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Synthesis of melampolides and *cis*,*cis*-germacranolides as natural herbicide models☆

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Abstract—The comparative study between the theoretical molecular properties of the starting materials and the yields in the transformation of melampolides to *cis*,*cis*-germacranolides using SeO_2/*t*-BuOOH (TBHP) as oxidant allows to establish a feasible relationship with their values of dipolar moment. Conditions for this transformation are optimized and some mechanistic considerations are made based in this finding. Cluster analysis of the phytotoxic activity of the melampolides and *cis*,*cis*-germacranolides obtained shows that the activity is greatly influenced by the spatial shape of the backbone, prevailing upon other factors such as the presence of reactive functional groups.
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1. Introduction

Selenium dioxide is a soft and widely used reagent useful to get access to allylic alcohols and unsatured carbonyl systems from alkenes, among others. The mechanism is thought to proceed through a first 'ene' reaction rate-determining step, followed by a [2,3] sigmatropic rearrangement, and breakdown of the resultant selenium ester.[1] This subject has been recently revisited, so as kinetic isotope effects and theoretical calculations strongly supports the idea of an ene concerted reaction with SeO_2 itself as active oxidant rather than an ene-concerted process with a selenous ester, or even an electrophilic attack by $HSeO_2^+$. However, the isolation of some minor products suggests the presence of a minor pathway involving a stepwise ene reaction through reversible formation of a zwitterion followed by rate-limiting proton transfer.[2] In spite of its usefulness, some environmental and safety concerns have arisen because wastes contain high amounts of the metal. Thus, metal-catalyzed oxidation methods with alkyl-hydroperoxides as stoichiometric oxidant have been developed and are commonly used. Among them, the system SeO_2/*tert*-butyl-hydroperoxide (TBHP) has found extensive application in natural products synthesis as a soft and stereoselective reactive.[3,4]

Currently, we are interested in developing new models of agrochemicals based on phytotoxic allelochemicals.[5] The increasing incidence of resistant weeds to important herbicides classes such as *s*-triazines[6] and dinitroanilines[7] in addition to environmental concerns are pulling-on research on this field. As an alternative, Allelopathy is able to offer new lead compounds to be used as herbicides and several classes of compounds such as phenolics, alkaloids, and terpenes have been reported as phytotoxic.[8] Among them, germacranolides (Fig. 1) constitute a very interesting group because of the high number of compounds reported and their wide spectrum of biological activities that includes pharmacological,[9] cytotoxic,[10] fungicidal,[11] antibacterial,[12] and allelopathic activities.[13] Consequently, an important effort to synthesize the germacranolide skeleton has been made.[14] These efforts have been centred, mainly, in *trans*,*trans*-germacranolides, but little has been done about the chemistry and biological activity in the other subgroups.

Going on with our previous work in sesquiterpene lactones (SL) as potential lead herbicides[4,5,15] here in we report the

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Keywords: Allylic oxidation mechanism; Synthesis of sesquiterpenes; Melampolides; *cis*,*cis*-Germacranolides; Structure–activity relationship study (SAR).

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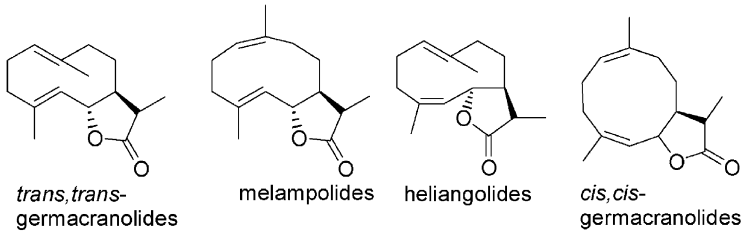


Figure 1. Main germacranolides backbones.

diasterospecific synthesis of several SL with melampolide and *cis*,*cis*-germacranolide skeletons using modified reaction conditions in the SeO_2–TBHP system[17] suitable for use in a Structure–Activity Relationship (SAR) study of their phytotoxicity.

2. Results and discussion

Metal-catalyzed oxidations with alkyl hydroperoxides can be divided into two categories depending on if the intermediate involved in the oxygen-transfer step is a peroxometal or an oxometal. In other words, the initial oxidizing agent could be the selenium dioxide itself, or the selenium-TBHP adduct. It is generally believed that this reaction proceeds through an initial ene reaction of SeO_2 with the olefin, followed by a [2,3] sigmatropic rearrangement, the resulting Se(II) being reoxidized by TBHP, and additional data supporting this vision has been published (Fig. 2).[16] In this case, only a catalytic amount of the metal is needed (typically, 2 mol%).[17]

When this reaction is carried out using the natural germacranolide costunolide (**1**) as substrate the amount of SeO_2 needed increases and the stereochemistry of the double bond changes in the final product from *E* to *Z* (compound **2**). In this case, a different mechanism with a bulky peroxometal intermediate *t*-BuOO–SeO_3H has been proposed (Fig. 3).[17]

This mechanism should certainly explain why the methyl group at C-10 reacts before the methylene position at C-9 in the germacranolide skeleton, instead of the accepted order $CH_2 > CH_3 > CH$.[16] The change in the stereochemistry of the double bond takes place through a rotation before the sigmatropic rearrangement occurs.

The reaction also yields as side-products the corresponding 14-oxoderivatives due to oxidation of the resulting hydroxyl group. This reaction has demonstrated to be useful in getting access to the melampolide and *cis*,*cis*-germacranolide skeletons, even though the yields obtained for *cis*,*cis*-germacranolides were low (around 10%).[5a]

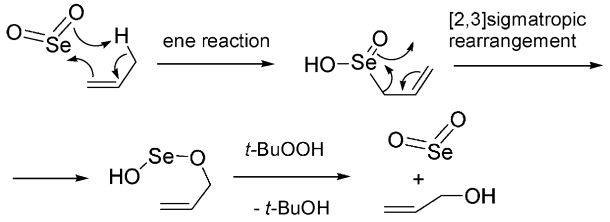


Figure 2. General mechanism of the SeO_2 allylic oxidation.

2.1. Synthesis and molecular modeling

In order to overcome these low yields and aiming also to perform a SAR study melampolides **2–8** were used as starting materials to obtain the corresponding *cis*,*cis*-germacranolides (Table 1) with different yields. Conditions for optimal yields were set to as SeO_2/TBHP (0.5:2 molar ratio) in a reflux of DCM using compound **4**. These conditions allow to minimize the amount of the 14-oxo-derivative. Best results were obtained with the 14-acetoxy- and 14-chloro-derivatives **4** and **5** respectively, followed by the oxo-melampolide **3** and the hydroxy-melampolide **2**. The 14-TBDMS derivative **6** gave the 14-oxoderivative **3** (53% yield) due to deprotection of the silyl group caused by the acid medium generated by the selenium salts and subsequent oxidation of the free hydroxyl. No change in the stereochemistry of the Δ(4–5) double bond was observed. Finally, lower or no yields were obtained with the epoxy-derivatives **7** and **8** (Table 1).

Theoretical ΔH_r° values as obtained using PM3 calculations (Table 2)[18] were in all cases about −42 Kcal/mol. This result supports the idea that the different reactivity observed for each substrate should not be related with the difference of energy between starting and final products. Consequently, factors affecting the transition states should arise as determining to explain the experimental data.

According to the mechanism proposed by Haruna and Ito[17] three transition states will consecutively take place during the reaction (Fig. 4): (a) a cyclic intermediate corresponding to the ene reaction step $E_1^\#$; (b) the eclipsed conformation of the selenium substituent and the double bond during the rotation step $E_2^\#$; and (c) that corresponding to the sigmatropic rearrangement $E_3^\#$. The conformational flexibility of the backbone in the different substrates and end products are mostly the same and are not influenced by substituents at the C-14 position. Otherwise, the reacting part of the melampolide (e.g. the double bond at C-4, C-5 and the C-15 methyl group) is the same for all starting compounds (**2–8**). Consequently, the activation energies corresponding to the rotation and rearrangement steps have to be similar in all cases and will not determine the different yields observed. Thus, we propose as a feasible working hypothesis that those factors affecting the energy of the first transition state ($E_1^\#$, ene reaction) would be crucial to explain the different results obtained and will also be in good agreement with the literature.[16]

A correlation between theoretical dipolar moments obtained

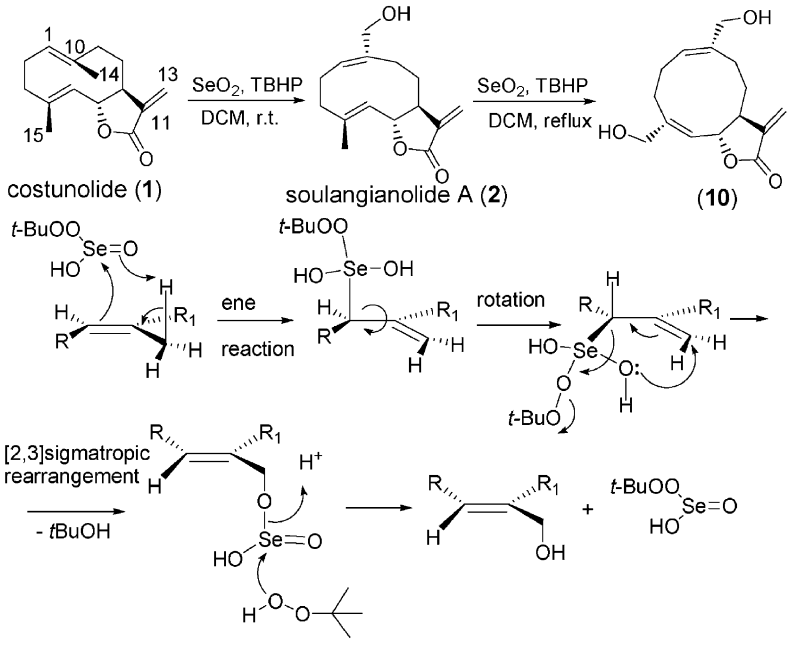
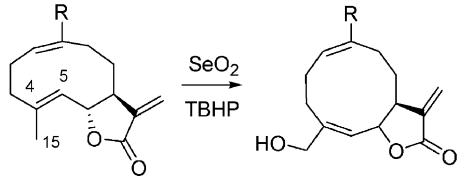


Figure 3. Mechanism proposed for the SeO_2-TBHP allylic oxidation.[17]

from PM3 calculations and the experimental yields can be observed (Table 1, Fig. 5): the lower the dipolar moment is, the higher the resulting yield. This correlation would be in good agreement with the hypothesis presented: the ene reaction is a concerted process where no new charges are generated; lower dipolar moments of starting material lead to lower dipolar moments in the transition state, and thus, transition states with lower polarities will be better dissolved in low polar solvents such as those used for these oxidations (DCM: $\mu = 1.60$ D; THF: $\mu = 1.63$ D). Accordingly, the solvation enthalpy contribution will be diminished and, consequently, the activation energy will also be lowered, thus favoring the reaction pathway. Compounds **4** and **5** present the lower dipolar moments and the higher yields while the free alcohol **2** and the 14-oxoderivative **3** render lower amounts of the *cis*,*cis*-germacranolide. Finally, the epoxiderivative **8**, with the highest value of dipolar moment, does not react at all (Table 1).



Compound **7** constitutes an exception to this correlation (Fig. 5), as the theoretical value of the dipolar moment does not match with the reactivity. However, experimental R_f values obtained by TLC show that real polarity of this compound should correspond to a value similar to those of compounds **2** and **8**, thus matching the correlation proposed.

The starting melampolide **2** was obtained from costunolide (**1**) using SeO_2/TBHP, along with the aldehyde **3** as side product.[5a,17] The acetylated derivative **4** was used as starting material for *cis*,*cis*-germacranolide large scale synthesis since it provided the best yields. Selective epoxidation using MCPBA at the C1–C10 (**18** and **19**) or the C4–C5 (**15**) double bonds in the *cis*,*cis*-germacranolide backbone was achieved through the introduction of a bulky steric demanding *tert*-butyl-dimethyl-silyl (TBDMS) group at C15 (**16**). However, steric hindrance induced by the C14-acetoxyl group in **16** did not allow direct epoxidation at C1–C10. So, selective deprotection of the acetoxy derivative at C14 was carried out using magnesium methoxide,[19] yielding the corresponding de-acetylated derivative (**17**) and traces of the enantiomerically pure Michael adduct at the unsatured lactone ring (**17b**). The stereochemistry of compound **17b** was unequivocally assigned as 11*S* through NOESY experiments. Epoxidation of the resulting free-hydroxyl compound **17** yielded both alpha (**18**) and beta

Table 1. Melampolide to *cis*,*cis*-germacranolides conversion using SeO_2/TBHP as oxidant system[a]

R	Starting material	Final product	Yield (%)	Dipolar moment (μ, D)
CH_2OH	**2**	**10**	10	4.34
CHO	**3**	**11**	24	4.45
CH_2OAc	**4**	**12**	57	3.78
CH_2Cl	**5**	**13**	43	4.04
$CH_2OTBDMS$[b]	**6**	**3**	53	4.70
1β,10β-Epoxy-derivatives				
CH_2OH	**7**	**14**	3	3.87
CH_2OAc	**8**	—	—	4.49

[a] SeO_2:TBHP molar relation (0.5:2), DCM, reflux.
[b] The reaction product was the carbaldehyde melampolide, with no change in the stereochemistry.

Table 2. Theoretical ΔH_f° and ΔH_r° (Kcal/mol) for some germacranolides as obtained from PM3 calculations[18]

Starting material	ΔH_f° (Kcal/mol)	End product	ΔH_f° (Kcal/mol)	ΔH_r° (Kcal/mol)
2	−94.8	**10**	−137.1	−42.3
3	−82.3	**11**	−124.4	−42.1
4	−136.6	**12**	−177.1	−40.5
5	−61.2	**13**	−101.2	−40.0
7	−106.8	**14**	−153.4	−46.6

(**19**) epoxide isomers. Finally, epoxidation of the double bond at C4–C5 only afforded one isomer (**15**) due to hindrance of the alpha face of the molecule by the lactone ring (Fig. 6). Epoxidation in the same position of the melampolide framework is not possible due to steric restrictions caused by the spatial disposition of the molecule.

2.2. Bioassays and SAR studies

The bioactive properties of sesquiterpene lactones have been attributed for a long time to the electrophilic properties of the α-methylene-γ-lactone moiety because it easily reacts with nucleophilic groups contained in important biomolecules.[20] A long discussion whether nucleophilic additions at the double bond of the lactone ring is the main factor causing bioactivity on sesquiterpene lactones is still on the literature. However, this feature might not be the only responsible of their bioactivity and we have recently reported positive bioactive sesquiterpene lactones without this functional group.[4,15a] The changes introduced in the basic melampolide and *cis*,*cis*-germacranolide frameworks attend to analyze the influence in the activity that the following criteria might have: (i) influence of reactive groups other than the α-methylene-γ-lactone group; (ii) influence of the spatial arrangement of the basic carbon framework; and (iii) importance of halogen-containing compounds, very common in pesticide and insecticide synthetic designs.[21] Consequently, compounds depicted in Figure 6 were synthesized according to the methodology described above and tested. Compounds **1** and **22–26** were previously tested[4] and have been introduced here by means of comparison and cluster analysis.

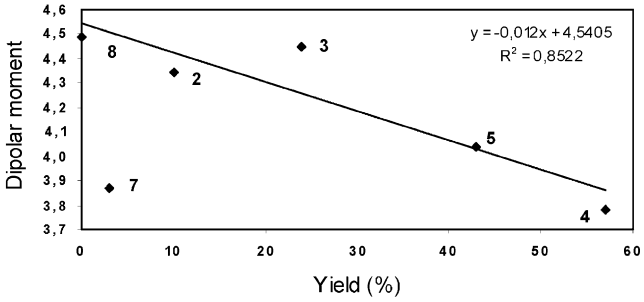


Figure 5. Correlation between dipolar moments of starting materials and the yields in the SeO_2/TBHP allylic oxidation.

Bioassays were carried out using the monocots *Triticum aestivum* L. and *Allium cepa* L., and the dicots *Lactuca sativa* L. and *Lepidium sativum* L. as target species, according to the methodology reported by our group[22] and

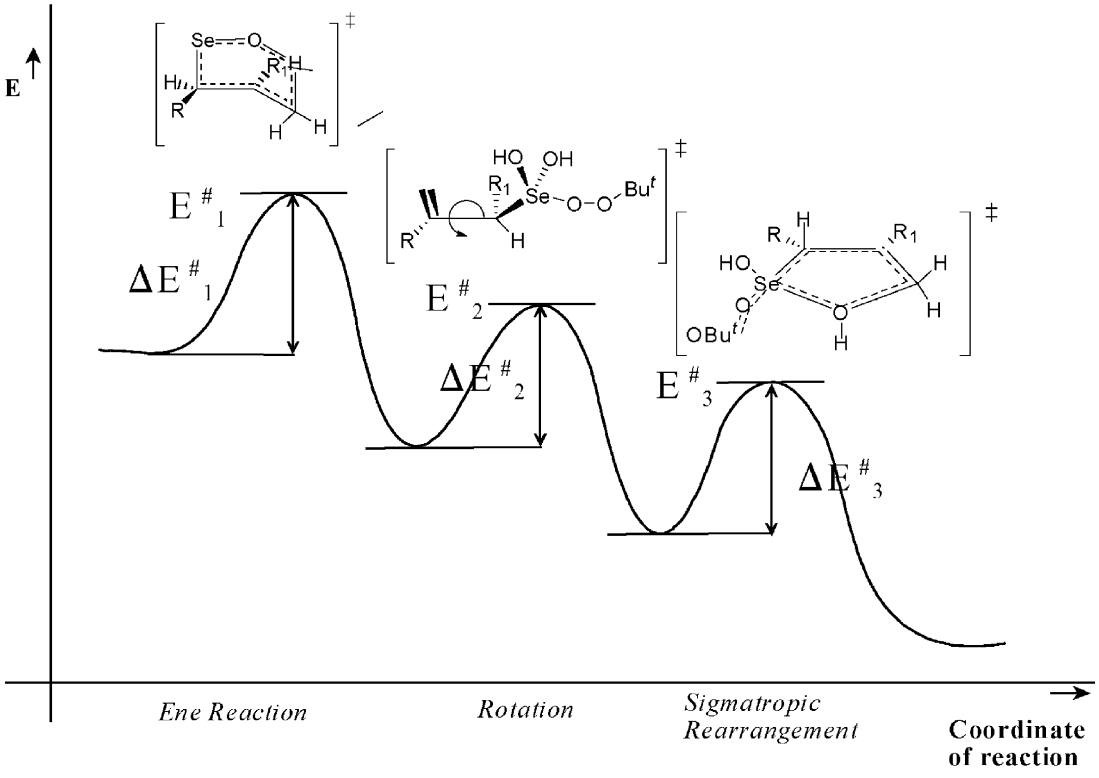


Figure 4. Activated states according to the proposed reaction mechanism.

Table 3. ^{1}H NMR data for listed compounds (400 MHz in $CDCl_3$, signal of the residual $CHCl_3$ centered at δ 7.25 ppm)[a]

H	3	4	5	6	8	9	10	11	12	12b
1	6.48 dd	5.48	5.59	5.42	2.90	4.48 m	5.49 dd	6.56	5.54	5.45
2α	2.40[b] m	2.12[b]	2.15[b]	2.11[b]	2.32 dddd	2.02[b] m	2.20[b]	2.76[b]	2.27[b]	2.40 dddd
2β	2.26[b] m	1.95[b]	1.99[b]	1.94[b]	1.19	1.56[b]	2.52[b]	2.54[b]	2.55[b]	2.60
3α	2.04 ddd	1.87 m	1.85 ddd	1.84 m	2.24 ddd	2.32[c] m	2.24[c] ddd	2.46 m	2.24[c]	2.51 ddd
3β	2.28 m	2.16	1.58	2.15	2.16	2.18[c] ddd	2.66[c] m	2.62 m	2.67[c]	2.83 ddd
5	4.97 brd	5.00	5.00	5.04	5.24 d	5.38 brd	5.48	5.50 d	5.49	6.32 brd
6	4.52 dd	4.54	4.58	4.58	4.59	4.62	5.19	5.07	5.18	5.35
7	2.26 ddddd	2.57	2.43	2.59	2.81	2.73	2.71 m	2.44	2.72	2.87
8α	2.86 dddd	2.26	2.36 m	2.33 dddd	2.16 m	2.28	2.08 dddd	2.42	2.07	2.07 m
8β	1.44 dddd	1.54	1.57	1.50	1.62	1.67	1.72	1.67	1.73	1.82
9α	2.38[c] m	2.17[c]	2.03[c]	2.18[c]	2.06	1.92[d]	2.60[d]	2.80	2.61	2.24 ddd
9β	1.95[c] m	2.07[c]	1.97[c]	2.01[c]	1.37 ddd	1.33[d]	2.30[d]	2.49 m	2.22	2.64
13a	5.41 d	5.39	5.46	5.40	5.41	5.42	5.58	5.58	5.39	5.69
13b	6.08 d	6.11	6.18	6.16	6.18	6.17	6.27	6.25	6.11	6.37
14	9.39 d	4.38	4.13	4.14	4.43	9.83 s	4.10 d	9.42	4.38	4.58
14′		4.55 d	3.99	4.00	3.91		4.05		4.55	4.43
15	1.83 d	1.83	1.84	1.84	1.85 s	1.91	3.99	4.04	3.98	9.38

H	13	14	15	16	17	17 b	18	19	20
1	5.61 dd	3.10 m	5.73 dd	5.54	5.48	5.48	3.17	3.09	6.47 ddd
2α	2.21[b] m	2.03[b]	2.19	2.17	2.17[b]	2.12 [b]	1.17	2.14	2.43 dddd
2β	2.54[b] m	1.76[b]	2.42	2.52	2.50[b]	2.46 [b]	2.43	2.61	2.26 dddd
3α	2.26 m	2.03[c]	2.28[b] ddd	2.20[b] m	2.18[c]	2.17 [b]	2.15	2.03	2.06 dd
3β	2.67 m	1.76[c]	1.88[b]	2.61[b]	2.54[c]	2.56 [b]	2.20	2.03	
5	5.49 d	5.52 dd	3.01 d	5.45 dd	5.44 brd	5.51 d	5.42	5.49	4.93
6	5.16 dd	5.20	4.28	5.17	5.18	5.07	5.04	5.19	4.58
7	2.66 m	2.83	2.86	2.70	2.68	2.20	2.76	2.82	1.67
8α	2.10 dddd	2.03 m	2.00 dddd	2.05 m	2.07 dddd	2.09	1.97	2.03	1.99 ddd
8β	1.74 dddd	1.76 m	1.80 dddd	1.72	1.71 dddd	1.63	1.76	1.74	1.42
9α	2.63 ddd	2.65 m	2.47 ddd	2.58 m	2.58 ddd	2.25	1.44	2.14	2.86 dddd
9β	2.44 m	2.21 ddd	2.24	2.25 m	2.31	2.52	2.38	2.61	2.33
13a	5.60 d	5.59	5.68	5.58	5.56	3.63	5.64	5.58	2.71 dd
13b	6.39 d	6.28	6.35	6.27	6.26	3.57	6.31	6.27	2.81 dd
14	4.09 d	3.80	4.62	4.59	4.10	4.05	3.90	3.81	9.43
14′	4.02 d	3.59	4.44	4.43	4.04	4.00	3.70	3.55	
15	3.99 s	4.03	3.78 d	3.96 s	3.96	3.98	4.07	4.00	1.86

4: CH_3–CO, δ 2.03, 3H, s; **6**: (CH_3)C–, δ 0.89, 9H, s; CH_3–, δ 0.05, 3H, s; CH_3–, δ 0.04, 3H, s; **8**: CH_3–CO, δ 2.09, 3H, s; **12**, **12b**: CH_3–CO, δ 2.03, 3H, s; **15**: 15′, δ 3.55, d, 1H; CH_3–CO, δ 2.03, 3H, s; **16–19**: (CH_3)C–, δ 0.90, 9H, s; CH_3–, δ 0.06, 3H, s; CH_3–, δ 0.05, 3H, s; **16**: CH_3–CO, δ 2.07, 3H, s; **17b**: OCH_3–, δ 3–34, 3H; (CH_3)C–, δ 0.89, 9H, s; CH_3–, δ 0.06, 3H, s; CH_3–, δ 0.05, 3H, s; **20**: *C*H_2–CH$_2$–N, δ 1.74, 4H, brs; H-11, δ 2.31, 1H, m; CH$_2$–*C*H_2–N, δ 2.61, 4H, brs.

[a] Multiplicities are not repeated if identical with the preceding column.
[b] Signals might be interchanged within the same column.
[c] Signals might be interchanged within the same column.
[d] Signals might be interchanged within the same column.

parameters analyzed were germination and root and shoot elongation.

Melampolides show, in general, low levels of phytotoxicity: only the root length of the dicots lettuce and cress was inhibited (Table 5). However, all values are below 20% inhibition. On the other hand, the aldehydes **3** and **9**, and the chlorinated compound **5** show good levels of stimulation of wheat germination. No significant activities where detected in onion.

Regarding *cis*,*cis*-germacranolides (Table 5), only the isomeric epoxides **18** and **19** present significant phytotoxic activity on lettuce germination. Other groups or substituents do not seem to increase these effects. The aldehyde **11** and the chlorinated derivative **13** present good levels of stimulation of wheat germination. Finally, the epoxide **18** and the acetoxy derivative **12** are the only *cis*,*cis*-germacranolides showing a weak inhibition on root lettuce, similar to those of melampolides.

According to these results and those previously published for similar sesquiterpene lactones with *trans*,*trans*-germacranolide structure,[15a] some conclusions can be established:

2.2.1. Influence of the conformation. Cluster analysis (or complete linkage analysis) is a powerful statistic tool used to compare and group data in categories attending to their similar bioactivity.[23] As a result, a hierarchy tree is obtained with those elements with lower differences of bioactivity among them falling closer or into the same subgroup. In our particular case, we combined data obtained for germination and growth of all three skeletal types (e.g. *trans*,*trans*-germacranolides, melampolides, and *cis*,*cis*-germacranolides) assayed with the four target species (lettuce, cress, onion, and wheat). The resulting grouping tree (Fig. 7) clearly separates previously tested *trans*,*trans*-germacranolides[15a] (compounds **1**, **22–24**, **26**, Fig. 7, group F_1) from melampolides and *cis*,*cis*-germacranolides (group F_2). The profiles of activity of the elements included in F_1 category are similar among them, and quite different from F_2. a general decreasing order of activity for germacranolides can be set to as *trans*,*trans*-germacranolides > melampolide > *cis*,*cis*-germacranolides.[24]

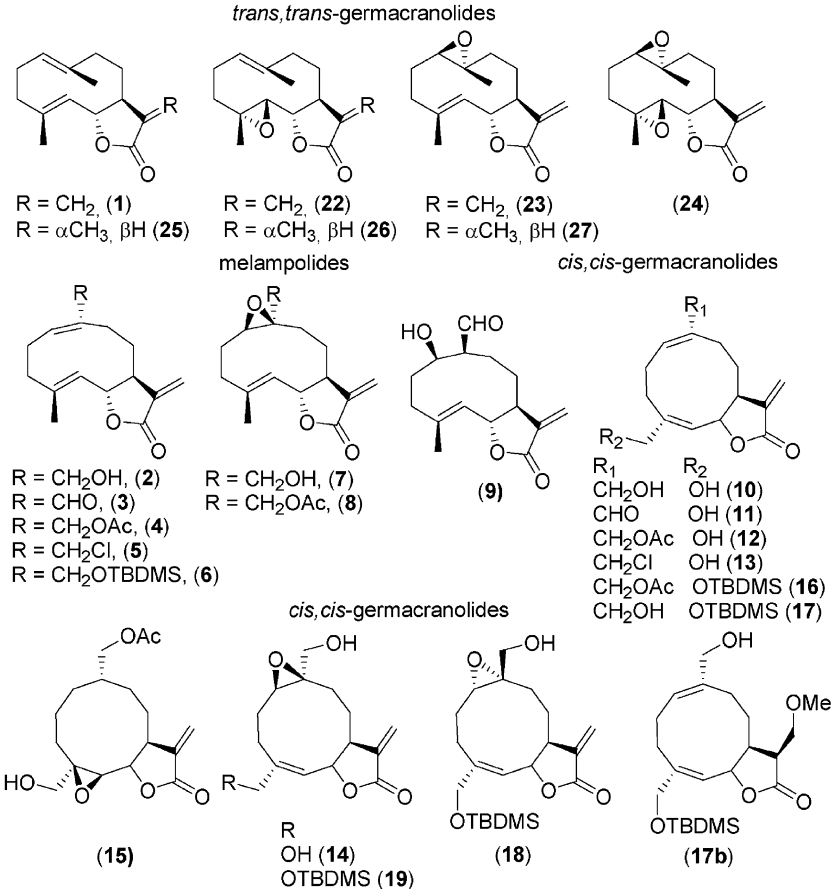


Figure 6. Germacranolides tested in the bioassays.

There is a clear correlation between the lost of activity and the change in the spatial disposition of the carbon framework—caused by the different stereochemistry in the two double bonds of the macrocycle. While *trans*,*trans*-germacranolides present a 'double-crown' like conformation (typical theoretical molecular volume $V=313$ Å^3), melampolides adopt a 'twisted' disposition (V(**2**)$=318$ Å^3), and *cis*,*cis*-germacranolides has a 'boat-like' conformation (V(**10**)$=335$ Å^3) (Fig. 8). Since all compounds present similar volumes, total steric demands are mostly the same for all of them. Thus, if the

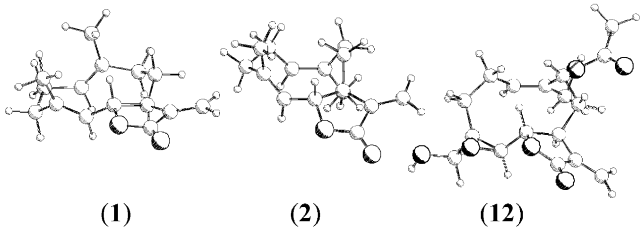


Figure 8. Minimum energy conformers as obtained by PM3 calculations.[21]

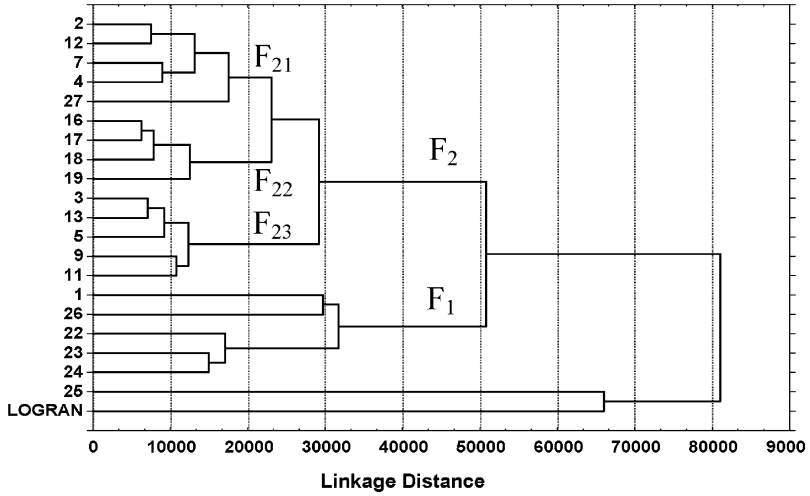


Figure 7. Complete linkage analysis (cluster) of the germacranolides tested.

Table 4. ^{13}C NMR data for listed compounds (100 MHz in $CDCl_3$, signal of the residual $CHCl_3$ centered at δ 77.0 ppm)[a]

C	3	4	5	6	8	9	10	11	12	12b
1	153.4 d	129.9	130.4	125.1[b]	62.6	71.4	125.5[b]	152.9	129.2	129.5
2	24.2[b] t	24.9	24.4	24.7	27.3	29.7[b]	24.3[c]	25.8[b]	24.6[b]	23.8[b]
3	37.1 t	38.1	38.1	38.5	34.3	35.5	26.5	25.6[b]	26.3	24.6
4	137.4 s	138.2	139.6 [b]	140.4	142.4	141.3	140.1[d]	138.4	140.1	141.0
5	126.1 d	125.3	125.3	125.0[b]	124.3	126.1	125.4[b]	126.3	126.5	150.0
6	80.7 d	80.6	80.3	80.8	79.3	79.6	78.1	77.9	77.9	77.3
7	45.5 d	45.3	45.4	45.5	46.0	44.1	43.8	43.6	43.8	42.9
8	26.1[b] t	25.1	25.1	25.2	24.1[b]	25.1	31.8	31.6	31.7	32.0
9	22.0 t	24.0	22.8	23.8	24.5[b]	17.4[b]	24.2[c]	22.1	24.4[b]	22.8[b]
10	144.7 s	135.8	137.6[b]	138.4	60.6	52.2 d	139.4[d] s	143.6	134.6	134.3
11	139.2 s	139.8	138.2	140.2	139.6	139.2	139.4[d]	139.4	139.5	138.1
12	170.2 s	170.7[b]	170.2	170.5	169.8[c]	171.1	170.1	170.1	170.1[c]	170.4[c]
13	119.3 t	118.8	119.2	118.6	119.2	119.3	122.6	123.3	122.7	123.9
14	195.6 d	67.5 t	48.9	66.7	67.1	203.8	66.2	195.3 d	67.3 t	67.2
15	16.9 q	17.1	17.2	17.2	17.4	16.9	66.2 t	66.3	66.3	194.1 d

C	13	14	15	16	17	17b	18	19	20
1	130.1 d	60.7	128.2	129.6	125.8	125.2	62.0	60.9	152.5
2	24.6[b] t	28.7	21.6	24.6	24.2[b]	24.4	22.4	30.3	25.4
3	26.2 t	23.6[b]	27.1	26.1	26.3	26.0	26.9[b]	23.5[b]	37.3
4	142.1 s			139.8	140.1	140.0	144.5	125.5	136.1
5	125.4 d	125.0	61.5	124.4	124.4	125.1	123.1	124.3	126.7
6	77.9 d	77.2	80.6	78.1	78.3	79.7	79.2	78.2	80.6
7	43.7 d	44.1	39.1	43.9	44.1	42.7	29.9	44.0	46.0
8	31.2 t	28.7	32.3	31.7	31.8	31.2	28.9	29.7	26.1
9	23.9[b] t	24.6[b]	25.1	24.6	24.4[b]	23.9	26.8[b]	24.7[b]	22.5
10	136.2 s	63.2	135.5	134.5	139.2[c]	139.6	63.9	63.2	145.4
11	139.5 s	139.2	138.3	139.6	139.8[c]	48.9 d	139.2 s	139.4	46.5 d
12	170.3 s	169.7	170.6[b]	170.8[b]	170.3	177.0	169.7	169.7	171.1
13	122.8 t	122.6	123.8	122.5	122.4	71.3	122.3	122.3	53.2
14	48.8 t	67.2	66.8	67.6	66.3	66.5	66.2	67.2	195.3 d
15	66.2 t	64.3	63.0	66.3	66.3	66.0	64.3	64.2	17.0 q

4: δ 20.9 q (CH_3–CO–); δ 170.3[a] s (CH_3–*C*O–); **6**: δ −5.2 q (CH_3–Si); δ −5.3 q (CH_3–Si); δ 25.9 q [$(CH_3)_3$C–Si)]; **8**: δ 20.7 q (CH_3–CO–); δ 170.5[b] s (CH_3–*C*O–); **12**: δ 20.9 q (CH_3–CO–); δ 171.1[b] s (CH_3–*C*O–); **12b**: δ 20.9 q (CH_3–CO–); δ 169.5[b] s (CH_3–*C*O–); **15**: δ 20.9 q (CH_3–CO–); δ 169.4[a] s (CH_3–*C*O–); **16**, **17**, **17b**, **18**, **19**: δ −5.3 q (CH_3–Si); δ −5.3 q (CH_3–Si); δ 18.4 s [$(CH_3)_3C$–Si)]; δ 25.9 q [$(CH_3)_3$C–Si)]; **16**: δ 20.9 q (CH_3–CO–); δ 170.0[a] s (CH_3–*C*O–); **17b**: δ 59.2 q (CH_3O–); **20**: δ 23.8 t [CH_2–CH_2–N]; δ 54.7 t [CH_2–CH_2–N].

[a] Multiplicities are not repeated if identical with the preceding column.
[b] Signals might be interchanged within the same column.
[c] Signals might be interchanged within the same column.
[d] Signals might be interchanged within the same column.

change in the shape of the backbone leads also to a change in the orientation of the reactive exocyclic double bond in the lactone ring (Fig. 8), this might be correlated with the decrease observed in the phytotoxicity.

2.2.2. Functional group influence. A second α,β-unsaturated carbonyl system at C-14 (compounds **3** and **11**) represents another possible reacting center for Michael nucleophilic additions. However, comparison of data from alcohols **2** and **10** and their corresponding aldehydes **3** and **11** show no increment in the phytotoxic activity. This is indicative that steric hindrance or stereoelectronic effects

Table 5. Bioactivity shown by melampolides and *cis*,*cis*-germacranolides in the Petri dish bioassay

	Lettuce			Cress			Wheat			Onion		
	G	R	S	G	R	S	G	R	S	G	R	S
Melampolides												
2		(−)	(−)	(−)	(−)	(+)	(−)	(+)	(+)		(−)(+)	(−)
3			(−)				+		(−)	(+)	(+)	
4		(−)	(+)	(−)	(−)	(+)					(−)(+)	
5							+		(−)	(+)	(+)	
7		(−)	(+)		(−)	+	(−)	+	(+)		(−)	
9			(−)				+			(+)	(+)	(+)
cis,*cis*-Germacranolides												
12		(−)	(+)	(−)	(−)	(+)		(+)	(+)			(+)
13		(−)	(+)				+		(−)	(+)		
15		(+)			+		(+)	−(+)	—	(+)		
16		(−)	(+)	(−)	(−)				(−)		(−)	
17			(+)	(−)	(−)	(−)	(−)		(−)	(−)	(−)	
18	—	—	(+)	(−)		(−)	(−)	(+)	—	(−)	(−)(+)	
19	—	(−)(+)	(+)	(−)		(−)		(+)	—		(+)	

G: Germination; R: Root Length; S: Shoot Length; blank: No active; (+), (−): stimulatory or inhibitory values below 20% at the maximum concentration tested (100 μM); +, −: stimulatory or inhibitory values between 20–40%.

dos not allow such a reaction. This hypothesis has been further tested trying to use pyrrolidine as molecular probe resembling amino groups of biomolecules. Reaction of pyrrolidine with compounds **3** and **11** led to the expected Michael adducts at the lactone ring (compounds **20** and **21**, respectively). However, no reaction products could be obtained in the unsaturated aldehyde system, thus supporting this idea. Note also that the non-conjugated aldehyde **9** remains inactive.

Halogen atoms are commonly present in pesticide formulation, where they have been usually introduced to increase the activity.[25] Otherwise, brominated and chlorinated derivatives are also frequently found in marine natural products showing high levels of biological activity.[26] However, the introduction of halogen atoms or even an epoxide ring do not add any increase of the phytotoxicity (compounds **5**, **7**, **8**, **14**, **15**, **18**, **19**), except for the isomeric epoxides *cis*,*cis*-germacranolide **18** and **19** which show an important increment of the phytotoxic activity, specially on lettuce germination. The different activity shown among epoxides at the C1–C10 in melampolides (**7**) and *cis*,*cis*-germacranolides (**18** and **19**) could relay on the different spatial disposition of the backbones, allowing an easier access in the last ones to the oxirane ring.

A deep look into the second group F_2, allows to differentiate three new subgroups: F_{22} includes only *cis*,*cis*-germacranolides with a TBDMS substituent at C-15, F_{23} groups both *cis*,*cis*-germacranolides and melampolides possessing a chlorine or carbaldehyde groups, and hydroxyl or acetoxyl containing compounds fall into the F_{21} category. Thus, the F_{22} subgroup resembles the sterically demanding TBDMS *cis*,*cis*-germacranolide derivatives.

3. Conclusions

We report an optimal relation SeO_2/TBHP to obtain *cis*,*cis*-germacranolides from melampolides as 1:4. Such a ratio is in good accordance with the proposed catalytic nature of SeO_2 in this reaction. Otherwise, to explain the change in the stereochemistry of the double bonds it is necessary to adopt a peroxometal intermediate reactive species in contrast to recently reported hypothesis,[2] at least for this system.

Also, a feasible relationship between the theoretical dipolar moment and the yield is observed, which mirrors the influence of the solvation energy in the first rate-determining ene reaction step. Compounds having low dipolar moments are better solvated in low polar solvents such as DCM or THF, thus favoring better yields.

Regarding the SAR study, it can be concluded as a general trend the decreasing order of activity *trans*,*trans*-germacranolides > melampolides > *cis*,*cis*-germacranolides. Such an order has been found to positively correlate with the progressive change in the carbon framework of the decalyne system and it is in good agreement with previous results.[15a] Cluster analysis has demonstrated to be a powerful tool to analyze profiles of activity and to group those compounds with similar bioactivity.

New nucleophilic reactive centers do not necessarily add additional biological inhibitory effects, unless they are quite accessible. In this sense, easily accessible oxirane rings seem to induce higher inhibiting activities (*cis*,*cis*-germacranolides **18** and **19**).

4. Experimental

4.1. General

All reagents and solvents were used as obtained from commercial suppliers, excepting compound **1**. Costunolide (**1**) was isolated by column chromatography from Costus Resin Oil (Pierre Chauvet, S. A.) and purified by recristallization from hexane/ethyl acetate mixtures. Solvents were distilled from glass prior to use. Column chromatography was performed on silica gel (35–75 mesh) and TLC analysis was carried out using aluminum-packed precoated silica gel plates. For HPLC, LiChrosorb silica 60 was used in the normal-phase mode with a differential refractometer (RI) in a Hitachi L-6020 HPLC instrument. ^1H and ^{13}C NMR spectra were recorded using a Varian UNITY-400 spectrometer at 400 MHz and 100 MHz, respectively, and using $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.25 ppm in the ^1H and δ_C 77.00 ppm for $CDCl_3$ in the ^{13}C spectra were used as internal references. Mass spectra were obtained by using a VG 1250 or a VG AUTOSPEC instruments at 70 eV. IR spectra were recorded on a Mattson 5020.

Soulangianolide A (**2**). 100 mg of costunolide (**1**) were dissolved in DCM (10 mL) and strongly stirred at room temperature with selenium dioxide (SeO_2, 24 mg, 0.5 equiv.). Then, *t*-butyl hydroperoxide (TBHP, 0.1 mL, 2 equiv.) was drop wise. After 1 h, filtering the reaction mixture through silica gel and the solvent evaporated under vacuum stopped the reaction. The crude of reaction was purified by CC using hexane/ethyl acetate mixtures as eluant, yielding **2** (62%) as a colorless oil and trace amounts of the 14-oxo-melampolide **3**. All spectral and physical data of 2 were in full agreement with those reported in the literature for 14-hydroxymelampolide.[5a]

4.1.1. 14-Oxomelampolide (3). ν_{max} (KBr) 1763 (carbonyl group), 1679 (aldehyde group), 1625 (double bond) cm^{-1}; HRMS: found $[M]^+$ 246.12549, $C_{15}H_{18}O_3$ requires 246.12560; FAB 246.137 $[M]^+$; EIMS, *m/z* (rel. int.): 231 $[M-CH_3]^+$ (1.1); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=8.4; 3α,3β=13.1; 3α,2β=11.7; 5,6=10.3; 6,7=9.6; 8α,8β=14.1; 8α,9β=12.8; 8α,9α=6.7; 8α,7=3.5; 8β,7=11.5; 8β,9β=5.9; 8β,9α=1.9; 13a,7=3.1; 13b,7=3.5; 14,9=1.7; 15,5=1.4; ^{13}C NMR data, see Table 4.

4.2. Acetylation

All acetylations were carried out by dissolving the compound in dry pyridine and adding an excess of acetic anhydride. After 24 h stirring, the reaction mixture was washed with a saturated aqueous solution of $CuSO_4$ to remove the excess of pyridine, yielding the corresponding acetyl derivatives quantitatively.

4.2.1. 14-Acetoxymelampolide (4). ν_{max} (KBr) 1768 (carbonyl group), 1730 (acetoxy carbonyl group), 1667 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 291.15991, $C_{17}H_{22}O_4$ requires 291.15963; EIMS, *m/z* (rel. int.): 291 $[M+1]^+$ (2.9), 231 $[M-AcOH]^+$ (100); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.6; 5,6=10.2; 6,7=9.4; 8α,8β=14.3; 8α,9β=13.1; 8α,9α=6.1; 8α,7=3.8; 8β,7=12.0; 8β,9β=4.5; 8β,9α=3.0; 13a,7=3.4; 13b,7=3.2; 14,14′=12.4; 15,5=1.3; ^{13}C NMR data, see Table 4.

4.2.2. (1*R*,10*S*)-14-Acetoxy-1,10-epoxymelampolide (8). ν_{max} (KBr) 1765 (carbonyl group), 1744 (acetoxy carbonyl group), 1678 (double bond) cm^{-1}; HRMS: found $[M]^+$ 306.14681, $C_{17}H_{22}O_5$ requires 306.14673; FAB 307.147 $[M+1]^+$; EIMS, *m/z* (rel. int.): 247 $[M-OAc]^+$ (36.0); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=4.3; 1,2β=10.1; 2α,2β=14.5; 2α,3α=1.6; 2α,3β=6.1; 3α,3β=12.7; 3α,2β=12.7; 5,6=10.5; 6,7=9.8; 8α,8β=17; 8β,7=12.4; 8β,9β=4.7; 8β,9α=3.1; 9α,9β=15.1; 9β,8α=13.8; 9β,8β=4.7; 13a,7=3.1; 13b,7=3.5; 14,14′=12.3; ^{13}C NMR data, see Table 4.

4.3. Chlorination of 14-hydroxymelampolide (2)

440 mg of compound **2** were dissolved in 4 ml of dried pyridine, followed by addition of tosyl chloride (TsCl, 414 mg, 1.2 equiv.) with stirring. After 22 h, the pyridine was removed under vacuum, and the crude was purified by CC, yielding 14-chloromelampolide **5** (80%).

4.3.1. 14-Chloromelampolide (5). ν_{max} (KBr) 1763 (carbonyl group), 1669 (double bond), 666 (C–Cl) cm^{-1}; HRMS: found $[M]^+$ 266.10748:266.10453, $C_{15}H_{19}ClO_2$ requires 266.10736:268.10441; FAB 266.097:268.564 $[M]^+$; EIMS, *m/z* (rel. int.): 251 $[M-CH_3]^+$ (3.0), 231 $[M-Cl]^+$ (4); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=9.3; 3α,3β=3α,2β=13.0; 3α,2α=1.9; 5,6=10.6; 6,7=9.4; 8α,8β=14.0; 8α,9β=13.8; 8α,9α=5.8; 8α,7=3.8; 8β,7=11.9; 8β,9β=4.7; 8β,9α=2.6; 13a,7=3.1; 13b,7=3.5; 14,14′=12.3; 15,5=1.0; ^{13}C NMR data, see Table 4.

4.4. Silylation of 14-hydroxymelampolide (2)

200 mg of **2** were dissolved in *N*,*N*-dimethylformamide (*N*,*N*-DMF, 4 mL), followed by addition of *t*-butyldimethylsilyl chloride (TBDMSCl, 273 mg, 2 equiv) and dry collidine (0.24 mL, 2 equiv.). After 24 h, the reaction was stopped by addition of water (4 mL) and the reaction mixture extracted with AcOEt (3×). The combined organic phases were dried with anhydrous sodium sulfate yielding 14-*t*-butyldimethylsilyloxy-melampolide **6** (99%).

4.4.1. 14-[*tert*-Butyl-dimethylsilyloxy]melampolide (6). ν_{max}/cm^{-1}: 1744 (carbonyl group), 1670 (double bond); HRMS: found $[M]^+$ 362.22759, $C_{21}H_{34}O_3Si$ requires 362.22772; FAB 361.232 $[M-1]^+$; EIMS, *m/z* (rel. int.): 305 $[M-C(CH_3)_3]^+$ (28.1), 277 $[M-C(CH_3)_3Si]^+$ (4.9); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=9.3; 5,6=10.6; 6,7=9.4; 8α,8β=14.0; 8α,9β=13.8; 8α,9α=5.8; 8α,7=3.8; 8β,7=11.9; 8β,9β=4.7; 8β,9α=2.6; 13a,7=3.1; 13b,7=3.5; 14,14′=12.3; 15,5=1.0; ^{13}C NMR data, see Table 4.

4.5. Epoxidations

All epoxidations were carried out as follows: to a solution of the starting compound (0.262 mmol) in sodium acetate buffered dried THF (8 mL) another solution of *m*-CPBA (0.290 mmol) in dried THF (4 ml) was drop wise while stirring. Reaction was monitored by TLC until no starting materials could be observed. Then, the reaction was stopped and the work-up was as follows: the reaction mixture was extracted with NaOH (aq) 5% (2×) and the organic phase washed with distilled water (2×). All the aqueous phases were re-extracted with ethyl acetate, and the combined organic phases were dried on anhydrous sodium sulfate. After separation by CC, (1*R*,10*R*)-1β,10β-epoxy-14-hydroxy-melampolide **7** was obtained from **2** in a crystalline form (62%). Spectral and physical data were in full agreement with those reported in the literature.[15a] Compound **15** was obtained from **12** with a 47% yield, and compounds **18** (42%) and **19** (56%) were obtained from **17**.

4.5.1. (4*R*,5*S*)-14-Acetoxy-4,5-epoxy-15-hydroxy-*cis*,*cis*-germacranolide (15). ν_{max} (KBr) 3483 (OH), 1762 (carbonyl group), 1738 (acetoxy carbonyl group), 1685 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 323.15048, $C_{17}H_{20}O_6$ requires 323.14946; EIMS, *m/z* (rel. int.): 323 $[M+1]^+$ (3.8), 263 $[M-AcO]^+$ (46.7), 245 $[M-AcO-H2O]^+$ (68.3); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=8.1; 3α,3β=14.1; 3α,2β=11.3; 3α,2α=7.4; 5,6=9.5; 6,7=3.5; 8α,8β=14.6; 8α,9β=12.9; 8α,7=5.0; 8α,9α=3.8; 8β,7=12.9; 8β,9β=4.9; 8β,9α=3.5; 9α,9β=14.8; 9β,8α=12.8; 13a,7=2.3; 13b,7=2.7; 14,14′=12.7; 15,15′=12.7; ^{13}C NMR data, see Table 4.

4.5.2. (1*S*,10*S*)-15-*t*-Butyldimethylsilyloxy-1,10-epoxy-14-hydroxy-*cis*,*cis*-germacranolide (18). ν_{max} (KBr) 3455 (OH), 1763 (carbonyl group), 1658 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 395.22762, $C_{21}H_{34}O_5Si$ requires 395.22538; EIMS *m/z* (rel. int.): 395 $[M+1]^+$ (8.8), 337 $[M-HC(CH_3)_3]^+$ (3.6); ^1H NMR data, see Table 3, *J* (Hz): 1,2α=11.0; 1,2β=3.2; 2α,2β=14.9; 2α,3α=2α,3β=4.3; 5,6=10.1; 6,7=3.6; 8α,8β=15.4; 8α,9β=9.6; 8α,7=3.7; 8α,9α=2.1; 9α,9β=15.1; 9α,8β=9.2; 13a,7=2.3; 13b,7=2.6; 14,14′=12.4; 14,8=5.1; 15,5=1.6; 15',5=1.9; ^{13}C NMR data, see Table 4.

4.5.3. (1*R*,10*R*)-15-*t*-Butyldimethylsilyloxy-1,10-epoxy-14-hydroxy-15-*cis*,*cis*-germacranolide (19). ν_{max} (KBr) 3477 (OH), 1764 (carbonyl group), 1660 (double bound) cm^{-1}; HRMS: found $[M+1]^+$ 395.22516, $C_{21}H_{34}O_5Si$ requires 395.22538; EIMS *m/z* (rel. int.): 395 $[M+1]^+$ (8.8), 337 $[M-HC(CH_3)_3]^+$ (3.6); ^1H NMR data, see Table 3, *J* (Hz): 2α,3α=2α,3β=4.4; 2α,2β=15.9; 5,6=11.3; 6,7=4.3; 13a,7=2.3; 13b,7=2.5; 14,14′=12.4; ^{13}C NMR data, see Table 4.

4.5.4. (1*R*,10*R*)-1,10-Epoxy-14-hydroxymelampolide (7) ring opening. 184 mg of compound **7** were dissolved in 10 ml of THF, followed by addition of boron trifluoride dihydrate ($BF_3 \cdot 2H_2O$, 0.15 mL, 2 equiv.). After 4 h, the reaction was stopped by adding water, the reaction mixture extracted with ethyl acetate (3×), and the combined organic phases were dried on anhydrous sodium sulfate. The crude

of reaction was purified by CC to afford (1*R*,10*S*)-1-hydroxy-14-oxomelampolide **9** (53%). The configuration at carbons C1 and C10 was assigned based on positive correlations found in the bidimensional NOESY experiment (H-5, H-7, H-10, H-1) that were in full agreement with the minimum energy conformer obtained by PM3 calculations.

4.5.5. (1*R*,10*S*)-1-Hydroxy-14-oxomelampolide (9). ν_{max} (KBr) 3463 (OH), 1759 (carbonyl group), 1722 (aldehyde group), 1665 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 265.14103, $C_{15}H_{20}O_4$ requires 265.14398; EIMS *m/z* (rel. int.): 265 $[M+1]^+$ (15.0), 247 $[M-H_2O]^+$ (46.9), 229 $[M-H_2O-CO]^+$ (89.8); ^{1}H NMR data, see Table 3, *J* (Hz): 1,10=11.8; 3α,3β=3β,2α=12.7; 5,6=6,7=10.1; 9α,9β=13.6; 9β,8α=13.6; 9β,8β=4.9; 13a,7=3.2; 13b,7=3.4; ^{13}C NMR data, see Table 4.

4.6. Oxidation of melampolides to *cis*,*cis*-germacranolides

Selenium dioxide (SeO_2, 0.5 equiv.) and TBHP (2 equiv.) were added to a DCM solution (7 mL) of compounds **2**–**8** (80 mg each). The reaction mixture was heated until reflux and strongly stirred over 8 h. Then, the reaction was stopped as it was for compound **2**, yielding **10** (10%), **11** (24%), **12** (57%), **13** (43%), and **14** (3%), respectively. Compound **8** did not react and compound **6** run deprotection of the silyl ether group and subsequent oxidation of the free hydroxyl group to yield **3** (53%). It was also possible to detect trace amounts of the 15-oxo-derivative **12b** using **4** as starting material. The low yields obtained for the rest of starting materials might be the cause for not detecting analogue compounds in the other systems assayed.

4.6.1. 14,15-Dihydroxy-*cis*,*cis*-germacranolide (10). ν_{max} (KBr) 3406 (OH), 1755 (carbonyl group), 1658 (double bond) cm^{-1}; HRMS: found $[M-H_2O]^+$ 246.12497, $C_{15}H_{18}O_3^+$ requires 246.12504; FAB 264.352 $[M]^+$; EIMS, *m/z* (rel. int.): 246 $[M-H_2O]^+$ (5.0), 228 $[M-2H_2O]^+$ (6.0); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.1; 5,6=9.5; 6,7=3.7; 8α,8β=14.3; 8α,9β=12.4; 8α,7=8α,9α=4.4; 8β,7=12.5; 8β,9β=4.7; 8β,9α=3.6; 9α,9β=14.4; 13a,7=2.4; 13b,7=2.7; 14,14′=12.6; ^{13}C NMR data, see Table 4.

4.6.2. 15-Hydroxy-14-oxo-*cis*,*cis*-germacranolide (11). ν_{max} (KBr) 3444 (OH), 1757 (carbonyl group), 1749 (aldehyde group), 1681 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 263.13237, $C_{15}H_{18}O_4$ requires 263.12833; EIMS, *m/z* (rel. int.): 263 $[M+1]^+$ (18.8), 245 $[M-H_2O]^+$ (100); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.1; 5,6=9.5; 6,7=3.8; 8α,8β=14.9; 8β,9β=4.8; 8β,9α=2.5; 13a,7=2.4; 13b,7=2.7; 14,1=1.3; ^{13}C NMR data, see Table 4.

4.6.3. 14-Acetoxy-15-hydroxy-*cis*,*cis*-germacranolide (12). ν_{max} (KBr) 3426 (OH), 1747 (carbonyl group), 1739 (acetoxy carbonyl group), 1636 (double bond) cm^{-1}; HRMS: found $[M]^+$ 306.14678, $C_{17}H_{22}O_5$ requires 306.14673; FAB 307.153 $[M+1]^+$; EIMS, *m/z* (rel. int.): 275 $[M-CH_2OH]^+$ (1.0), 246 $[M-CH_2OH-AcOH]^+$ (27.0); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.5; 5,6=9.6; 6,7=3.8; 8α,8β=14.5; 8α,9β=12.5; 8α,9α=8α,7=4.9; 8β,7=12.9; 8β,9β=4.9; 8β,9α=3.7; 13a,7=2.3; 13b,7=2.7; 14,14′=12.5; ^{13}C NMR data, see Table 4.

4.6.4. 14-Acetoxy-15-oxo-*cis*,*cis*-germacranolide (12b). ν_{max} (KBr) 1768 (aldehyde group), 1747 (carbonyl group), 1731 (acetoxy carbonyl group), 1689 (double bond) cm^{-1}; HRMS: found $[M]^+$ 304.13065, $C_{15}H_{20}O_5$ requires 304.13107; EIMS, *m/z* (rel. int.): 304 $[M]^+$ (1.4), 261 $[M-AcO]^+$ (1.7); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.8; 2α,2β=2α,3β=14.7; 5,6=9.2; 6,7=3.7; 8α,8β=14.5; 8α,9β=12.2; 8α,9α=5.5; 8β,7=12.6; 8β,9β=4.9; 8β,9α=5.4; 9α,9β=14.9; 13a,7=3.1; 13b,7=3.4; 14,14′=12.9; 15,5=1.3; ^{13}C NMR data, see Table 4.

4.6.5. 14-Chloro-15-hydroxy-*cis*,*cis*-germacranolide (13). ν_{max} (KBr) 3461 (OH), 1761 (carbonyl group), 1687 (double bond) cm^{-1}; HRMS: found $[M-Cl]^+$ 247.13336, $C_{15}H_{19}O_3Cl$ requires 247.13342; EIMS, *m/z* (rel. int.): 282 $[M]^+$ (1.0), 247 $[M-Cl]^+$ (46.7), 251 $[M-CH_2OH]^+$ (34.2); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=9.7; 5,6=9.6; 6,7=3.9; 8α,8β=14.5; 8α,9β=12.4; 8α,7=4.9; 8α,9α=4.9; 8β,7=12.7; 8β,9β=4.8; 8β,9α=3.7; 9α,9β=14.7; 13a,7=2.5; 13b,7=2.7; 14,14′=11.4; ^{13}C NMR data, see Table 4.

4.6.6. 14,15-Dihydroxy-1β,10β-epoxide-*cis*,*cis*-germacranolide (14). ν_{max} (KBr) 3504 (OH), 1763 (carbonyl group), 1660 (double bond) cm^{-1}; HRMS: found $[M]^+$ 280.13112, $C_{15}H_{20}O_5$ requires 280.13108; FAB 280.659 $[M]^+$; EIMS, *m/z* (rel. int.): 252 $[M-H_2O]^+$ (4.0), 244 $[M-2H_2O]^+$ (6.0); ^{1}H NMR data, see Table 3, *J* (Hz): 5,6=9.3; 6,7=4.4; 9α,9β=14.5; 9α,8β=9α,8α=9β,8β=4.3; 13a,7=2.3; 13b,7=2.6; 14,14′=12.4; ^{13}C NMR data, see Table 4.

4.7. Silylation of 12

30 mg of **12** were dissolved in N,N-dimethylformamide (3 mL), followed by addition while stirring of TBDMSCl (2 equiv., 25 mg) and collidine (2 equiv., 0.04 mL). After 24 h, the reaction was stopped as for compound **2** to afford the corresponding 14-acetoxy-15-*t*-butyldimethylsilyloxy-*cis*,*cis*-germacranolide **16** (99%).

4.7.1. 14-Acetoxy-15-*t*-butyldimethylsilyloxy-*cis*,*cis*-germacranolide (16). ν_{max} (KBr) 1767 (carbonyl group), 1730 (acetoxy carbonyl group), 1682 (double bond) cm^{-1}; HRMS: found $[M+1]^+$ 420.23107, $C_{21}H_{30}O_5Si$ requires 420.23320; EIMS, *m/z* (rel. int.): 420 $[M]^+$ (3.8), 361 $[M-AcO]^+$ (27.9), 303 $[M-AcO-HC(CH_3)_3]^+$ (12.6); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.8; 5,6=9.5; 6,7=3.7; 8α,8β=14.2; 8β,7=12.6; 8β,9β=4.7; 8β,9α=4.0; 13a,7=2.3; 13b,7=2.7; 14,14′=12.4; ^{13}C NMR data, see Table 4.

4.8. Deacetylation of 16

30 mg of **16** were dissolved in dried methanol (1 mL) under nitrogen atmosphere. Afterwards, magnesium methoxide (0.08 mL, dilution 7.4% wt. in MeOH) was added. After 24 h, the reaction was stopped by adding water, the reaction

mixture extracted with AcOEt (4×), and the combined organic phases washed with brine (4×) and dried with anhydrous sodium sulphate. The solvent was removed in vacuum. 15-*t*-Butyldimethylsilyloxy-14-hydroxy-*cis*,*cis*-germacranolide **17** was obtained with a 74% yield, along with traces of the corresponding Michael adduct **17b**.

4.8.1. 15-*t*-Butyldimethylsilyloxy-14-hydroxy-*cis*,*cis*-germacranolide (17). ν_{max} (KBr) 3442 (OH), 1755 (carbonyl group) cm^{-1}; HRMS: found $[M]^+$ 378.22259, $C_{21}H_{34}O_4Si$ requires 378.22264; FAB 379.124 $[M+1]^+$; EIMS, *m/z* (rel. int.): 361 $[M-H_2O]^+$ (3.8), 323 $[M-HC(CH_3)_3]^+$ (5.1); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=7.2; 5,6=9.6; 6,7=3.7; 8α,8β=14.3; 8β,7=12.7; 8β,9α=4.2; 13a,7=2.4; 13b,7=2.7; 14,14′=12.6; ^{13}C NMR data, see Table 4.

4.8.2. (11*S*)-15-*t*-Butyldimethylsilyloxy-11,13-dihydro-14-hydroxy-13-methoxy-*cis*,*cis*-germacranolide (17b). ν_{max} (KBr) 3446 (OH), 1768 (carbonyl group), 1658 (double bond) cm^{-1}; FAB 409.226 $[M-1]^+$; EIMS, *m/z* (rel. int.): 393 $[M-OH]^+$ (4.7), 353 $[M-C(CH_3)_3]^+$ (3.0); ^{1}H NMR data, see Table 3, *J* (Hz): 1,2α=1,2β=6.6; 5,6=9.2; 6,7=5.2; 8α,8β=14.1; 8β,7=12.9; 8β,9α=4.0; 8β,9β=4.6; 9α,9β=14.9; 9β,8α=4.0; 13,13′=9.3; 13,11=5.5; 13′,11=3–7; ^{13}C NMR data, see Table 4. The stereochemistry at C-11 was assigned based on positive nOe correlations found in the bidimensional NOESY experiment between H-6 and H-13.

4.9. Michael additions of pyrrolidine to compounds 3 and 11

0.15 mmol each of compounds **3** and **11** were dissolved in dry THF (0.5 mL) and the solution cooled in the freezer for 15 min. Pyrrolidine solution (21 mg, 0.3 mmol) in dry THF (0.5 mL) was added to the cooled solution and the mixture kept at room temperature during 20 h. The solvent was evaporated in vacuum and the crude of reaction purified on a Al_2O_3 column chromatography using DCM/MeOH (97:3) as eluant, to yield 75% of **20** and **21** in each case.[27] The stereochemistry of the adduct at C-11 was established as *R* for compound **20** since a positive nOe effect was observed for H-11 when the signal corresponding to H-6 was irradiated (% nOe: 2.8% for H-11, 2.8% for H-9, 1.2% for H-15, and 3.1 for H-8β). No positive nOe effects could be observed on the side chain signals when H-5 was irradiated (% nOe: 1.2% for H-1, 6.3% for H-2β, and 8.4% for H-7).

4.9.1. (11*R*)-14-Oxomelampolide pyrrolidine mono adduct (20). ν_{max} (KBr) 1768 (carbonyl group), 1658 (double bond) cm^{-1}; HRMS: found $[M]^+$ 317.196936, $C_{19}H_{26}NO_3$ requires 317.199094; EIMS, *m/z* (rel. int.): 317 $[M]^+$ (100.0), 288 $[M-CHO]^+$ (9.1), 246 $[M-C_4H_8N]^+$ (4.6); data, see Table 3, *J* (Hz): 1,2α=9.1; 1,2β=7.1; 2α,2β=12.4; 2α,3β=6.0; 2α,3α=3.1; 2β,3α=7.0; 2β,3β=2.1; 5,6=6,7=10.1; 8α,8β=14.2; 8α,9β=12.5; 8β,9β=1.9; 9α,9β=13.9; 13,13′=13.2; 13,11=4.0; 13′,11=5.2; ^{13}C NMR data, see Table 4.

4.9.2. 14-Oxo-15-hydroxy-*cis*,*cis*-germacranolide pyrrolidine mono adduct (21). ν_{max} (KBr) 3446 (OH), 1768 (carbonyl group), 1658 (double bond) cm^{-1}; HRMS: found $[M]^+$ 333.194748, $C_{19}H_{26}NO_4$ requires 333.194009; EIMS, *m/z* (rel. int.): 333 $[M]^+$ (5.0), 317 $[M-O]^+$ (100.0), 288 $[M-O-CHO]^+$ (10.7), 261 $[M-C_4H_9N]^+$ (6.9); ^{1}H NMR δ_H (400 MHz, CDCl3) 9.40 (d, 1H, *J*=1.4 Hz, H-14), 5.18 (brd, 1H, *J*=9.8 Hz, H-6), 5.65 (d, 1H, *J*=10.1 Hz, H-5), 6.54 (dd, 1H, *J*=8.3 Hz, H-1).

4.10. Germination and seedling growth bioassays[22]

Seeds of *Lactuca sativa* L. cv. Roman (lettuce), *Lepidium sativum* L. cv. Común (cress), *Allium cepa* L. cv. Valenciana (onion), and *Triticum aestivum* L. cv. Cortex (wheat) were obtained from FITÓ, S.L. (Barcelona, Spain). All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity. Bioasays were carried out in 9 cm Ø plastic Petri dishes, using Whatman #1 filter paper as support. The general procedure for seedling bioassay was as follows: 25 seeds of each species were placed per dish, excepting *Triticum aestivum* (10 seeds per dish), with 5 mL of test soln, and incubated in the dark at 25 °C. Four replicates for each concentration were set up. Germination and growth time varied for each plant species: *L. sativum*, 3 days; *L. sativa* and *T. aestivum*, 5 days; and *Allium cepa*, 7 days. Test mother solutions (10^{-2} M) were prepared using dimethyl sulfoxide (DMSO) and then diluted to 10^{-4} M using 10 mM MES (2-[*N*-morpholino]ethanesulphonic acid). Following solutions were obtained by dilution maintaining the 0.5% DMSO percentage. Parallel controls were performed. All pH values were adjusted to 6.0 before bioassay. 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.

4.11. Statistical treatment

Germination and root and shoot length were tested by Welch's test,[28] the differences between test solutions and controls being significant with $P<0.01$. Cluster analysis was performed using the Statistica package.[29] The analyses were recorded to all compounds tested using as variables germination index, and root and shoot growth.

4.12. Molecular modeling

Minimum energy conformations and molecular properties were obtained using MMX and PM3 calculations (PCMODEL ver 6.0, Serena Software, Bloomington, IN; MOPAC, ver. 6.00), respectively. Conformers were obtained using the randomize command in PCMODEL and the local minimum energy structures obtained were used for further semiempiric minimization with MOPAC using PM3 method. For semiempiric calculations the parameters PRECISE, GEOOK, and *T*=86400 were used. Theoretical ΔH_f° values produced by MOPAC allowed to discriminate among conformers and to obtain the minimum energy conformer in each case.

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