 1,2α = 4.1; 1,2β = 10.1; 5,6 = 10.1; 5,15 = 1.0; 6,7 = 9.7; 7,8α = 2.8; 7,8β = 9.0; 7,13a = 7,13b = 3.2; 8α,8β = 12.0; 8α,9β = 15.5; 8α,9α = 4.4; 8α,7 = 3.1; 8β,9α = 12.9; 9α,9β = 13.9; 9β,8β = 4.4; 14,14′ = 12,2; **6**: 1,2α = 1,2β = 7.6; 5,6 = 9.6; 6,7 = 9.4; 7,13a = 3.2; 7,13b = 3.4; 8α,8β = 13.4, 8α,9β = 12.8; 8α,9α = 8α,7 = 4.3; 14,14′ = 12.7; **7**: 5,6 = 3.9; 6,7 = 9.3; 7,13a = 1.7; 7,13b = 2.1; 14,14′ = 15,15′ = 16.8; **9**: 3,2α = 3,2β = 6.7; 5,6 = 6,7 = 9.3; 7,13a = 3.1; 7,13b = 3.5; 15,15′ = 1.7; **11**: 1,2α = 4.2; 1,2β = 8.5; 3,15 = 1.9; 3,15′ = 1.5; 6,7 = 8.7; 7,13a = 3.1; 7,13b = 3.6; **12**: 2α,2β = 14.6; 2α,3β = 7.6; 2β,3β = 5.6; 3β,15 = 1.9; 3β,15′ = 1.6; 6,7 = 8.6; 7,13a = 3.2; 7,13b = 3.6; 9α,8β = 7.8.

Table 3. ^{13}C NMR data of compounds **4–7**, **9**, **11** and **12** (50.30 MHz, $CDCl_3$, signal centred at δ77.00 ppm)*

C	4	5	6	7	9†	11	12‡
1	126.1 *d*	62.5	127.2	125.5[a]	49.6	53.2	79.0 *s*
2	23.3 *t*	27.1	23.5	26.4[b]	36.6	37.4	45.5
3	38.3 *t*	34.4	36.7	26.9[b]	74.6 *d*	74.6	70.2
4	140.8 *s*	142.5	60.1	115.9	154.3	156.4	154.7
5	125.2 *d*	124.4	63.6	125.8[a]	45.6	80.2 *s*	81.6
6	80.7 *d*	79.3	81.2	78.1	84.9	85.9	84.4
7	45.3 *d*	46.3	42.7	43.9	44.2	40.0	37.7
8	24.9 *t*	24.0[a]	25.5	24.3	31.3	31.3	30.6
9	24.6 *t*	24.0[a]	23.9	31.8	39.8	38.6	26.6
10	138.7 *s*	63.1	138.9	115.9	139.6	139.3	139.9
11	140.1 *s*	139.8	139.6	139.7	148.6	147.4	148.7
12	170.8 *s*	170.2	169.8	—	169.7	170.4	170.8
13	118.8 *t*	119.3	120.2	122.7	120.5	121.2	120.6
14	66.1 *t*	64.5	65.7	66.3	113.2	114.1	115.3
15	17.0 *q*	17.3	17.8	66.3 *t*	113.3	114.7	116.9

*Degree of protonation and assignments were obtained by ^{13}C–^{1}H correlation; multiplicities are not repeated if identical with those in the preceding column.

†The degree of protonation was obtained by APT heteronuclear multipulse programs.

‡Spectrum in $CDCl_3$–MeOD (1:1).

[a,b]Paired values in the same column may be interchanged.

one. So, the curve observed corresponds to the combination of the different activity curves for each site of action.

Although the only parameter that is usually reported when evaluating the allelopathic activity is the germination effect, we want to point out the significance that radical and shoot length parameters could have as indicators of future plant development. An insignificant effect on germination need not be related with an inactive

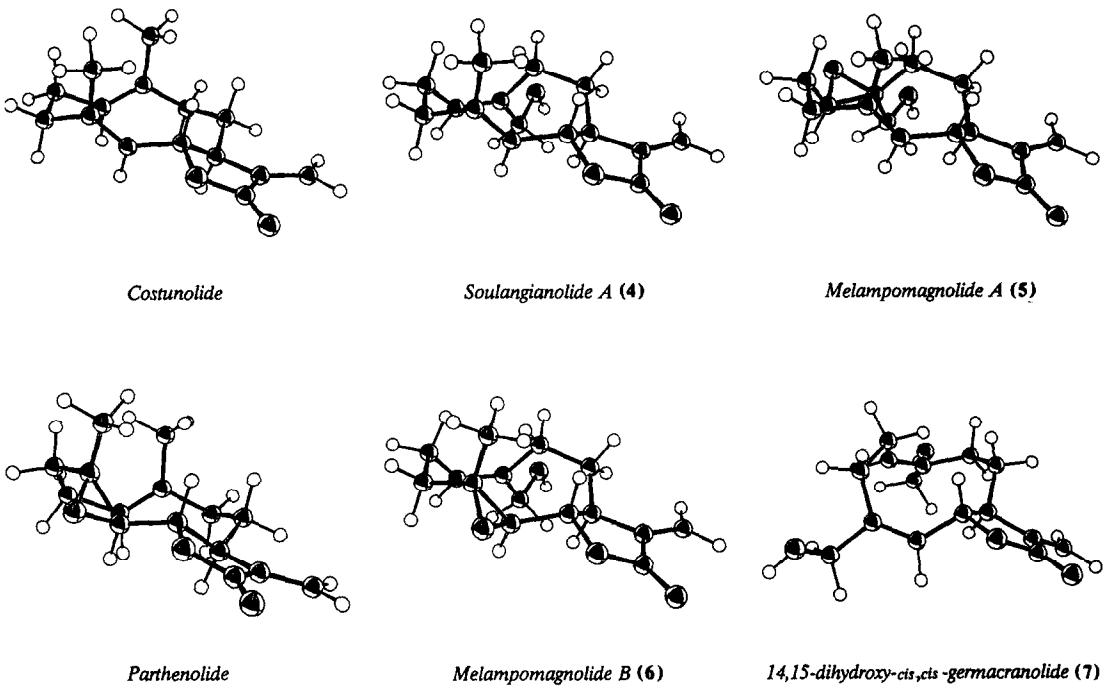


Fig. 6. Minimum energy conformers in solution of costunolide, parthenolide and compounds 4–7 obtained using MMX calculations.

Table 4. Selected dihedral angles of costunolide, parthenolide and compounds 4–7 obtained using MMX calculations and their correlation with some ^{1}H NMR data

	Φ (C_{10}–C_{14}, C_5–H)	Φ (H-5, H-6)	Φ (H-7, H-13a)	$J_{exp(5,6)}$	$J_{exp(6,7)}$	$J_{exp(7,13a)}$ / $J_{exp(7,13b)}$
Costunolide	174.81°	160.21°	94.69°	8.8	8.6	3.1 3.6
4	−45.03°	173.54°	95.53°	10.2	9.7	3.2 3.4
5	−43.84°	174.03°	95.60°	10.1	9.7	3.2 3.2
Parthenolide	160.82°	155.24°	87.55°	9.0	9.4	3.2 3.5
6	−41.20°	−164.23°	90.31°	9.6	9.4	3.2 3.4
7	18.64°	−136.82°	63.81°	3.9	9.3	1.7 2.1

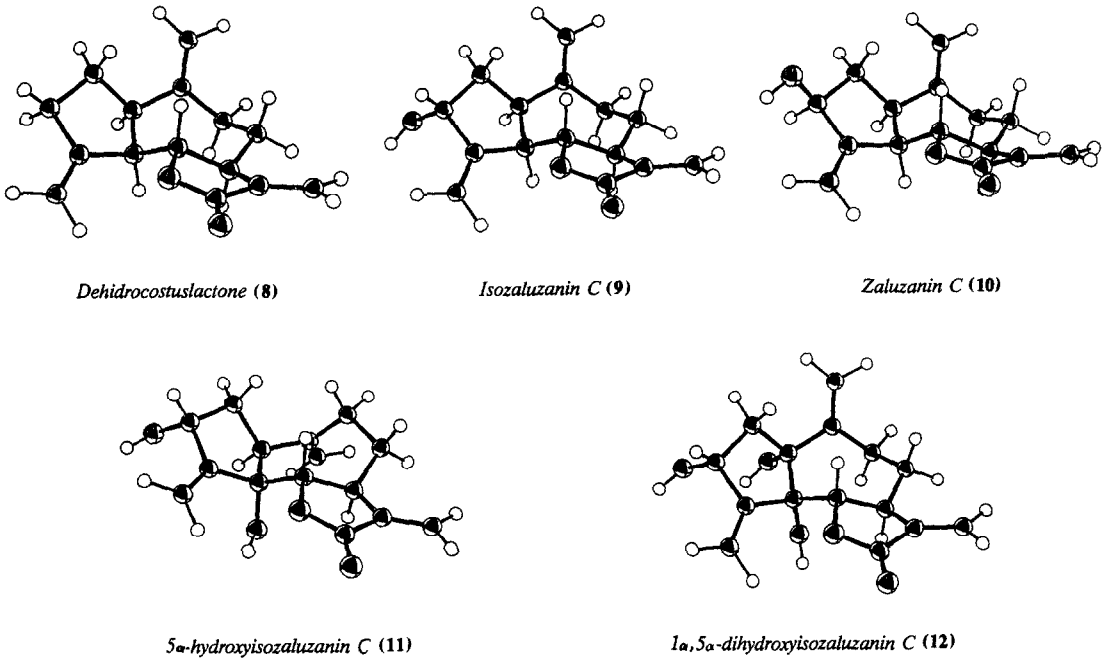
extract or compound if it has the capability to induce malformations in the future plant (e.g. abnormally large shoots or rachitic radicals). Thus 10^{-9} M **11** has no strong effect on germination, but has a powerful inhibiting effect over radical and shoot length (−67 and −72%, respectively). 10^{-6} M **1** does not affect germination (−3%) or growth of the shoot (−16%), but radical growth is powerfully inhibited (−44%) (Table 1).

EXPERIMENTAL

Sesquiterpene lactones. α-Santonin (**1**) was purchased from Sigma. 1,2-Dihydro-α-santonin (**2**) was obtained by reduction of **1** with $[Ph_3P]_3RhCl$ in a H_2 atmosphere; 3β-hydroxy-1,2-dihydro-α-santonin (**3**) was obtained by reduction of **2**, protection, sepn and deprotection according to the methods previously described [18].

Soulangianolide A (**4**) was prepared from costunolide as follows: SeO_2 and *tert*-butylhydroperoxide was added to a soln of costunolide in dry CH_2Cl_2 under an atmos. of N_2 (0.5:2.0:1.0, molar). The reaction mixture was separated by CC on silica gel using petrol–EtOAc (3:2) yielding **4** as a major product (40%) and a small amount of the 14-aldehyde derivative. Compound **4**: $C_{15}H_{20}O_3$; gum; IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3417 (OH, st), 1752 (γ-lactone); MS (70 eV) *m/z* (rel. int.): 248 $[M]^+$ (1.4), 230 $[M-H_2O]^+$ (14.1), 215 $[M+1-MeOH]^+$ (9.4); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

A large excess of *tert*-butylhydroperoxide (0.5:4.0:1.0, molar) gave melampomagnolide A (**5**) as major product in 70% yield,



Dehidrocostuslactone (**8**) *Isozaluzanin C* (**9**) *Zaluzanin C* (**10**)

5α-hydroxyisozaluzanin C (**11**) *1α,5α-dihydroxyisozaluzanin C* (**12**)

Fig. 7. Minimum energy conformers in solution of compounds **9–12** obtained using MMX calculations.

Table 5. Selected dihedral angles of compounds **8**, **9**, **11** and **12** obtained using MMX calculations and their correlation with some ^{1}H NMR data

	ΔE_{tC-tB} (kcal mol^{-1})	Φ (C_1–R_1, C_5–R_2)	Φ (H-7, H-13a)	Φ (H-8α, H-9α) Φ (H-8α, H-9β)	$J_{exp(2\alpha,3\beta)}$ $J_{exp(2\beta,3\beta)}$	$J_{exp(5,6)}$	$J_{exp(6,7)}$
		$R_1=R_2=H$					
8	−0.66	30.65°	93.42°	−67.88° −48.00°	— —	9.6	9.6
		$R_1=R_2=H$					
9	−0.87	32.24°	93.97°	68.32° −47.47°	6.7 6.7	9.3	9.3
		$R_1=H$, $R_2=OH$					
11	+0.07	36.76°	82.95°	−37.02° −150.71°	7.0 7.0	—	8.7
		$R_1=R_2=OH$					
12	−1.81	30.18°	93.16°	68.40° −47.00°	7.6 5.6	—	8.6

*$E_{tC-tB} = E_{twist\ chair-twist\ boat}$

and the corresponding 14-aldehyde derivative. Compound **5**: $C_{15}H_{20}O_4$; crystal, mp 178–179° (from EtOAc); IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3465 (OH, st), 1756 (γ-lactone), 1260 (C–O–C, epoxy); MS (70 eV) *m/z* (rel. int.): 265 $[M+1]^+$ (0.3), 247 $[M+1-H_2O]^+$ (0.8), 229 $[M+1-H_2O-O]^+$ (1.9); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Treatment of parthenolide in the same conditions as described for costunolide afforded melampomagnolide B (**6**) in 80% yield. Compound **6**: $C_{15}H_{20}O_4$; crystals, mp 174–176° (from $CHCl_3$EtOAc), IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3419 (OH, st), 1755 (γ-lactone), 1256 (C–O–C, epoxy); MS (70 eV) *m/z* (rel. int.): 264 $[M]^+$ (0.6), 246 $[M-H_2O]^+$ (0.9), 228 $[M-H_2O-O]^+$ (0.7); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Reaction of **4** with SeO_2 and *tert*-butylhydroperoxide (1.0:2.0:2.0, molar) in dried CH_2Cl_2 under N_2 atmos., after 3 hr at 50°, gave 14,15-dihydroxy-*cis*-*cis*-germacranolide (**7**) (43%). Compound **7**: $C_{15}O_4H_{20}$; gum; IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3393 (OH, st), 1745 (γ-lactone); MS (20 eV) *m/z* (rel. int.): 264 $[M]^+$ (0.4), 246 $[M-H_2O]^+$ (5.7), 228 $[M-2H_2O]^+$ (8.0); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3.

Dehydrocostuslactone (**8**) was isolated from costus resin oil (Pierre Chauvet, S. A., France) by CC on silica gel using hexane–EtOAc (95:5) as eluent.

Treatment of **8** as described for **4** afforded isozaluzanin C (**9**) and 5α-hydroxyisozaluzanin C (**11**), as major products (44% and 35% yield, respectively). Compound **9**: $C_{15}O_3H_{18}$; gum; IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3422 (OH, st), 1755 (γ-lactone); MS (70 eV) *m/z* (rel. int.): 246 $[M]^+$ (4.0), 228 $[M-H_2O]^+$ (12.4); ^{1}H NMR: Table 2; ^{13}C NMR: Table 3. Compound **11**: $C_{15}O_4H_{18}$; crystals, mp 166–168° (from EtOAc); IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3403 (OH, st),

1752 (γ-lactone); MS (70 eV) *m/z* (rel. int.): 262 $[M]^+$ (1.4), 244 $[M-H_2O]^+$ (7.3), 226 $[M-2H_2O]^+$ (4.1); ^1H NMR: Table 2; ^{13}C NMR: Table 3.

Lactone **9** (100 mg) was dissolved in dried pyridine and treated with mesyl chloride to obtain the mesyl derivative. The reaction mixt. was stirred with aq. 5% NaOH for 24 hr, neutralized with aq. 4% HCl and extracted with EtOAc (× 5). The purification of the extract by CC gave zaluzanin C (**10**) in 90% yield.

Reaction of **11** as described for **4** gave, after 12 hr, 1α,5α-dihydroxy-isozaluzanin C (**12**) in a 28% yield. Compound **12**: $C_{15}O_5H_{18}$; gum; IR $\nu_{max}^{neat,KBr}$ cm^{-1}: 3420 (OH, st), 1755 (γ-lactone); MS (70 eV) *m/z* (rel. int.): 279 $[M+1]^+$ (0.4), 260 $[M-H_2O]^+$ (1.7), 243 $[M-H_2O-OH]^+$ (5.7), 242 $[M-2H_2O]^+$ (5.0), 227 $[M-2H_2O-OH]^+$ (2.6), 226 $[M-H_2O-2OH]^+$ (2.1), 224 $[M-3H_2O]^+$ (1.7); ^1H NMR: Table 2; ^{13}C NMR: Table 3.

The spectroscopic data of synthetic compounds are in agreement with those previously reported as natural products (**4** [19], **5** and **6** [20], **9** [21, 22], **10** [22]). The unambiguous assignments of ^1H and ^{13}C NMR of **4–6** and **9** are presented in Tables 2 and 3.

Lettuce seed germination bioassay. Seeds of lettuce, *Lactuca sativa* var. *nigra*, 1989 crop, were obtained from Rancho La Merced, Junta de Andalucía, Jerez, Spain. All undersized and damaged seeds were discarded and the assay seeds were selected for uniformity of size.

Germination bioassays consisted of germinating 25 lettuce seeds for 5 days (3 for germination and 2 for root and shoot growth) in the dark at 25° in 9-cm plastic petri dishes containing a 10-cm sheet of Whatman no. 1 filter paper, and 10 ml of a test or control soln. Test solns (10^{-4} M) were prepared using DMSO (0.1% v/v) as initial solubilizing agent. Test solns 10^{-5}–10^{-9} M were obtained by diluting the previous soln. Parallel controls consisted of deionized H_2O with the same DMSO concn. There were 3 replicates of each treatment and of parallel controls. The number of seeds per replicate, time and temp. of germination were chosen in agreement with a number of preliminary experiments, varying the number of seeds, vol. of test soln per dish and the incubation period. All the pH values were adjusted to 6.5–7.0 before the bioassay.

Statistical treatment. The germination, root and shoot length values were tested by the Student's *t*-test, being the differences between the experiment and the control significative with a value of $P=0.01$.

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