JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

SAR Studies of Sesquiterpene Lactones as *Orobanche cumana* Seed Germination Stimulants

JUAN C. G. GALINDO,[†] ALEJANDRO P. DE LUQUE,[‡] JESÚS JORRÍN,*[,§] AND FRANCISCO A. MACÍAS*[,†]

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, c/ República Saharaui s/n, Apartado 40, 11510 Puerto Real, Cádiz, Spain; Institute of Sustainable Agriculture, IAS-CSIC, 14080 Córdoba, Spain; and Departamento de Bioquímica y Biología Molecular, ETSIAM, Universidad de Córdoba, Apartado 3048, 14080 Córdoba, Spain

Studies of the structure−activity relationship (SAR) directed to evaluate the effect of several sesquiterpene lactones (SL) as germination stimulants of three *Orobanche* spp. (*O. cumana*, *O. crenata*, and *O. ramosa*) have been achieved. Results are compared with those obtained in the same bioassay with an internal standard, the synthetic analogue of strigol GR-24. A high specificity in the germination activity of SL on the sunflower parasite *O. cumana* has been observed, and a relationship between such activity and the high sunflower SL content is postulated. Molecular properties of the natural and synthetic germination stimulants (GR-24, GR-7, and Nijmegen-1) and SL have been studied using MMX and PM3 calculations. Consequently, comparative studies among all of them and their activities have been made. SL tested present similarities in molecular properties such as the volume of the molecule and the spatial disposition of the carbon backbone to the natural germination stimulant orobanchol. These properties could be related to their biological activity.

KEYWORDS: Sesquiterpene lactones; guaianolides; germacranolides; eudesmanolides; SAR studies; germination stimulants; allelopathy; parasitic weeds; *Orobanche cumana*; Orobanchaceae

INTRODUCTION

Parasitic angiosperms of the genus *Orobanche* (commonly known as broomrapes) affect important dicotyledoneous crops, such as legumes, tomato, tobacco, and sunflower, causing important yield losses (*1*, *2*). Parasitic angiosperms have a complex life cycle, in which specific steps (separated both spatially and temporally) can be differentiated: diaspore, after-ripening, conditioning, germination, haustorial induction, attachment, penetration and installation, development, emergence, and flowering (*1*, *2*). There is a continuous exchange of chemical information between the host and the parasite during the latter's life cycle. This aspect is partly known for *Striga* spp. (*3*−*5*), but little is known about molecular signaling in other parasitic species, including *Orobanche*.

The germination of root parasitic plant seeds depends on chemical exudates from the root of the host plants. To date, the chemical nature of several germination stimulants is known in the case of *Striga* (*3*), but no information is available for other parasitic plants, with the exception of two papers describing alectrol and orobanchol as germination stimulants of *Orobanche minor* (*6*, *7*) (**Figure 1**). All of these compounds isolated from different hosts of *Striga* and *Orobanche* are generally known as strigolactones. At the same time, a whole collection of strigolactone analogues named the GR family have been developed and tested with the aim of obtaining greater chemical stability and easy multigram-scaling synthesis (**Figure 1**) (*8*−*10*).

Several approaches have received attention in attempts to control parasitic weeds. These methods include the use of resistant genotypes (if any) and manual (i.e., hand-pulling), chemical (i.e., herbicides), or biological (i.e., the use of catch or trap crops, pathogenic fungi or insects) methods (*1*). In general, there has been limited success in the control of the parasitic plants by using the above-mentioned techniques (*11*−*13*). "Suicidal germination" by using germination stimulants could be one of the most promising approaches, and it constitutes the framework in which the GR family of synthetic inductors has been developed. This approach assumes presowing treatment with an exogenously applied stimulant should lead to a "suicidal" germination in the absence of any host. Besides sorgolactones, several sesquiterpene lactones (SL) have been tested as potential inductors of the germination of *Striga* spp. (*14*, *15*), and some fungal metabolites have also been tested on *Striga* and *Orobanche* (*16*) with promising results.

Following this approach, we successfully tested several SL as possible inductors of *Orobanche cumana* (sunflower parasite),

* Authors to whom correspondence should be addressed [(F.A.M.) telephone +34 (956) 016370, fax +34 (956) 016193, e-mail famacias@uca.es; (J.J.) telephone +34 (957) 218439, fax +34 (957) 218563, e-mail bf1jonoj@uco.es].
[†] Universidad de Cádiz.
[‡] Institute of Sustainable Agriculture, Cordoba.
[§] Universidad de Córdoba.

10.1021/jf0110809 CCC: $22.00
Published on Web 02/14/2002

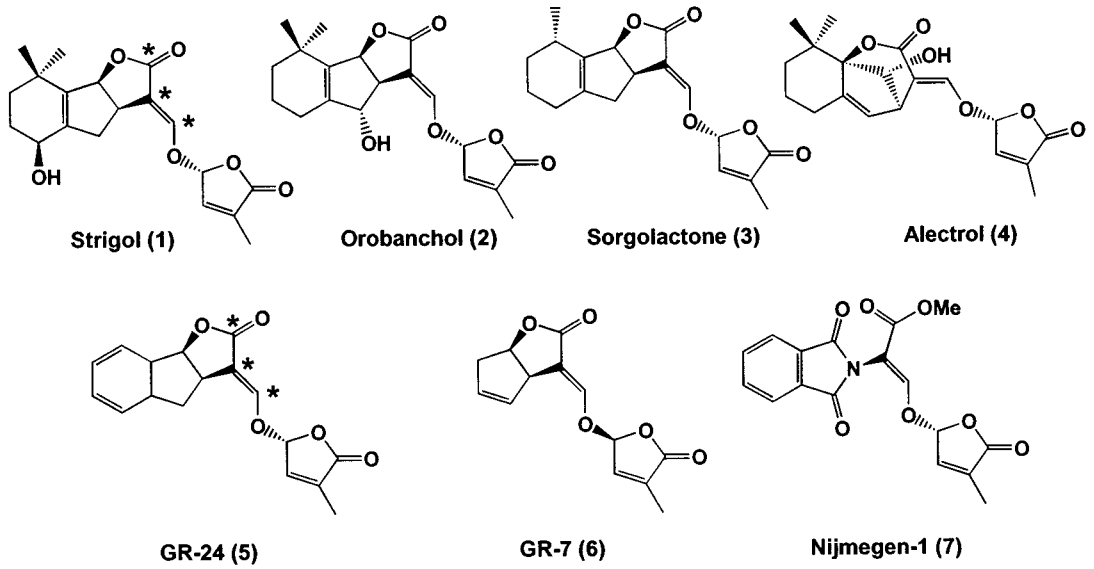

Figure 1. Natural and synthetic inductors of germination in parasitic weeds.

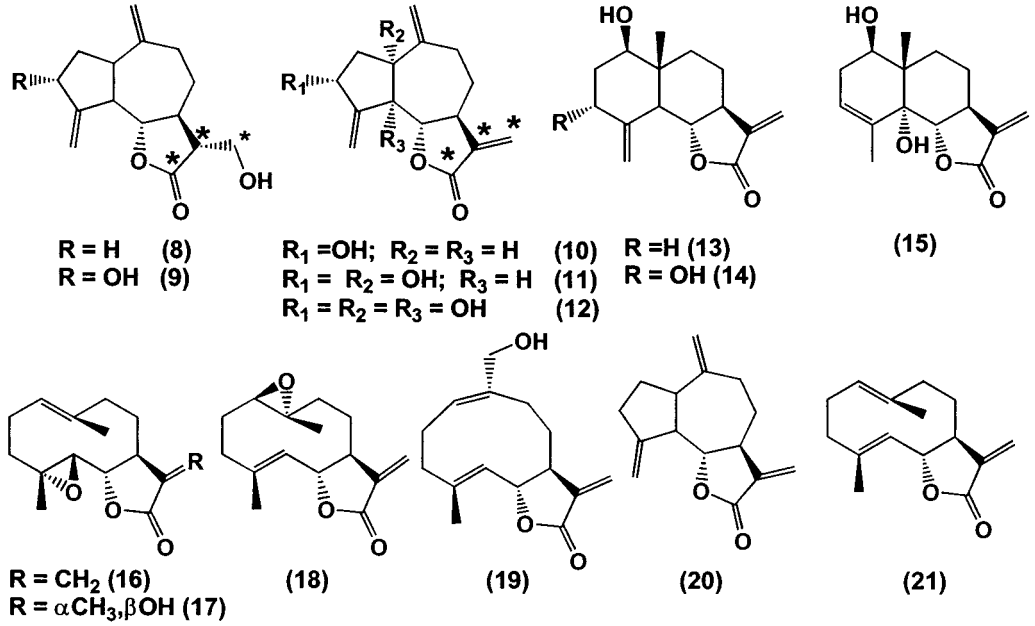

Figure 2. Tested SL: guaianolides **8**, **9**, and **12**; eudesmanolide **13**; and *trans*,*trans*-germacranolides **17** and **18**. Compounds **19**–**21** were previously tested (*7*), and their results are included by means of making suitable comparisons.

Orobanche crenata (pea parasite), and *Orobanche ramosa* (tobacco and tomato parasite) seed germination (*17*). Those lactones bearing features common to many SL isolated from sunflower were able to induce germination of the specific sunflower parasite *O. cumana* at lower concentrations than the positive control GR-24. Moreover, active compounds did not exert any inducing germination activity on the rest of the *Orobanche* species tested. Going on with this study and on the basis of the previous reported results, we have tested a collection of six additional SL with guaianolide, *trans*,*trans*-germacranolide, and eudesmanolide backbones (**Figure 2**, compounds **8**, **9**, **12**, **13**, **17**, and **18**). According to the literature (*18*), the enol-γ-lactone moiety has been proposed as the part of the molecule responsible for the observed bioactivity. This part might react with a hypothetic nucleophile in the enzyme receptor's cavity through a Michael addition reaction (**Figure 3**). Thus, tested SL have been selected with the aim of verifying two different hypotheses: (i) the importance of the presence of Michael acceptors, because such a mechanism has been proposed to be responsible for the activity in strigolactones; and (ii) the influence of the type of backbone.

MATERIALS AND METHODS

Starting Materials. Dehydrocostuslactone (**20**) and costunolide (**21**) were obtained from crude costus resin oil (*Saussurea lappa* roots extract) by previous column chromatography (CC) separation and purified by crystallization from hexane/ethyl acetate mixtures and high-performance liquid chromatography (HPLC); parthenolide (**16**) was obtained from *Magnolia grandiflora* leaf extract by CC separation and HPLC purification. All of the structures of the natural and synthetic compounds were confirmed by comparison of their spectroscopical data (IR, EIMS, and ^{1}H and ^{13}C NMR) with those in the literature. GR-24 was supplied by B. Zwanenburg (University of Nijmegen, Netherlands).

Synthetic Procedures. Compounds **9** and **12** were obtained by allylic oxidation of **8** and dehydrocostuslactone (**20**) using SeO_2/*tert*-butyl-hydroperoxide according to the previously published methods (*19*, *20*).

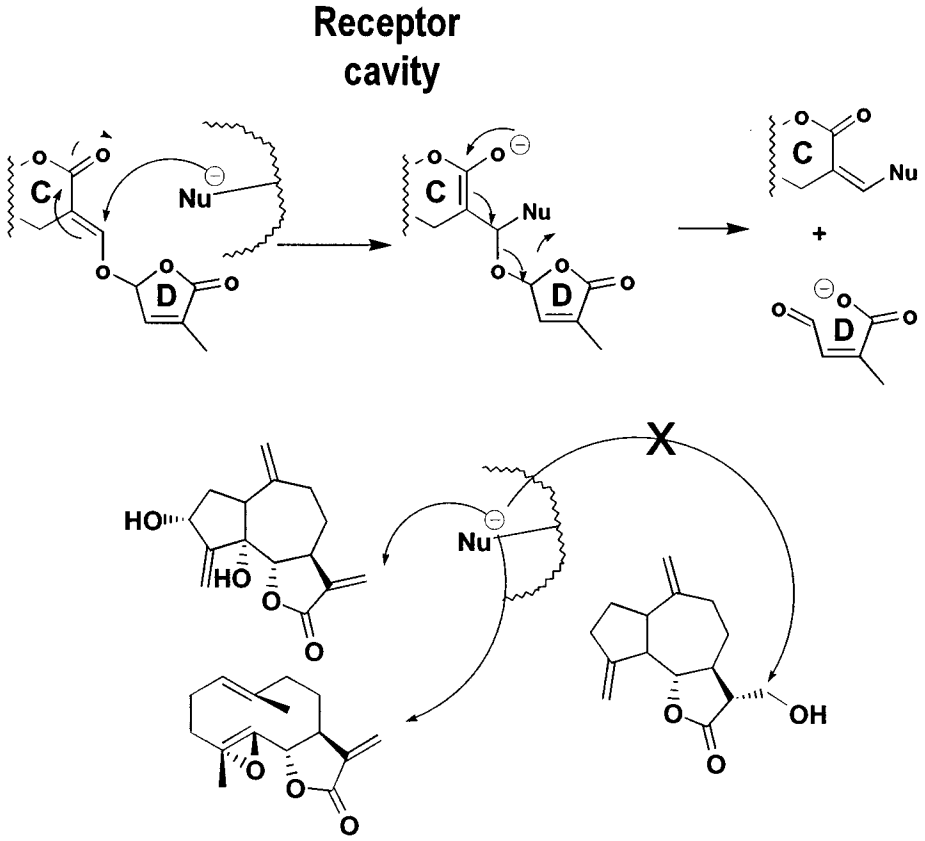

Figure 3. Proposed mechanism of action in strigolactones (top) according to Mangnus (*8*) and proposed hypothesis for sesquiterpene lactones (bottom).

Compound **8** was obtained from dehydrocostuslactone (**20**) by treatment with Na_2CO_3/HMPA (*21*). Reynosin (**13**) and **18** were obtained from costunolide (**21**) by biomimetic cyclicization and selective buffered epoxidation with *m*-chloroperbenzoic acid (MCPBA), respectively (*22*). Finally, compound **17** was obtained by reduction of parthenolide (**16**) with $NaBH_4$ (MeOH, 0 °C) (*23*).

All products were purified prior to the bioassay using HPLC equipped with a refractive index detector. Minimum degree of purity was 99% as extracted from the chromatograms.

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra were recorded at 399.952 and 100.577 MHz, respectively, on a Varian UNITY-400 spectrometer using $CDCl_3$ as solvent. The resonances of residual chloroform at δ_H 7.25 and δ_C 77.00 were used as internal reference for ^{1}H and ^{13}C spectra, respectively. Mass spectra were obtained by using a VG 1250 or a Kratos MS-80-RFA instrument at 70 eV. The infrared (IR) spectra were recorded on a Bio-Rad FTS-7. CC was performed on silica gel (35−75 mesh), and thin-layer chromatography (TLC) analysis was carried out using aluminum-packed precoated silica gel plates. For HPLC, LiChrosorb silica 60 was used in the normal-phase mode using a differential refractometer (RI) and a UV detector, with a Hitachi L-6020A HPLC instrument. All solvents were of spectral grade or distilled from glass prior to use.

***Orobanche* Species Germination Bioassays.** Two populations of *O. cumana*, named 7 and 21, with different degrees of virulence, were collected from infected experimental sunflower fields by Dr. González Carrascosa (Semillas Cargill, Spain). Seeds of *O. ramosa* were provided by Dr. D. M. Joel (Department of Weed Research, Agricultural Research Organization, Newe-Ya'ar Research Center, Israel), and those of *O. crenata* by Dr. D. Rubiales (CSIC, Córdoba, Spain). The *Orobanche* germination bioassay on filter paper was used as previously reported (*17*, *24*). Seeds were preconditioned in darkness [20 °C, 11 days, and 0.3 mM (2-[*N*-morpholino]ethanesulfonic acid (MES) buffer] and germinated in the presence of GR-24 or SL **8**, **9**, **12**, **13**, **17**, and **18** (darkness, 20 °C, 6 days). Germination was observed under a dissection microscope (30×). Seeds were considered to be germinated when the radicle was at least 0.2 mm long. Germination was expressed as percentage of the total seeds.

Stock solutions were prepared with acetone and diluted with 0.3 mM MES to obtain a 10 μM (0.1% acetone) solution; 0.1 and 1 μM solutions were prepared by dilution with 0.3 mM MES (0.1% acetone aqueous solutions).

Molecular Modeling. PCModel 7.5 was used to obtain basic minimum energy structures. Then, GMMX command was used to generate other low-energy local minima. These conformers were further minimized using PM3 calculations (MOPAC) (*25*) to obtain more accurate theoretical ΔH_f values that allow the real minimum energy conformer to be obtained. Volume, saturated and unsaturated areas, polar areas, dipole moment, and other molecular parameters were also obtained from PCModel.

RESULTS

Preliminary dose−response assays were performed with GR-24, the universal standard stimulant utilized to evaluate germination of parasitic plants (**Figure 4**). Low percentage of germination (<10%) or no germination was observed in the absence of added GR-24 (data not shown). Differences in germination between *Orobanche* species and populations were observed, the percentage of germination being dependent on the concentration of GR-24 added. Germination was observed at doses as low as 0.01 μM GR-24 for *O. ramosa*, *O. crenata*, and *O. cumana* cv. 7 and 10 μM for *O. cumana* cv. 21. With the exception of *O. cumana* 7, maximum percentage of germination took place at 100 μM GR-24 (values close to 80% germination for *O. crenata*, 65% for *O. ramosa*, and 50% for *O. cumana*). Sixty-five percent of the *O. cumana* 7 seeds germinated at 10 μM GR-24.

When the activity of the SL was assayed, stimulatory effect was considered with a percentage of germination higher than the observed in the absence of any added compound (seeds incubated in MES buffer, corresponding to 10% or lower values). Thus, *O. cumana* seeds germinated in the presence of

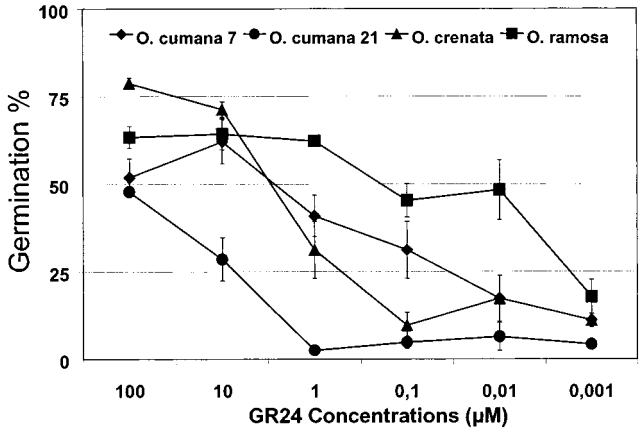

Figure 4. Effect of different concentrations of GR-24 (10^{-4}–10^{-9} M) on the germination of three different *Orobanche* spp. expressed in percent of total seeds.

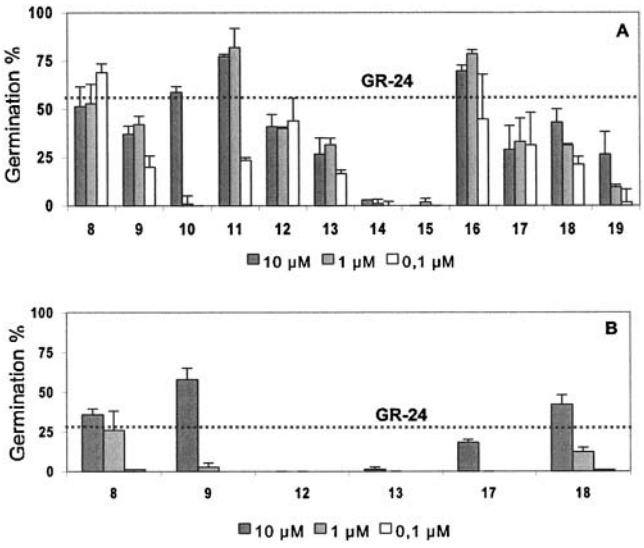

Figure 5. Effects of SL **8**–**19** over *O. cumana* cv. 7 (A) and cv. 21 (B) germination expressed in percent of total seeds. Dashed lines represent germination level of *O. cumana* at 10 μM GR-24.

tested SL (**Figure 5**), with the exception of populations 21 and 7 treated with **12** and **13** and with **14** and **15**, respectively. All SL assayed failed to induce germination of *O. crenata* and *O. ramosa* seeds, with percent germination for these two species being close to zero (data not shown).

Differences in germination were observed between *O. cumana* populations, the different SL, and the concentration tested. Comparison of the newly obtained data and those previously reported (*17*) revealed that the percentage of germination was higher for population 7 than for population 21, compounds **8**, **11**, and **16** were most active for population 7, and compounds **8**, **9**, and **18** were the most potent inductors for population 21. With these compounds the germination stimulant effect was higher than that obtained with GR-24. In the case of *O. cumana*, six lactones, **9**, **12**, **13**, and **17**−**19**, were moderate stimulants with germination values lower than those obtained with GR-24. Otherwise, compounds **12** and **13** were inactive and **17** a moderate stimulant in the case of *O. cumana* 21. Finally, depending on the compound, maximum effect was obtained at concentration values of 10 or 1 μM.

Molecular modeling of all 12 tested compounds (**8**−**19**) and those described in our previous paper (*17*) allowed us to obtain the minimum energy conformation for each one and some theoretical molecular properties as well. Similar calculations were made also for natural (**1**−**4**) and synthetic (**5**−**7**) strigolactones. Parameters studied were dipole moment, heat of formation, total volume, total area, saturated and unsaturated areas, and polar and nonpolar areas. Results of selected parameters can be found in **Table 1**.

Table 1. Selected Molecular Properties Calculated by Means of MMX and PM3 Force Field Calculations Using PCModel and MOPAC Programs

compound	dipole moment (μ)	vol (Å^3)	total area	% unsatured area	% polar area	activity[a]
strigolactones						
1	6.413	435.6	441.0	8.9	27.8	++[b]
2	5.078	438.4	436.9	8.7	28.1	++[c]
3	6.320	387.1	378.7	9.9	26.0	++[d]
4	5.639	412.4	401.8	9.3	29.1	++[b]
5	6.103	352.8	339.3	15.9	28.7	++
6	6.184	290.5	278.0	14.6	35.3	+
7	6.074	380.9	360.7	18.6	41.5	+[b]
sesquiterpene lactones						
8	4.256	318.3	325.4	9.0	20.2	++
9	3.63	322.3	323.9	8.6	25.9	++
10	4.359	309.0	312.2	13.6	21.4	++
11	4.701	315.0	318.4	13.3	28.5	++
12	2.65	318.3	319.3	13.9	31.1	+
13	3.524	315.1	318.4	9.2	20.3	+
14	4.498	320.3	334.0	8.9	27.4	0
15	3.065	314.6	316.	9.0	20.0	0
16	5.234	322.5	330.2	9.0	17.8	++
17	5.675	333.0	341.8	4.1	17.5	+
18	2.212	323.6	329.9	8.9	18.2	++
19	3.775	323.6	332.9	12.7	20.1	+

[a] ++, % germination > 40%; +, 40 > % germination > 0; 0, no activity. Germination induction obtained at 10 μM using *O. cumana* seeds. [b] Values obtained using *O. minor* seeds and 0.001 μM (*6*). [c] Values obtained using *O. minor* seeds and 0.001 μM (*30*). [d] Values obtained using *O. crenata* seeds (*10*).

DISCUSSION

The *Striga* germination stimulants reported, isolated from both host and nonhost plants, belong to different chemical groups. However, most of the compounds reported belong to the group of the sesquiterpenes named strigolactones (*3*). To date, no germination inductors of *O. cumana* germination have been reported from sunflower. Otherwise, no germination stimulants of *O. cumana* or other *Orobanche* spp. have been isolated and characterized from their hosts with the exception of *O. minor* (*6*). Additionally, sunflower presents a high content in SL, mainly related with defensive roles (allelopathic, fungicidal, and insecticidal) (*26*).

In this and a previous paper (*17*) we describe the stimulant germination activity of those SL with structures very similar to those reported from sunflower. Most of the SL tested induce germination of *O. cumana*, but not of *O. crenata*, *O. ramosa*, or *O. aegyptiaca*. The high specificity shown by these SL for the specific sunflower parasite could be a clue that these compounds might be related with recognition processes between *O. cumana* and its host. The percentages of germination depend on the compound, concentration, and population. Differences in germination among populations within *O. cumana* could be related to the existence of different stimulants or receptors; thus, some compounds, such as **12** and **13**, were inactive for population 21 but not for population 7, and SL **9** was more active for population 21 than for population 7. In any case, a hypothesis such as negative allelopathic interactions among individuals through other chemicals cannot be discarded and needs further experimental confirmation.

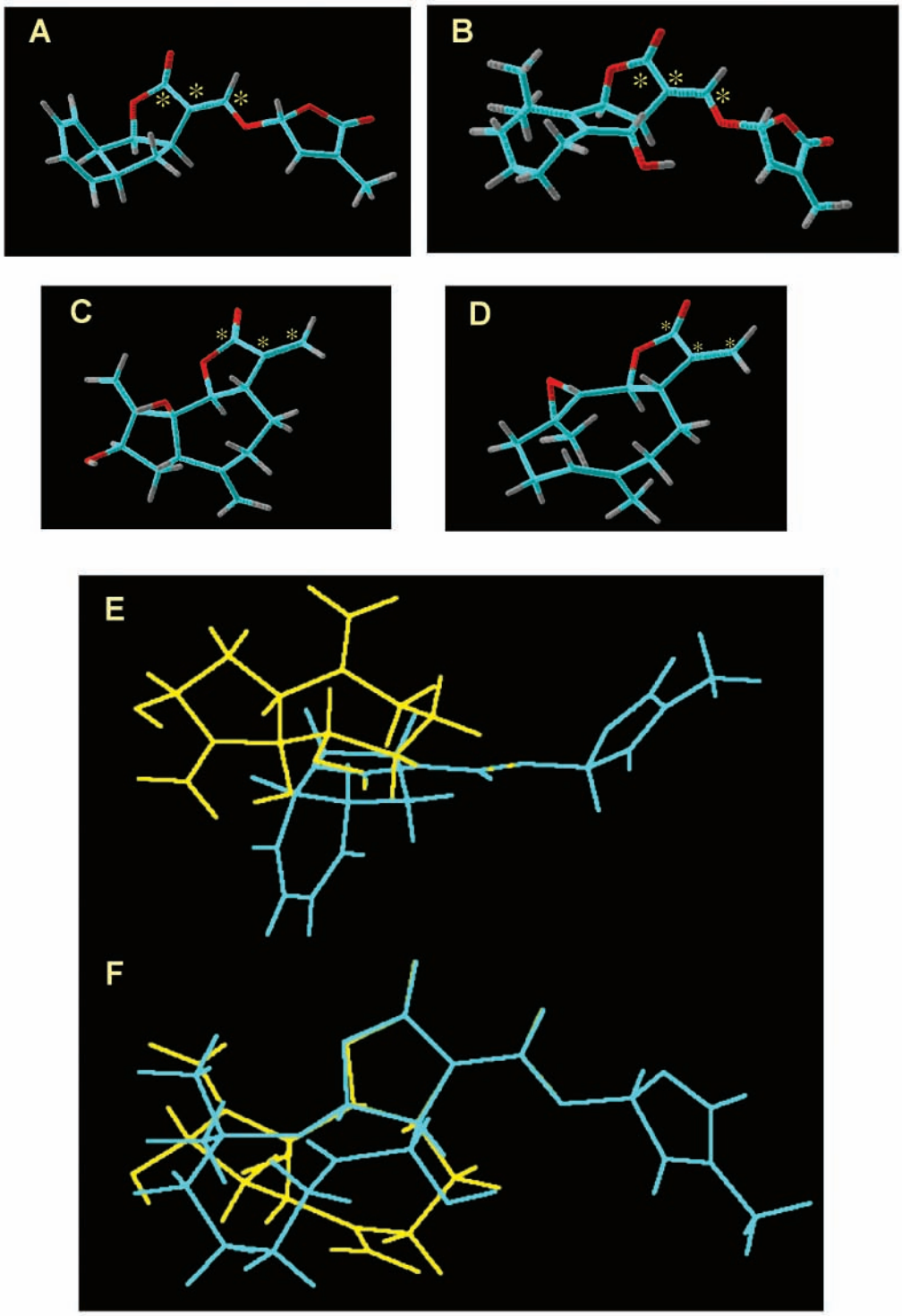

Figure 6. Minimum energy conformers obtained by using MMX calculations for GR-24 (**5**) (A), orobanchol (**2**) (B), 5-hydroxyisozaluzanin C (**11**) (C), and parthenolide (**16**) (D). Marked atoms were used for overlapping structures for comparison of SL with GR-24 (**5**) (E) and with orobanchol (**2**) (F).

The most active compounds are those with guaianolide (**1**−**3**) and *trans,trans*-germacranolide (**4**−**6**) backbones. Previously, SL with the same backbones have been reported to stimulate the germination of *Striga* spp. (*14*, *15*, *27*). These are compounds commonly present in sunflower chemical composition, whereas melampolides and eudesmanolides (much less active) are not so abundant or not present. This could indicate that SL present in sunflower roots might be responsible for host recognition mechanisms in *O. cumana*. Of course, this hypothesis needs to be further confirmed by a bioassay-directed chemical search. However, we cannot disregard the possibility that a positive correlation between the specific action of SL on sunflower parasite and sunflower chemical composition can be made.

Regarding the different backbones, it can be stated as a general trend that guaianolides (**9**−**13**) and *trans,trans*-germacranolides (**16**−**18**) are the most active, followed by melampolides (**19**) and eudesmanolides (**13**−**15**). However, no other correlations could be made with the activity on this basis.

The molecular properties of all strigolactones (natural and synthetic) showed similar values for all theoretical parameters (**Table 1**, entries **1**−**7**), even though GR-7 (**6**) and Nijmegen-1 (**7**) show much lower activities than the rest on several species

of *Orobanche* and *Striga*, as has been reported in the literature (*10*). Comparison of marked atoms shows a perfect overlapping of the lactone-enol-γ-lactone moiety in all natural and synthetic inductors (**Figure 6**). Moreover, all of these compounds show a common polar area localized in this part of the molecule that might be recognized by the receptor. Only one difference can be found among natural and synthetic strigolactones, and this is regarding the orientation of the carbon backbone in the nonpolar part of the molecule. Whereas natural strigolactones occupy the same space, the GR-24 carbon backbone does not: the strigolactone carbon backbone is bent down and that of GR-24 is bent up (panels A and B, respectively, of **Figure 6**).

Returning to SL molecular properties, a comparison among tested compounds (**8**−**19**, **Table 1**) and strigolactones shows that all of them present a volume only a little lower than that of GR-24 (**5**, **Table 1**) or orobanchol (**2**, **Table 1**). All SL would fit into the hypothetic receptor cavity postulated for strigolactones. They also present similar values of polar and unsatured areas with respect to the overall area of the molecule, and the polar area is located in a similar position compared with strigolactones. Thus, they could be recognized by the same receptor as strigolactones. Moreover, overlapping of the common marked atoms (**Figure 6**,**D**) shows how SL present a similar spatial orientation of the carbon backbone in comparison with orobanchol (**2**) (**Figure 6F**). On the other hand, whereas the backbone of synthetic GR-24 is bent up (**Figure 6A**), those of all active SL are bent down, the same as natural strigolactones (**Figure 6E**). Such similarity could be related with the high specificity of SL with *O. cumana* germination induction.

Regarding chemical properties, it has been reported (*18*) that the enol moiety in strigolactones is crucial for the induction. Removal of the double bond or replacement of the oxygen atom by a sulfur or nitrogen atom results in no activity. Most of tested SL (**10**−**16**, **18**−**19**) fit the first requirement, because they present a double bond attached to the lactone ring, thus allowing a Michael addition reaction in a similar way as has been proposed for strigolactones (**Figure 2**). Such reaction is commonly described in the literature for SL (*28*, *29*). However, the striking point is that compounds **8**, **9**, and **17**, without such a double bond and with no possibility of rendering any Michael addition reaction, are active. Moreover, under mild natural or cell conditions (pH values not lower than 5.5), there is no possibility to regenerate the double bond through elimination reactions. Compounds **8** and **9** present an oxygen atom in a similar disposition as strigolactones do, and the whole hydroxymethylene-lactone moiety might be recognized by the target, even though a Michael addition reaction cannot take place. However, this cannot be applied to compound **17**, lacking both the exocyclic double bond and the oxygen atom. Thus, another recognition mechanism or other target site might be triggered in this case, but this is another hypothesis that needs further confirmation.

In conclusion, our bioassays show that SL are useful inductors of *O. cumana* seed germination in the range of 10^{-5}−10^{-7} M, some compounds presenting higher values of activity than GR-24.

Second, the high specificity shown by SL in their activity on the sunflower *Orobanche* parasite could be related with a host recognition mechanism, because SL are common constituents of sunflower and are not present in other plants parasitized by different species of *Orobanche* (e.g., green peas, sweet clover, or tobacco). Differences in germination between populations could suggest the existence of different germination stimulants or receptors.

Regarding the molecular properties, SL present values of polar and unsatured area similar to those of strigolactones. Moreover, overlapping of the lactone moieties of both SL and strigolactones shows a similar spatial disposition in both families, but different when compared with the synthetic derivative GR-24. The meaning of this is still uncertain but might be related to the specificity of action of SL.

Finally, although the enol-γ-lactone moiety is crucial for strigolactone activity, this is not the case for SL: compounds lacking the lactonic methylene double bond or the oxygen atom are still active. Thus, those SL fitting the strigolactone structural requirements could act through the same target site, and those that do not should have a different target site or a different recognition mechanism.

ABBREVIATIONS USED

SL, sesquiterpene lactones; HMPA, hexamethylphosphorus triamide; MES, 2-[*N*-morpholino]ethanesulfonic acid; SAR, structure−activity relationship; MCPBA, *m*-chloroperbenzoic acid; CC, column chromatography; TLC, thin layer chromatography; HPLC, high-performance liquid chromatography.

ACKNOWLEDGMENT

We thank Dr. González Carrascosa (Semillas Cargill, Spain), Dr. D. M. Joel (Department of Weed Research, Agricultural Research Organization, Newe-Ya'ar Research Center, Israel), and Dr. D. Rubiales (CSIC, Córdoba, Spain) for kindly providing *Orobanche* seeds.

LITERATURE CITED

(1) Parker, C.; Riches, C. R. In *Parasitic Weeds of the World: Biology and Control*; CAB International Press: Wallingford, Oxon, U.K., 1993.

(2) Press, M. C.; Graves, J. D. *Parasitic Plants*; Chapman and Hall: London, U.K., 1995.

(3) Butler, L. G. Chemical communication between the parasitic weed *Striga* and its crop host—A new dimension in allelochemistry. In *Allelopathy: Organisms, Processes and Application*; Inderjit et al., Eds.; ACS Symposium Series 582; American Chemical Society: Washington, DC, 1995; pp 158−168.

(4) Lynn, D. G.; Chang, M. Phenolic signals in cohabitation: implications for plant development. *Annu. Rev. Plant Physiol.* **1990**, *41*, 497−526.

(5) Estabrook, E. M.; Yoder, J. I. Plant-plant communications: rhizosphere signaling between parasitic angiosperms and their hosts. *Plant Physiol.* **1998**, *116*, 1−7.

(6) Yokota, T.; Sakai, H.; Okuno, K.; Yoneyama, K.; Takeuchi, Y. Alectrol and Orobanchol, germination stimulants for *Orobanche minor*, from its host red clover. *Phytochemistry* **1998**, *49*, 1967−1973.

(7) Mori, K.; Matsui, J.; Yokota, T.; Sakai, H.; Bo, M.; Takeuchi, Y. Structure and Synthesis of Orobanchol, the Germination Stimulant for *Orobanche minor*. *Tetrahedron Lett.* **1999**, *40*, 943−946.

(8) Mangnus, E. M.; Dommerholt, F. J.; De Jong, R. L. P.; Zwanenburg, B. Improved synthesis of strigol analogue GR 24 and evaluation of the biological activity of its diastereomers. *J. Agric. Food Chem.* **1992**, *40*, 1230−1235.

(9) Nefkens, G. H. L.; Thuring, J. W. J. F.; Beenakkers, M. F. M.; Zwanenburg, B. Synthesis of phthaloylglycine-derived strigol analogue and its germination stimulatory activity towards seeds of the parasitic weeds *Striga hermonthica* and *Orobanche crenata*. *J. Agric. Food Chem.* **1997**, *45*, 2273−2277.

(10) Wigchert, S. C. M.; Kuiper, E.; Boelhouwer, G. J.; Nefkens, G. H. L.; Verkleif, J. A. C.; Zwanenburg, B. Dose−Response of Seeds of the Parasitic Weeds *Striga* and *Orobanche* toward the Synthetic Germination Stimulants GR24 and Nijmegen 1. *J. Agric. Food Chem.* **1999**, *47*, 1705−1710.

(11) Cubero, J. I.; Pieterse, A. H.; Khalil, S. A.; Sauerborn, J. Screening techniques and sources of resistance to parasitic angiosperms. *Euphytica* **1994**, *73*, 51−58.
(12) Jurado-Expósito, M.; García-Torres, L.; Castejón-Muñoz, M. Broad Bean and Lentil seed treatments with imidazolinones for the control of broomrape (*Orobanche crenata*). *J. Agric. Sci.* **1997**, *129*, 307−314.
(13) Qasem, J. R. Chemical Control of Branched Broomrape (*Orobanche ramosa*) in Glasshouse Grown Tomato. *Crop Prot.* **1998**, *17*, 625−630.
(14) Fischer, N. H.; Weidenhamer, J. D.; Riopel, J. L.; Quijano, L.; Menelaou, M. A. Stimulation of witchweed germination by sesquiterpene lactones: a structure−activity study. *Phytochemistry* **1990**, *29*, 2479−2483.
(15) Rugutt, J. K.; Rugutt, K. J. Stimulation of *Striga hermonthica* Seed Germination by 11β,13-Dihydroparthenolide *J. Agric. Food Chem.* **1997**, *45*, 4845−4849.
(16) Yoneyama, K.; Takeuchi, Y.; Ogasawara, M.; Konnai, M.; Sugimoto, Y.; Sassa, T. Cotylenins and Fusicoccins stimulate seed germination of *Striga hermonthica* (Del.) Benth and *Orobanche minor* Smith. *J. Agric. Food Chem.* **1998**, *46*, 1583−1586.
(17) Pérez de Luque, A.; Galindo, J. C. G.; Macías, F. A.; Jorrín, J. Sunflower sesquiterpene lactone models induce *Orobanche cumana* seed germination. *Phytochemistry* **2000**, *53*, 45−50.
(18) Mangnus, E. M.; Zwanenburg, B. Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. *J. Agric. Food Chem.* **1992**, *40*, 1066−1070.
(19) Macías, F. A.; Galindo, J. C. G.; Massanet, G. M. M. Potential allelopathic activity of several sesquiterpene lactone models. *Phytochemistry* **1992**, *21*, 1969−1977.
(20) Macías, F. A.; Galindo, J. C. G.; Velasco, R. F.; Castellano, D. Sesquiterpene Lactones with Potential Use as Natural Herbicide Models (II): Guaianolides. *J. Agric. Food Chem.* **2000**, *48*, 5288−5296.
(21) Macías, F. A.; Galindo, J. C. G.; Massanet, G. M.; Gómez-Madero, J. F.; Fronczek, F. R. Synthesis of 13-hydroxylated and 11,13-dihydroxylated sesquiterpene lactones: Michael addition of a nucleophilic hydroxyl group. *J. Nat. Prod.* **2001**, submitted for publication.
(22) Rodríguez, A. A. S.; García, M.; Rabi, J. Facile biomimetic synthesis of costunolide-1,10-epoxide, santamarin, and reynosin. *Phytochemistry* **1978**, *17*, 953−954.
(23) Macías, F. A.; Galindo, J. C. G.; Velasco, R. F.; Castellano, D. Sesquiterpene lactones with Potential Use as Natural Herbicide Models (I): *trans,trans*-Germacranolides. *J. Agric. Food Chem.* **1999**, *47*, 4407−4414.
(24) Serghini, K.; Perez de Luque, A.; Castejón-Muñoz, M.; García-Torres, L.; Jorrín, J. Sunflower response to broomrape parasitism: induced synthesis and excretion of 7-hydroxylated simple coumarins. *J. Exp. Bot.* **2001**, *52*, 2227−2234.
(25) MOPAC 7.5, J. J. P. Stewart and F. J. Seiler, 1990.
(26) Macías, F. A.; Molinillo, J. M. G.; Varela, R. M.; Torres, A.; Galindo, J. C. G. Bioactive compounds from the Genus *Helianthus*. In *Recent Advances in Allelopathy. Vol. I. A Science for the Future*; Macías, F. A., Galindo, J. C. G., Molinillo, J. M. G., Cutler, H. G., Eds.; Servicio de Publicaciones de la UCA: Cádiz, Spain, 1999; pp 121−148.
(27) Fischer, N. H.; Weidenhamer, J. D., J. D.; Bradow, J. M. Dihydroparthenolide and other sesquiterpene lactones stimulate witchweed germination. *Phytochemistry* **1989**, *28*, 2315−2317.
(28) Galindo, J. C. G.; Hernandez, A.; Dayan, F. E.; Tellez, M. R.; Macías, F. A.; Paul, R. N.; Duke, S. O. Dehydrozaluzanin C, a natural Sesquiterpenolide, causes rapid plasma membrane leakage. *Phytochemistry* **1999**, *52*, 805−813.
(29) Hejchman, E.; Haugwitz, R. D.; Cushman, M. Synthesis and Cytotoxicity of Water-Soluble Ambrosin Product Candidates. *J. Nat. Prod.* **1995**, *38*, 3407−3410.
(30) Matsui, J.; Yokota, T.; Bando, M.; Takeuchi, Y.; Mori, K. Synthesis and Structure of Orobanchol, the Germination Stimulant for *Orobanche minor*. *Eur. J. Org. Chem.* **1999**, *9*, 2201−2210.

Received for review August 10, 2001. Revised manuscript received December 31, 2001. Accepted January 1, 2002. This research was supported by the Dirección General de Investigación Científica y Técnica, Spain (DGICYT; Project PB98-0575, F.A.M., J.C.G.G.), Junta de Andalucía, and Universidad de Córdoba Ministerio de Ciencia y Tecnologia, Spain (AGL-2001-2420) (J.J.). J.C.G.G. acknowledges a fellowship from the University of Cádiz.

JF0110809