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Facile synthesis of anhydrojudaicin and 11,13-dehydroanhydrojudaicin, two eudesmanolide-skeleton lactones with potential allelopathic activity

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ABSTRACT

Natural product anhydrojudaicin (**7**) is a eudesmanolide that has been synthesized from costunolide (**1**) in three steps with good yield, simplifying procedures available in the literature. The key step was the efficient oxidation and rearrangement using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). Following the same methodology, 11,13-dehydroanhydrojudaicin (**8**) was prepared for the first time. All compounds were characterized and tested in three specific bioassays: phytotoxicity bioassays on wheat coleoptile and on weed seeds (*Amaranthus viridis*, *Echinochloa crus-galli* and *Panicum maximum*); as well as bioassay of germination of parasitic weed seeds of broomrape (*Orobanchaceae*). The final products showed better activity profiles than the starting eudesmanolide in all bioassays. The data allow us to propose compounds **7** and **8** as leads for new natural product-based agrochemicals.

1. Introduction

Eudesmanolides constitute a large family of sesquiterpene lactones that includes compounds with different types of bioactivities, comprising antibacterial (Talbi et al., 2015), phytotoxic (Da Silva et al., 2017), cytotoxic and antiviral (Hui et al., 2018). This fact explains the biological activities of numerous plants that produce eudesmanolides as allelochemicals. The presence of the α-methylene-γ-lactone group in their structure is usually correlated with some of the activities shown by sesquiterpene lactones (Padilla-Gonzalez et al., 2016).

Many eudesmanolides are produced as secondary metabolites by plants, and consequently their isolation usually does not provide enough yields to study properly their possibilities as bioactive compounds. Organic synthesis gives a solution to this limitation, when efficient synthetic routes let obtain those minor compounds isolated from natural sources.

Anhydrojudaicin (**7**) is a eudesmanolide isolated from the aerial parts of *Artemisia canariensis*, and it is possible to obtain only 28 mg from 10 kg of plant for its isolation (Mansilla and Palenzuela, 1999). The synthesis of **7** in nine steps from α-santonin has been published. Compound **7** is an intermediate in the synthesis of several bioactive guaianolides (Yuuya et al., 1999) but activity has not been reported for compound **7** itself.

For these reasons, the first aim of the study presented here is the development of an efficient method to obtain **7** by organic synthesis. This procedure will also allow to prepare for the first time 11,13-dehydroanhydrojudaicin (**8**), using costunolide (**1**) as starting material (Fig. 1).

Considering good activities for eudesmanolides in the literature, the second aim of the study is the evaluation of bioactivity of compounds prepared. Three specific bioassays are selected, focusing on the search of new lead compounds for agrochemicals based on natural products.

Firstly, the etiolated wheat coleoptile bioassays was selected as a quick and easy general phytotoxicity bioassay. Second bioassay will study the phytotoxicity of synthetized compounds on three problematic weed species: *Amaranthus viridis* L. (green amaranth), which can affect crops like soybean (Kaspary et al., 2017), *Echinochloa crus-galli* L. (barnyard grass), which harms rice crops (Talbert and Burgos, 2007), and *Panicum maximum* Jacq. (guinea grass), which is harmful on coffee plantations (Da Silva et al., 2017).

Bioactivities of all the compounds will be also evaluated as suitable allelochemicals to control parasitic weed infestations. Parasitic plants differ from common plants in relevant biological aspects. A main particularity are their seeds, which only start their germination mechanism when they detect a specific chemical signal generated by a nearby possible host plant. This distinctive feature leads the search of compounds that stimulate the parasitic seeds germination, in order to apply the on growing suicidal germination strategy, which is also known as

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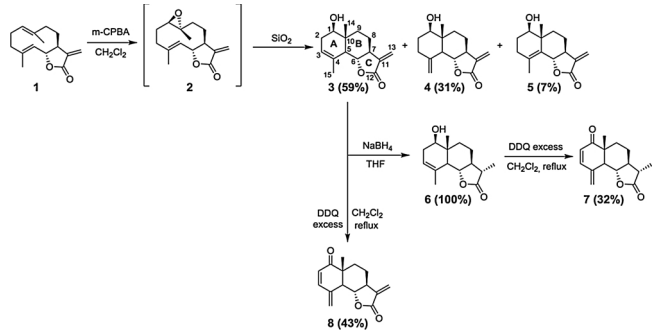

Fig. 1. Synthetic route to obtain anhydrojudaicin (**7**) and 11,13-dehydroanhydrojudaicin (**8**) from costunolide (**1**).

the honeypot strategy, to prevent the infestations of parasitic weeds (Mejías et al., 2018). Three of the most harmful parasitic weeds species of broomrape were selected for the study, as they damage different relevant crops: *Phelipanche ramosa* (Le Corre et al., 2014), *Orobanche cumana* (Yang et al., 2017) and *Orobanche crenata* (Rubiales and Fernández Aparicio, 2012).

As all compounds synthetized are structurally related, the structure-activity relation (SAR) study will be the third objective of the research reported herein. Bioassays carried out will be useful to identify the features that influence the activity of the molecules for each application.

2. Results and discussion

2.1. Synthesis of anhydrojudaicin (7) and 11,13-dehydroanhydrojudaicin (8)

The synthetic route for compounds **7** and **8** is shown in Fig. 1. The starting material costunolide (**1**) was isolated from *Saussurea lappa* and the structure was confirmed by the ^{1}H NMR and ^{13}C NMR spectra, which corresponded with the published data (Park et al., 2010).

The first step involved the intramolecular oxidative cyclization of the germacranolide ring of **1** by treatment with 3-chloroperbenzoic acid (*m*-CPBA) in dichloromethane as solvent at room temperature. The reaction was complete in two hours to generate epoxide **2** (Lu and Fischer, 1996), which was found to be unstable. The opening of the epoxide occurred on silica gel during the chromatographic separation to provide three eudesmanolides in different yields: santamarine (**3**, 59%), reynosin (**4**, 31%) and magnolialide (**5**, 7%). The spectroscopic data for compounds **3**–**5** were identical to those published for santamarine and reynosin (Ogura et al., 1978) and for magnolialide (El-Feraly et al., 1979), respectively.

Compound **3** was used to prepare the target products. The preparation of **7** required the reduction of the methylene of the lactonic ring and this step was carried out with $NaBH_4$ in THF at room temperature to give **6** in quantitative yield in one hour. In the ^{1}H NMR spectrum of **6** the signal for H-13 appears as a doublet at δ 1.22, rather than two deshielded doublets, and a new multiplet signal for H-11 was observed at δ 2.28. The spectroscopic data correspond with those published in the literature for 11,13-dihydrosantamarine (Clark and Hufford, 1979).

The final step was the isomerization and oxidation of **6** by treatment with excess DDQ in refluxing CH_2Cl_2 for 24 h. This reaction gave **7** in 32% yield after chromatographic separation. The IR spectrum showed the absence of the peak assigned to the hydroxy group and the presence of a new peak at 1672 cm^{-1}, which corresponds to a new carbonyl group. The ^{1}H NMR spectrum showed modified couplings and shifts for H-2 (from δ 2.38 and 1.94 to δ 5.86, *d*), H-3 (from δ 5.33 to δ 7.04, *dd*) and H-15 (from δ 1.81, *s*, to δ 5.82 and 5.54, both *dd*), and the lack of the signal for H-1 at δ 3.64. The NMR data obtained for **7** (Table 1) correspond with those available for 1-oxo-5α*H*,6,11β-eudesm-2,4(14)-dien-6,13-olide (Ando and Takase, 1977), which is also known as anhydrojudaicin (Saber et al., 1964). The *S* configuration at C-11 was determined by NOE 1D NMR spectroscopy, with H-6 showing a positive NOE effect with H-11, which implies a β-orientation of H-11 (Fig. 2).

Table 1
NMR spectroscopic data [^{1}H (500 MHz) and ^{13}C (125 MHz)] for products **7** and **8** in $CDCl_3$ (δ in ppm, *J* in Hz).

Product	7		8	
Position	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C
1	–	202.4	–	202.4
2	5.86 (1H, d, J = 9.9)	125.2	5.88 (1H, d, J = 10.0)	125.2
3	7.04 (1H, dd, J = 9.9, 0.6)	146.6	7.06 (1H, dd, J = 10.0, 0.7)	146.7
4	–	139.7	–	139.5
5	2.82 (1H, d, J = 10.7)	49.5	2.96 (1H, d, J = 10.8)	49.9
6	4.16 (1H, dd, J = 10.7, 10.7)	79.4	4.15 (1H, dd, J = 10.8, 10.8)	79.7
7	1.65 (1H, m)	52.0	2.57 (1H, ddddd, J = 11.2, 11.2, 3.2, 3.2, 3.2)	49.2
8a	2.06 (1H, m)	31.5	2.09 (1H, m)	31.2
8b	1.55 (1H, m)		1.66 (1H, br ddd, J = 13.8, 13.0, 3.8)	
9a	1.97 (1H, dd, J = 12.8, 3.3)	22.4	2.18 (1H, ddd, J = 13.1, 2.8, 2.8)	20.8
9b	1.50 (1H, dd, J = 12.8, 3.3)		1.60 (1H, m)	
10	–	47.2	–	47.3
11	2.35 (1H, dq, J = 10.0, 6.9)	40.6	–	138.1
12	–	178.9	–	170.3
13a	1.25 (3H, d, J = 6.9)	12.4	6.15 (1H, d, J = 3.2)	118.0
13b			5.48 (1H, d, J = 3.2)	
14	1.07 (3H, s)	17.8	1.06 (3H, s)	17.8
15a	5.82 (1H, dd, J = 1.4, 0.6)	122.1	5.88 (1H, d, J = 1.3)	122.4
15b	5.54 (1H, dd, J = 1.4, 0.6)		5.56 (1H, dd, J = 1.3, 0.7)	

The final step of the synthetic procedure to produce **8** involved treatment of **3** with excess DDQ to give **8** in 43% yield. A new band due to carbonyl group was observed at 1672 cm^{-1} in the IR spectrum. Comparison of the ^{1}H NMR spectrum with that of compound **7** showed that similar signals were displayed for the protons of the carbocyclic moiety. These spectra only differed in the presence of two signals at δ 6.14 and 5.48 for H-13 (instead of δ 1.25) and the absence of a signal for H-11, which indicates the presence of a methylene rather than a methyl group in the lactonic ring. The NMR data for **8** are listed in Table 1.

In summary, compounds **7** has been prepared in three easy steps with an overall yield of 19%, and this represents an improvement on the published synthesis of **7** (Yuuya et al., 1999), which requires nine steps from α-santonin. The synthesis of **8** is also provided by the use of

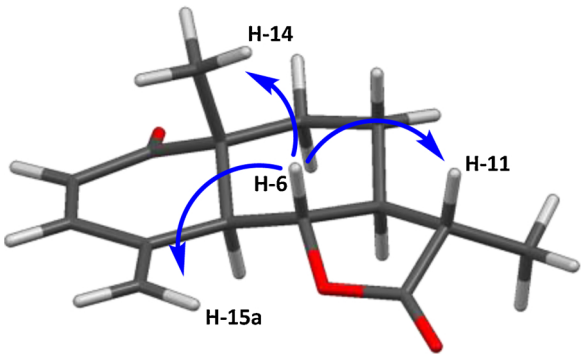

Fig. 2. 3D structure using PM6 calculation of **7** and NOE 1D effects of H-6 attended to confirm β-orientation of H-11.

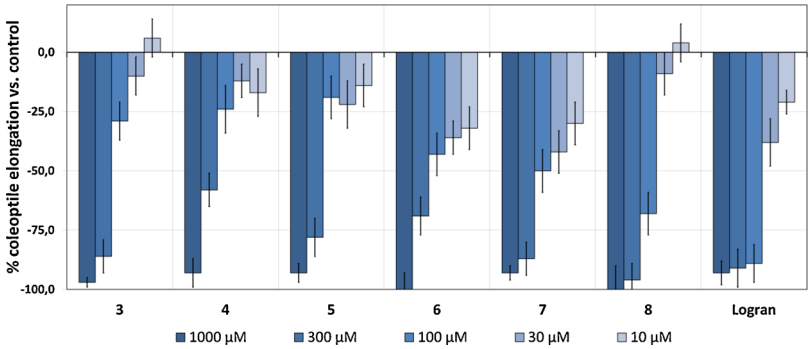

Fig. 3. Profiles obtained for products in the etiolated wheat coleoptile bioassay. Positive values indicate stimulation of growth vs. the control, and negative values indicate inhibition.

DDQ for the oxidation and isomerization to obtain an exodienone system. Compound **8** has been synthesized for the first time and this will facilitate the study of the structure-activity relationships and the influence on the bioactivity of the presence of a methyl or methylene group in the lactonic ring of the eudesmanolide, along with the presence of an exodienone system in the ring A.

The availability of the starting material and the synthetic yields obtained allowed the test of bioactivities of all eudesmanolides prepared (**3**–**7**) in three different bioassays, in an effort to evaluate the relationship between activity and structure, which was the third objective of this study.

2.2. Wheat coleoptile bioassay

This bioassay is a useful tool as a first approach to evaluate the phytotoxicity, as this is a quick and easy assay. Results are given as percentage coleoptile elongation in relation to that in the negative control. The commercial herbicide Logran® was used as a positive control (Macías et al., 2000). The profiles obtained are shown in Fig. 3.

All compounds showed high inhibition activities at the two highest concentrations and it is worth highlighting **3**, **7** and **8**, which have activities similar to, or higher than, the commercial herbicide with inhibition values close to 90% or above, even in the second dilution. Compounds **7** and **8** showed better retained activity with dilution, especially **7** at the lowest concentrations – with inhibition values of 42 and 30% at 30 and 10 µM, respectively. At 100 µM, compound **8** was the most active product, with an inhibition of 68%.

Regarding the IC_{50} values of the products (Table 2), compounds **7** and **8** got the best results, 67.6 and 71.9 µM respectively, and these values are close to that of the commercial herbicide used as a positive control in the bioassay.

Compounds **3** and **6** (139.6 and 146.4 µM, respectively) showed very similar values and this indicates the small influence of a methylene group in the lactonic ring. A similar effect can be observed in the cases of **7** and **8** (67.6 and 71.9 µM respectively). This similarity indicates the small contribution that the saturation of the bond between C-11 and C-13 has on the activity of the molecules in this bioassay.

Table 2
Lipophilicity of products, calculated as clog*P*, and IC_{50} values calculated with results of the wheat coleoptile bioassay.

Compound	clog*P*	IC_{50} Wheat (µM)	R^2
3	1.18	139.8	0.969
4	1.18	284.1	0.952
5	1.38	191.7	0.941
6	1.05	146.4	0.934
7	0.95	67.6	0.954
8	1.02	71.9	0.955
Logran®	–	40.1	0.947

The feature that proved to be effective in improving the activity was the modification of ring A. In this way, compounds **7** and **8**, which contain the exodienone system, present lower IC_{50} values (by around 50%) than compounds **3** and **6**.

Additionally, calculated clog*P* values (Table 2) are generally similar, so the structural modifications made to the molecules do not have a significant influence on their lipophilicity. A clear relationship between clog*P* and IC_{50} was not observed, but it is worth noting that the compound with lowest clog*P*, **7**, was the product with the lowest IC_{50} value. This fact reflects a possible improvement on the activity when structural features provide a decrease on the lipophilicity of the eudesmanolide, being easier the transport to the active site though cell membranes. Deeper studies should be carried out to confirm a trend in the activity of eudesmanolides connected with the lipophilicity of the molecule.

2.3. Phytotoxicity bioassay on weed species

All tested compounds were active and they were therefore subjected to the phytotoxicity bioassay on weed species. Weed species selected were *E. crus-galli*, *A. viridis* and *P. maximum*. These species were chosen since the starting material for our synthesis, i.e., santamarine (**3**), as well as reynosin (**4**), showed high phytotoxicity on these weeds in a previous study of our research group (Da Silva et al., 2017).

The activity profiles obtained are shown in Fig. 4, where negative differences from control indicate inhibition of growth. All compounds showed inhibitory effects at the highest concentrations, except for **3** and **6** on shoot growth of *E. crus-galli*. The activities of the final products **7** and **8** were higher than that of Logran® at 1000 µM on both shoots and roots, with inhibition values over 75% in most cases. These activities were retained at the second concentration and, in the case of *A. viridis*, at the third concentration, with values above 55% inhibition in all cases.

The IC_{50} values of **7** and **8** (Table 3) are better than those of the commercial herbicide in many cases and they are the only compounds that have significant inhibition values for *E. crus-galli* shoots. Furthermore, higher IC_{50} values than the commercial herbicide were obtained for **8** on *E. crus-galli* roots (205.4 vs. 314.3 µM); **6** on *A. viridis* shoots (50.0 vs. 60.9 µM); **7** on *P. maximum* shoots (256.6 vs. 341.7 µM); and for all compounds on *P. maximum* roots. The IC_{50} of the commercial herbicide on *P. maximum* roots would correspond with a value for a concentration higher than those tested in the bioassay, i.e., over 1000 µM.

Comparison of the IC_{50} values of **3** vs. **6** and **7** vs. **8** highlights the effects of some structural changes. The presence of a methyl group in the lactonic ring in **6** improves activity in relation to a methylene group in **3**, especially on *A. viridis* (from 240.2–39.0 µM in root and 287.3–50.0 µM in shoot), whereas **7** and **8** show different trends depending on the weed species. Better values were obtained on *E. crus-galli* (from 343.5–205.4 µM in root and 631.7–587.6 µM in shoot) for

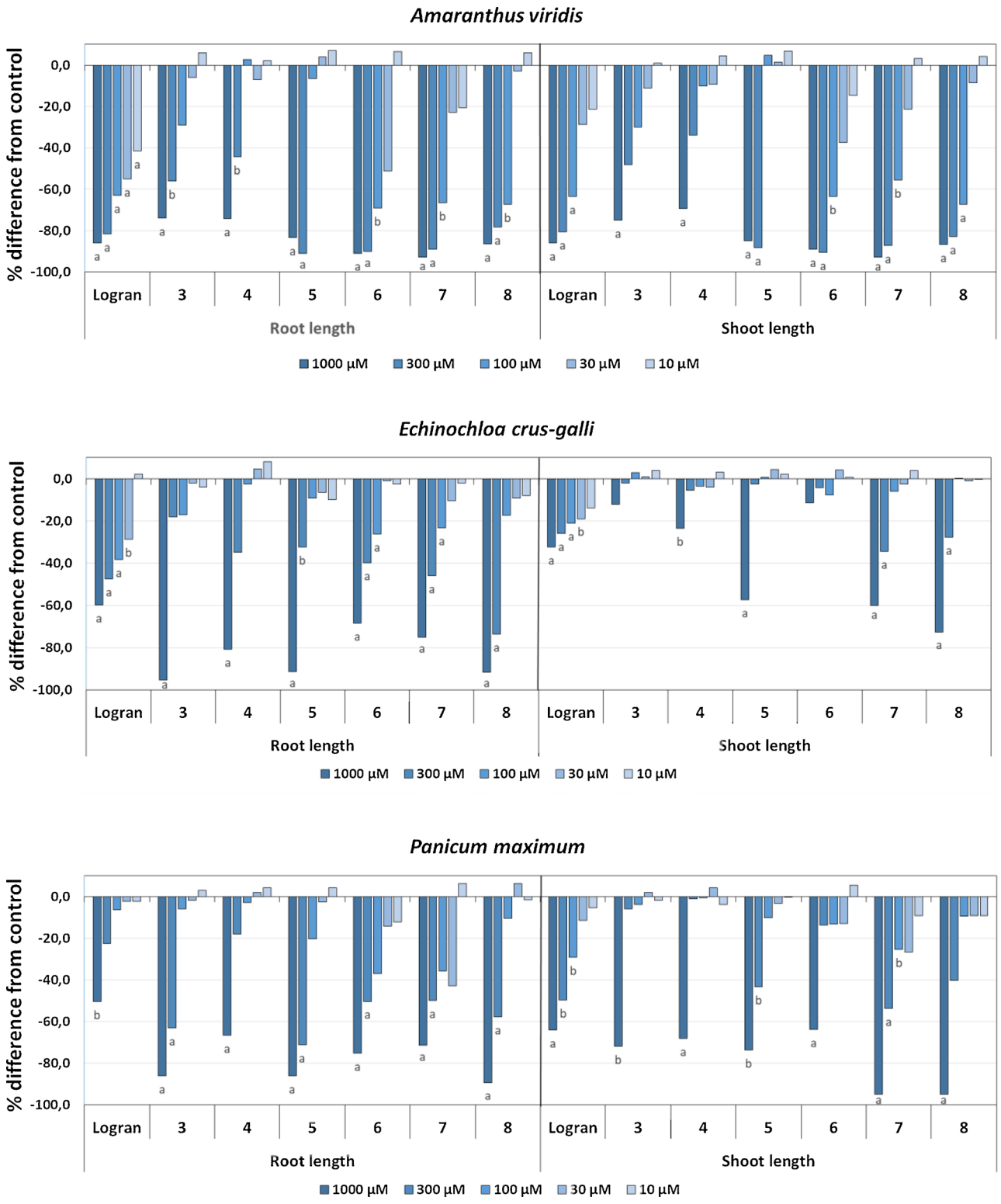

Fig. 4. Graphs for phytotoxicity weed bioassay. Positive values indicate stimulation of growth vs. the control, and negative values indicate inhibition. Significance levels $p < 0.01$ (a), or $0.01 < p < 0.05$ (b).

the molecule with a methylene group (**8**), whereas the presence of the methyl group in **7** improves on the IC_{50} value of **8** on *P. maximum* (from 322.5–235.1 µM in root and 415.3–256.6 µM in shoot), with *A. viridis* being only slightly sensitive to this difference.

The final products **7** and **8** are the most phytotoxic eudesmanolides tested on *E. crus-galli* growth and they show significantly improved activities when compared to santamarine (**3**). Compounds **6–8** also have enhanced phytotoxicity when compared to **3** on *A. viridis*, with IC_{50} values close to those of Logran®, from 240.2 (roots) and 287.3 µM (shoots) to 39.0 and 50.0 µM, respectively, in the best cases (**6**). Regarding *P. maximum*, the highest inhibitory activity was shown by **7**, which has an IC_{50} of 256.6 µM on shoots and the second highest IC_{50} on roots (235.1 µM) – i.e., only exceeded by that of **5** (199.7 µM).

Finally, cluster analysis was carried out in order to resolve which

Table 3
IC_{50} values of products for root and shoot growth for the three weed species tested.

Compound	IC_{50} *A. viridis* root (μM)	R^2	IC_{50} *A. viridis* shoot (μM)	R^2	IC_{50} *E. crus-galli* root (μM)	R^2	IC_{50} *E. crus-galli* shoot (μM)	R^2	IC_{50} *P. maximum* root (μM)	R^2	IC_{50} *P. maximum* shoot (μM)	R^2
3	240.2	0.955	287.3	0.955	476.9	0.906	a	–	271.7	0.956	a	–
4	442.3	0.968	539.9	0.992	462.0	0.970	a	–	739.1	0.977	930.3	0.920
5	165.7	0.865	205.0	0.872	475.9	0.914	a	–	199.7	0.973	411.5	0.992
6	39.0	0.942	50.0	0.992	439.7	0.987	a	–	271.3	0.934	869.2	0.947
7	63.2	0.980	77.9	0.984	343.5	0.999	631.7	0.994	235.1	0.934	256.6	0.962
8	75.6	0.878	72.1	0.932	205.4	0.959	587.6	0.974	322.5	0.932	415.3	0.911
Logran®	18.9	0.946	60.9	0.988	314.3	0.910	a	–	a	–	341.7	0.984

a = IC_{50} value over 1000 μM or not calculable.

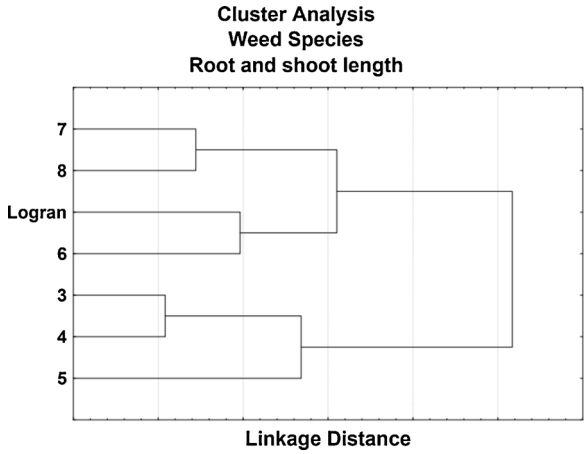

Fig. 5. Cluster analysis for weeds bioassay.

compounds are as effective as commercial herbicide Logran®. For this analysis was used root and shoot length activity data of all species, showed graphically in Fig. 4, so results of this bioassay are summarized in the cluster. The results of the cluster analysis are shown in Fig. 5, where products are arranged attending to activity similarities. Most active compounds are placed on the top of the figure, being **7** the most and **5** the least active. It can be seen that compounds **7** and **8** are ranked in the group that exceeds the activity of the commercial herbicide, with Logran® being more similar to compound **6** in terms of activity. This analysis supports the results discussed on previous paragraphs, denoting the potential of both anhydrojudaicin (**7**) and 11,13-dehydroanhydrojudaicin (**8**) to be proposed as lead compounds for new herbicides based on natural products.

2.4. Parasitic plant bioassay

This bioassay is a good tool to study the ability of products to stimulate the germination of seeds of parasitic plants so that the compounds can be used in preventive herbicides through the suicidal germination strategy. This technique is employed to control parasitic weed infestations on crops, and requires products that are able to stimulate the germination of weed seeds. These compounds are applied on the field prior to sowing, so the parasitic seeds in question would then start their germination but would die since there is no host plant for the parasite.

The results obtained were dependent on the species studied. In all cases (Fig. 6), compound **7** gave better profiles than **3** (eudesmane precursor) and **8** (methylene analog).

The results (Fig. 6) indicate that *O. crenata* and *O. cumana* are very selective species in terms of the type of compound that causes germination of their seeds. In the case of *O. crenata* germination, compounds **3** and **7** are remarkable as they can stimulate more than 15% germination of the seeds. The lower values obtained at the highest concentrations can be explained by the phytotoxicity that these products may have at these relatively high doses. No activity was detected for **8**.

O. cumana seeds were not affected by any of the compounds except for **7**, which showed 75% stimulation of germination at 100 μM, and **8**, which gave 25% stimulation at 100 μM. These results again highlight the potential of these compounds.

The tested compounds showed good profiles on *P. ramosa* seeds. The presence of the exodienone system (**7** and **8**) led to an improvement in the activity and **7** and **8** are more active than **3** and **6**, respectively. Compound **8** is the most active product evaluated on *P. ramosa* seeds. The most active compound at lower concentrations was the natural product reynosin (**4**) and this was the most active compound at the lowest dose (0.1 μM), with an excellent stimulation of 64% observed on seeds.

In summary, regarding synthetic considerations, the strategy used to obtain anhydrojudaicin (**7**) from costunolide (**1**) represents a marked improvement on the previously published method that synthesizes this product from α-santonin. Furthermore, the synthesis of 11,13-dehydroanhydrojudaicin (**8**) is reported for the first time in only 2 steps from costunolide.

Considering SAR, exodienone system of targeted products (**7** and **8**) improved significantly the activity in all bioassays of starting molecules, that only have one double bond in ring A. Thus, IC_{50} of **7** and **8** were among the best obtained in all cases, being the only compounds active on *O. cumana* germination.

Attending to the presence of a methyl or methylene group at the ring C, slight differences were observed on wheat coleoptile bioassay. However, no clear relationship could be observed on the phytotoxicity on weeds or parasitic seed germination, except on *O. cumana* where the absence of a double bond between C-11 and C-13 (compound **7**) provokes a relevant enhancement of activity.

Results detailed in this study let conclude that the target synthetic products, **7** and **8**, could be appropriate for use as lead compounds for new herbicides to control weeds of *A. viridis*, *E. crus-galli* and *P. maximum* and also as components in preventive herbicides to control infestations of the parasitic plants *O. cumana* and *P. ramosa* on crops by the suicidal germination strategy.

3. Experimental

3.1. General experimental procedures

All procedures and complete elucidation of compounds were carried out using equipment available at the University of Cadiz. Agilent spectrometers at 400/100 and 500/125 MHz were used to record NMR spectra. $CDCl_3$ (ProlaboTM, VWR) was the solvent for samples and the residual peak was used as internal reference at δ 7.26 in ^1H and δ 77.0 in ^{13}C NMR. The exact masses of compounds were measured on a UPLC-QTOF ESI (Waters Synapt G2, Manchester, UK) high-resolution spectrometer (HRTOFESIMS), with mass spectra recorded by the negative- or positive-ion method in the *m/z* range 100–2000, mass resolution of

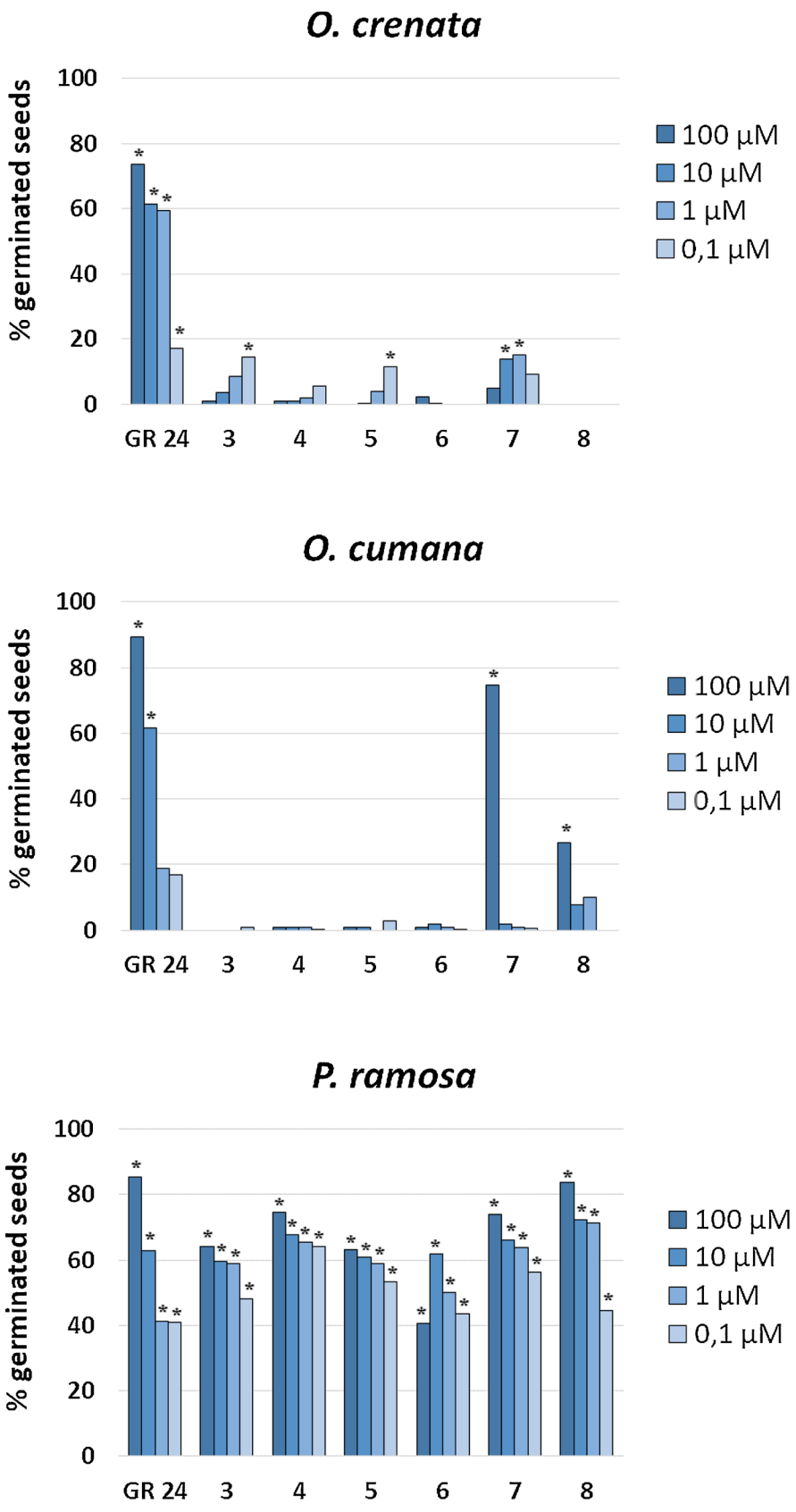

Fig. 6. Graphs for parasitic weed bioassay. Significant differences with control are marked with an asterisk.

20,000 and an acceleration voltage of 0.7 kV. A Perkin-Elmer Spectrum TWO IR spectrophotometer was used to obtain FTIR spectra. Optical rotations were measured on a JASCO P-2000 polarimeter, using $CHCl_3$ as solvent.

Saussurea lappa root extract was purchased from Pierre Chauvet S.A. (Seillans, France). Reagents were supplied by Merck (Darmstadt, Germany) or Sigma-Aldrich Co. (St. Louis, Missouri). Silica gel Geduran® Si 60 (0.063–0.200 mm) was used for column chromatography. HPLC was carried out on a Merck-Hitachi system (Tokyo, Japan) with a refractive index detector (Elite LaChrom L-2490). A semipreparative LiChrospher 10 µm 250-10 Si 60 (Merck) column was employed with a flow rate of 3 mL/min.

3.2. Synthesis of anhydrojudaicin (7) and 11,13-dehydroanhydrojudaicin (8)

Compounds **7** and **8** were synthesized from compound **1** in three and two steps, respectively.

3.2.1. Isolation of costunolide (1)

Column chromatography was used to purify compound **1**, eluting first with 0.7 L of hexane and then 6 L of hexane/EtOAc 19:1, from 53.2 g of a root extract of *Saussurea lappa*.

3.2.2. Synthesis of eudesmanolides (3–5)

Compound **1** (180 mg, 0.77 mmol) was dissolved in CH_2Cl_2 (2 mL) in a round-bottomed flask and mCPBA (199 mg, 1.15 mmol) was added under magnetic agitation. After 2 h at room temperature, all starting material had been consumed. One product was observed by TLC (hexane/EtOAc 7:3) and this was believed to be epoxide **2**. The mixture was neutralized with a 0.5 M aqueous solution of NaOH. The organic phase was extracted three times with dichloromethane, dried with anhydrous Na_2SO_4, filtered through filter paper and concentrated under reduced pressure. The crude product was loaded onto a chromatography column by absorption onto silica gel and purified using a gradient of hexane/AcOEt 100:0–80:20 to give two fractions: a mixture of three compounds and one pure compound (product **4**). The mixture was subsequently purified by HPLC (hexane/EtOAc 1:1) to give three compounds with different yields, in order of elution: magnolialide (**5**, 7%), santamarine (**3**, 59%) and reynosin (**4**, 31%).

3.2.3. Synthesis of dihydrosantamarine (6)

$NaBH_4$ (14 mg, 0.37 mmol) was added to a stirred solution of compound **3** (53 mg, 0.21 mmol) in THF (5 mL) in a round-bottomed flask. According to TLC (hexane/acetone 70:30) the reaction had finished after 1 h. The mixture was solved in ethyl acetate and poured into water. The aqueous phase was extracted three times with ethyl acetate. The organic layer was dried with anhydrous Na_2SO_4, filtered through filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/acetone gradient 100:0–50:50) to give compound **6** with quantitative yield as a white solid.

3.2.4. Synthesis of anhydrojudaicin (7)

The reaction was carried out in a two-necked round-bottom flask. A solution of **6** (59 mg, 0.24 mmol) in CH_2Cl_2 (6 mL) was added to DDQ (240 mg, 1.04 mmol). The reaction mixture was heated under reflux (45 °C) for 24 h under magnetic agitation. The mixture was allowed to cool to room temperature, dried with anhydrous Na_2SO_4, filtered through filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/acetone gradient 100:0–60:40) to give **7** as a colorless oil in 32% yield. Calculated *m/z* for $[C_{15}H_{19}O_3]^+$ 247.1334, obtained 247.1340; $[\alpha]_D^{25}$ = +16.3° (c 0.044, $CHCl_3$); IR (film) $\hat{u}_{max}$ cm^{-1} 1783 and 1672 (carbonyl groups). For NMR data see Table 1.

3.2.5. Synthesis of 11,13-dehydroanhydrojudaicin (8)

A solution of **3** (35 mg, 0.14 mmol) in CH_2Cl_2 (6 mL) was added to DDQ (252 mg, 1.09 mmol) in a two-necked round-bottomed flask equipped with a Liebig condenser. The reaction mixture was heated under reflux (45 °C) for 24 h with stirring. The mixture was allowed to cool, dried with anhydrous Na_2SO_4, filtered through filter paper and concentrated under reduced pressure. The crude product was purified by column chromatography (hexane/acetone gradient 100:0–50:50) to give **8** as a colorless oil in 43% yield. Calculated *m/z* for $[C_{15}H_{17}O_3]^+$ 245.1178, obtained 245.1188; $[\alpha]_D^{25}$ = +4.6° (c 0.116, $CHCl_3$); IR (film) $\hat{u}_{max}$ cm^{-1} 1772 and 1672 (carbonyl groups). For NMR data see Table 1.

3.3. Bioassays

3.3.1. Wheat coleoptile bioassay

The bioassay was carried out by optimized procedures established by our research group (Macías et al., 2004). To obtain the coleoptiles, wheat seeds (*Triticum aestivum* L. cv. Catervo, provided by FITÓ S.A., Barcelona, Spain) were sown in deionized water-moistened Whatman® paper in Petri dishes with a diameter of 14 cm. The samples were introduced into an incubation chamber, in the dark to avoid photosynthesis, at 25 °C for 4 days. Under a green safelight the roots and caryopses were removed from the shoots. 4 mm lengths of the shoots were removed using a Van der Weij guillotine to remove the apical 2 mm, thus obtaining the coleoptiles. The products tested were predissolved in DMSO (0.5%) and diluted in buffer to the final concentrations required for the bioassay (1000, 300, 100, 30 and 10 µM). The buffer was a phosphate-citrate buffer containing 2% sucrose and adjusted to pH 5.6.

The commercial herbicide Logran® was used as positive control. Logran® is a mixture of N^2-*tert*-butyl-N^4-ethyl-6-(methylsulfanyl)-1,3,5-triazine-2,4-diamine (terbutryn, 59.4%) and 2-(2-chloroethoxy)-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)carbamoyl]benzene-1-sulfonamide (triasulfuron, 0.6%). Buffered aqueous solutions without any product were used as negative controls and these contained the same percentage of DMSO as the samples.

The bioassays were carried out in test tubes. Each concentration had three replicates and one test tube was used per replicate. In each tube five coleoptiles were introduced together with 2 mL of solution. The tubes were placed horizontally in a roller tube apparatus at 0.25 rpm in the dark for 24 h at 25 °C. Finally, coleoptiles were digitally photographed on a template to measure their elongation. Data were analyzed by Welch's test and results are presented as percentage elongation against the negative control.

3.3.2. Phytotoxicity bioassay on weeds

Seeds were placed on moistened Whatman® paper of diameter 50 mm in Petri dishes with 1.0 mL of buffer solution (2-(N-morpholine) ethanesulfonic acid (MES) 10 mM, pH adjusted to 6.0) containing products at concentrations of 1000, 300, 100, 30 or 10 µM. Twenty seeds were placed in each Petri dish, with five replicates per concentration tested, with seeds of *Amaranthus viridis* L., *Echinochloa crus-galli* L. and *Panicum maximum* Jacq. Buffer solution without any tested compound was used as negative control and the herbicide Logran® as positive control, as in the coleoptile bioassay.

Dishes were kept in darkness at 25 °C in a germination chamber: *E. crus-galli* and *P. maximum* (11 days), *A. viridis* (13 days). After these periods, the dishes were placed in a freezer for 24 h.

Measurement of the shoots and roots was performed after placing seedlings on a plastic film, with the lengths measured with a Fitomed® digitizing table using a light pen. Statistical analysis was performed by Welch's test, with significance levels of 0.01 and 0.05 established.

3.3.3. Parasitic seeds bioassay

The following bioassay was developed by our research group (Cala

et al., 2017; Rial et al., 2018) using seeds of parasitic species of *O. cumana* (provided by Dr. Leonardo Velasco, Instituto de Agricultura Sostenible (CSIC), University of Cordoba, Spain), *O. crenata* and *P. ramosa* (both provided by Prof. Maurizio Vurro, Istituto di Scienze delle Produzioni Alimentari (ISPA-CNR), University of Bari, Italia). Seeds were placed on water-moistened glass fiber filter papers of diameter 10 mm in Petri dishes and samples were stored in darkness for 10 days. The products were dissolved in acetone (1% v/v) and then diluted in type I water to obtain solutions of 100, 10, 1 and 0.1 μM. For each concentration, four samples were replicated, with 80 μL of solution added per sample. A solution of water/acetone (99:1 v/v), without any product, was employed as negative control. Synthetic strigolactone GR24 (provided by Prof. Binne Zwanenburg, Radboud University, Nijmegen, Netherlands) was tested in the same was as a product to provide a positive control. The results were obtained after samples had been stored for 7 days in darkness.

The percentages of germinated seeds were measured using a binocular camera. Values were approximated to a normal frequency distribution by means of an angular transformation, and subjected to an analysis of variance (ANOVA) with SPSS software (SPSS Inc., Chicago, IL). Two-sided Dunnett's tests were used to evaluate significant mean differences between values of samples and negative-control values. Rejection of null hypothesis was defined at level of 0.05.

3.4. Calculation of IC_{50} and logP

The IC_{50} values for products were calculated by fitting activity data to a sigmoidal dose-response model using GraphPad Prism v.5.00 software. ChemBioDraw Ultra 17.0 software was used for the calculation of clog*P*.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2019.04.014.

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