Natural and Synthetic Podolactones with Potential Use as Natural Herbicide Models†

Francisco A. Macías,*,‡ Ana M. Simonet,‡ Pedro C. Pacheco,‡ Alejandro F. Barrero,§ Eduardo Cabrera,§ and David Jiménez-González§

Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, C/República Saharaui s/n, Apartado 40, 11510 Puerto Real, Cádiz, Spain, and Departamento de Química Orgánica, Instituto de Biotecnología, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071 Granada, Spain

A collection of 11 natural and synthetic podolactones have been tested as allelochemicals in a range between 10^{-4} and 10^{-9} M, and their potential use as natural herbicide models is discussed. Their effects on the germination and growth of the dicots *Lactuca sativa* (cv. Nigra and cv. Roman), *Lepidium sativum*, and *Lycopersicon esculentum* and the monocots *Allium cepa*, *Hordeum vulgare*, and *Triticum aestivum* as standard target species have been studied. An important inhibitory effect on the germination and growth of all tested species (average = 90%) was produced by compounds **9**−**11** at 10^{-4} M. The specific structural requirements related to their activities are studied. On the basis of these results, their use as potential natural herbicide models is proposed.

Keywords: *Podolactones; diterpenes; allelopathy; natural herbicide models; Lactuca sativa; Lycopersicon esculentum; Lepidium sativum; Allium cepa; Hordeum vulgare; Triticum aestivum; Logran*

INTRODUCTION

Podolactones and other related compounds of natural origin present certain interesting biological properties such as termiticidal activity, effects on the development of housefly larvae, cytotoxicity, antileukemia activity, plant growth inhibitory activity, antifeedant activity, and antibiotic activities (Ellestad et al., 1970; Hayashi et al., 1979; Zhang et al., 1992). Nevertheless, their allelopathic activity has not been previously tested.

As part of our research on bioactive natural products (coumarins, terpenoids, flavonoids, and phenolics), we are conducting a systematic study of their activity as allelopathic agents (Macías et al., 1992, 1993, 1997, 1999b) and their potential use as natural herbicide models.

We present here the effects of a series of aqueous solutions in the range from 10^{-4} to 10^{-9} M of compounds of the podolactone family (Figure 1), the naturally occurring LL-Z12271α (**10**), isolated from *Acrostalagmus* species (Ellestad et al., 1970), and its analogues (**1**−**9** and **11**) on the germination and growth of *Lactuca sativa*, *Lepidium sativum*, and *Lycopersicon esculentum* (dicotyledon species) and *Allium cepa*, *Hordeum vulgare*, and *Triticum aestivum* (monocotyledon species) as standard target species (STS) (Macías et al., 2000).

A commercial herbicide has been included in the bioassay to serve as an internal standard and to evaluate the potential use of these natural and synthetic podolactones in comparison with the herbicides currently on the market and, consequently, to propose them as lead compounds.

EXPERIMENTAL METHODS

Tested Compounds. The synthesis of compounds **1**−**11** (Figure 1) is described in Barrero et al. (1999).

Bioassays. Seeds of *Lac. sativa* L. (lettuce) cv. Nigra and cv. Roman, *Lep. sativum* L. (cress), *Lyc. esculentum* L. (tomato), *A. cepa* L. (onion), *H. vulgare* L. (barley), and *T. aestivum* L. (wheat) were obtained from FITÓ, S.A. (Barcelona, Spain). All undersized and damaged seeds were discarded, and the assay seeds were selected for uniformity. Bioassays were carried out in Petri dishes of 90 mm diameter, with Whatman No. 1 filter paper as support. Aqueous solutions were buffered with 10 mM 2-[*N*-morpholino]ethanesulfonic acid (MES)/1 M NaOH. The pH values were measured with a Crison micropH 2001. Each concentration of each compound was tested by using 100 seeds (4 dishes × 25 seeds for smaller seeds and 10 dishes × 10 seeds for larger seeds). Seeds were incubated in the dark at 25 °C in a Memmert ICE 700 controlled chamber for 5 days except for *Lep. sativum* L. (3 days). Parallel controls were performed for each concentration.

Data are presented as percent differences from control in Figures 2−4, and a summary of their bioactivity is given in Table 1. Zero represents the control, positive values represent stimulation of the studied variable, and negative values represent inhibition.

Statistical Analysis. Welch's test with a significance of $P < 0.01$ was applied to ensure the absence of differences between two blocks. The mean values for every parameter (germination average and root and shoot elongation) and their population variance within each Petri dish were calculated (Macías et al., 2000).

RESULTS

It has been reported that the presence of an alkylating α,β-unsaturated carbonyl function is related to the

* Author to whom correspondence should be addressed (e-mail famacias@uca.es; telephone +34-956.016370; fax +34-956.016288).

† Part 12 in the series "Natural Products as Allelochemicals"; for part 11 see Macías et al. (1999b).

‡ Universidad de Cádiz.

§ Universidad de Granada.

10.1021/jf990321y CCC: $19.00
Published on Web 06/23/2000

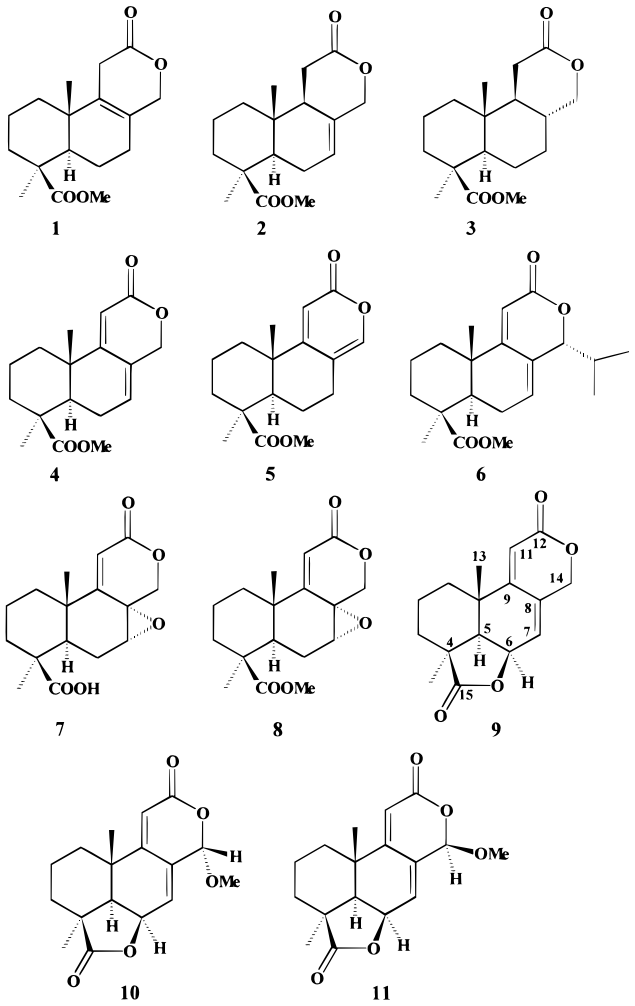

Figure 1. Natural and synthetic podolactones tested for allelopathic activity.

potential allelopathic activity of several compounds (Fischer, 1986). Macías et al. (1992) reported that the presence of an α-methylene-γ-lactone group in sesquiterpene lactones is related to the germination and growth activity of *Lac. sativa*, but this effect is strongly determined by conformational changes. Otherwise, the presence of an α,β-unsaturated δ-lactone moiety integrated in an aromatic system (coumarins) is related to a significant inhibition of germination and development of *Lac. sativa* only at concentrations $>10^{-4}$ M (Macías et al., 1993).

In this study we have evaluated the phytotoxicity of 11 podolactone-related compounds, some of them having an α,β-saturated or unsaturated δ-lactone ring (**1**−**3**, **7**, and **8**), others having an α,β,γ,δ-unsaturated δ-lactone ring (**4**−**6** and **9**−**11**), whereas some (**9**−**11**) have an additional γ-lactone ring (Figure 1). Our objective was to establish the structural requirements needed for this bioactivity and, consequently, the possibilities for proposing them as natural herbicide models.

Germination and Growth Effects. The most relevant effect observed was a strong inhibition of germination and growth (∼−90%) over all tested species produced by compounds **9**−**11** at 10^{-4} M.

Lac. sativa cv. Nigra and cv. Roman (Figure 2; Table 1). The germination is greatly inhibited by compounds **10** and **11** at 10^{-4} M, **11** being the more potent on the germination of cv. Roman (−83%, 10^{-4} M). The growth of both lettuce varieties is also strongly inhibited by

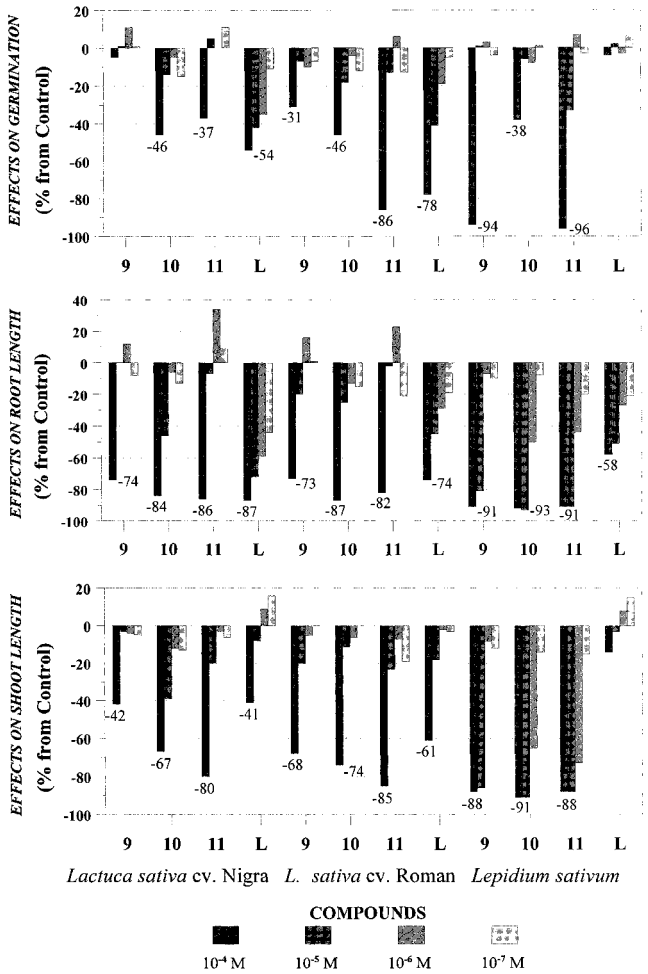

Figure 2. Effects of compounds **9**−**11** in comparison with Logran (L) on the germination and root and shoot lengths of *Lac. sativa* cv. Nigra, *Lac. sativa* cv. Roman, and *Lep. sativum*. Main values are presented as percentage differences from the control (e.g., +16% means 116% compared with the control): *, $0.01 < P < 0.05$; other values, $P < 0.01$.

9−**11**. (Cv. Nigra: shoot, **9**, −42%; 10^{-4} M; **11**, −80%, 10^{-4} M. Cv. Roman: root, **9**, −73%, 10^{-4} M; **11**, − 82%, 10^{-4} M.) The overall effect of the other compounds exhibits <20% difference from control and can be considered relatively inactive in comparison with these compounds.

A very strong inhibitory effect can be observed for *Lep. sativum* (Figure 2; Table 1) by **9**−**11** at 10^{-4} M (**11**: germination, −96%; root, −91%; shoot, −88%) as well as for *Lyc. esculentum* (Figure 3; Table 1). Those compounds maintain this effect at 10^{-5} M. Indeed, compounds **10** and **11** are still able to strongly inhibit the growth of *Lac. sativum* at 10^{-6} M (**11**: root, −44%; shoot, −73%) and of *Lyc. esculentum* at 10^{-4}−10^{-5} M (**9**: root, −90%, 10^{-4} M; −77%, 10^{-5} M); compound **11** showed a strong activity even at 10^{-6} M (root, −45%; shoot, − 44%). The overall germination effect of the other compounds shows <10% difference from control for *Lac. sativum*, and the growth is moderately inhibited (∼20%), whereas compounds **1**−**3**, **5**, and **6** stimulate the tomato root growth (**1**, +56%, 10^{-9} M; **5**, +58%, 10^{-7} M; **3**, +60%, 10^{-8} M).

The inhibition effect produced by compounds **10** and **11** over germination of *A. cepa*, *T. aestivum* at 10^{-4} M (Figure 3; Table 1), and *H. vulgare* (Figure 4; Table 1) is a very strong inhibition (**11**, *A. cepa*, −85%; *T.*

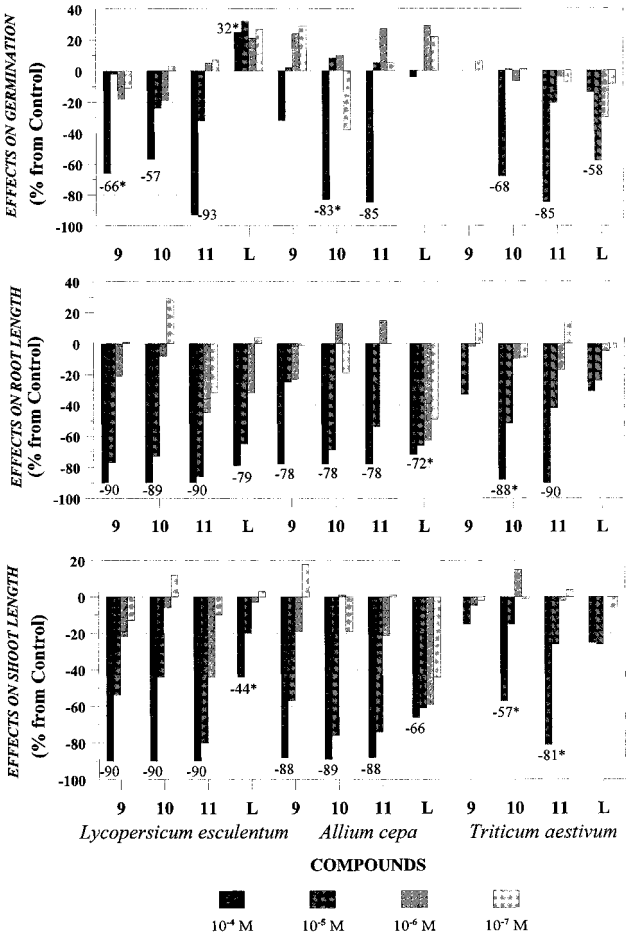

Figure 3. Effects of compounds **9**−**11** in comparison with Logran (L) on the germination and root and shoot lengths of *Lyc. esculentum*, *A. cepa*, and *T. aestivum*. Main values are presented as percentage differences from the control: *, $0.01 < P < 0.05$; other values, $P < 0.01$.

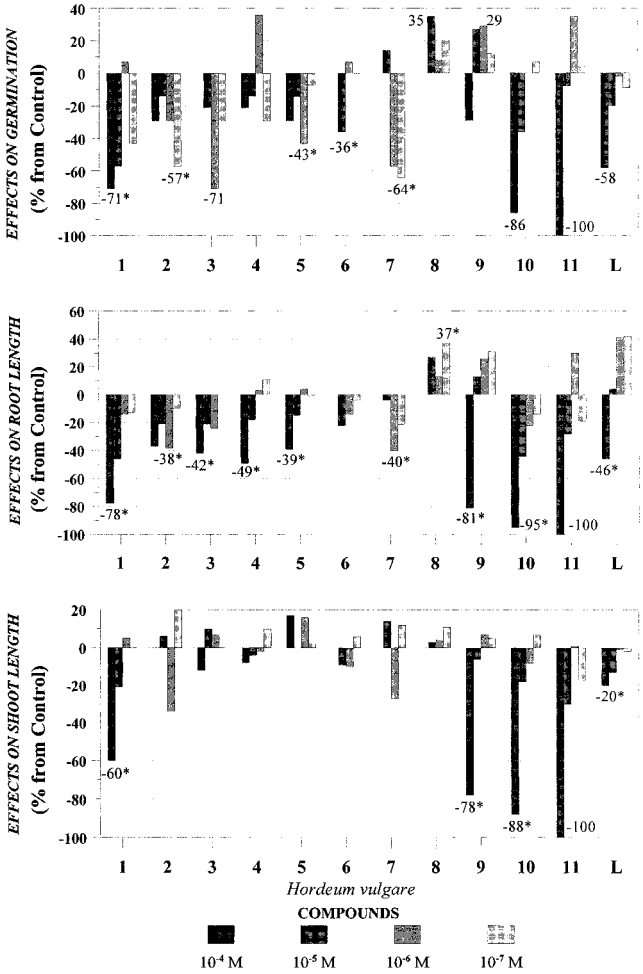

Figure 4. Effects of compounds **1**−**11** in comparison with Logran (L) on the germination and root and shoot lengths of *H. vulgare*. Main values are presented as percentage differences from the control: *, $0.01 < P < 0.05$; other values, $P < 0.01$.

aestivum, −85%; *H. vulgare*, −100%, 10^{-4} M), whereas **1**, **3**, and **9** show strong inhibition (**1**, −45%, 10^{-8} M; **3**, −46%, 10^{-6} M) of *A. cepa*. Compounds **1**−**3** and **7** also inhibit germination of *H. vulgare* (**1**, −71%, 10^{-6} M; **3**, −71%, 10^{-4} M; **7**, −72%, 10^{-8} M) and can be considered inactive for *T. aestivum*.

Growth of monocots is strongly inhibited at 10^{-4} and 10^{-5} M by compounds **9**−**11.** (**11**: *A. cepa*, root, −78%, 10^{-4} M; −54%, 10^{-5} M; shoot, −88%, 10^{-4} M, −74%, 10^{-5} M. **11**: *T. aestivum*, root, −90%, 10^{-4} M; −42%, 10^{-5} M; shoot, −81%, 10^{-4} M, dropping off with the dilution. **11**: *H. vulgare*, root and shoot, −100%, 10^{-4} M; the activity is, in general, not significant at lower concentrations.) Compounds **1**−**4** and **6** show a moderate inhibitory activity over *A. cepa* (average = −30%) and a strong inhibition of *H. vulgare* by compound **3** (at 10^{-4} M: root, −79%; shoot, −60%). The rest of the compounds can be considered inactive over *T. aestivum*.

DISCUSSION

Structure−**Activity Relationship.** The tested molecules can be divided according to their structural features into four groups. Those compounds with an α,β-saturated or an unsaturated δ-lactone ring (**1**−**3**), with an epoxide functionalization (**7** and **8**), or having an α,β,γ,δ-unsaturated δ-lactone ring (**4**−**6**) are generally inactive. The remaining group, compounds **9**−**11** having a 7,9-diene-12,14-δ-lactone and a 6,15-γ-lactone, are in general very active. The presence of a 7,9-diene-12,14-δ-lactone functional group in compounds **9**−**11** cannot be considered the only factor responsible for this activity because this functionalization is also present on inactive compounds **4**−**6**. The key difference between active and nonactive compounds comes from the presence or not of a second γ-lactone ring located between C-4 and C-6. This ring fixes a specific conformation between the A and B rings of compounds **9**−**11**, as shown in Figure 5 for compound **11**. Also, the presence of a methoxy group at C-14 enhances the inhibitory activity (**10** and **11**) at low concentrations (*Lep. sativum*, shoot, 10^{-6} M; average, −70%), this effect being even more pronounced when the methoxy group is β-oriented. This observation may be related to the possibility of this part of the molecule reacting by opening this special δ-lactone ring.

Comparison with the Commercial Herbicide Logran. The use of a commercial herbicide, Logran (terbutryn plus triasulfuron, pre- and postherbicide, L in Figures 2−4 and Table 1) as positive control is common in other biological studies and has been recently proposed for bioassays of phytotoxicity (Macías et al., 2000). The use of an internal standard allows comparison of the results obtained from a certain compound with those from a commercial product and also comparison of results of different bioassays.

It is important to note that compounds **9**−**11** present the same or higher activity as Logran in both lettuces

Table 1. Summary of the Bioactivity Data of Compounds 1−11 and Logran[a]

		lettuce cv. Nigra	lettuce cv. Roman	cress	tomato	onion	wheat	barley
Logran	germination	=	=	0	+	+	=	=
	root length	=	=	=	=	=	−	=
	shoot length	=	=	(−)	=	=	−	−
1	germination	0	(+)	0	(−)	−	0	=
	root length	0	0	(−)	++	0	0	=
	shoot length	(−)	(−)	(−)	(+)	(−)	0	=
2	germination	(−)	(+)	0	(+)	−	(−)	=
	root length	(−)	(−)	0	++	0	0	−
	shoot length	(−)	(−)	(−)	+	(−)	0	0
3	germination	−	0	0	0	−	0	=
	root length	(−)	(+)	(−)	++	(−)	(−)	=
	shoot length	−	−	−	−	−	0	0
4	germination	−	(−)	0	0	(−)	0	−
	root length	(−)	0	−	0	−	0	=
	shoot length	(−)	(−)	−	0	−	0	0
5	germination	(−)	0	0	0	(−)	(−)	−
	root length	0	0	−	++	0	(−)	−
	shoot length	(−)	0	(−)	(+)	(−)	0	(+)
6	germination	(−)	(−)	0	(+)	−	0	−
	root length	0	(+)	−	++	−	(−)	(−)
	shoot length	(−)	(−)	(−)	(+)	−	0	0
7	germination	(−)	(−)	0	0	0	0	=
	root length	(−)	0	(−)	+	0	0	−
	shoot length	(−)	(+)	−	(+)	(−)	0	0
8	germination	0	(−)	0	−	(+)	0	+
	root length	0	0	0	(−)	(+)	+	+
	shoot length	0	(+)	0	−	(−)	0	(+)
9	germination	0	−	=	=	−	0	+
	root length	=	=	=	=	=	−	=
	shoot length	=	=	=	=	=	(−)	=
10	germination	=	=	−	=	=	=	=
	root length	=	=	=	=	=	=	=
	shoot length	=	=	=	=	=	=	=
11	germination	−	=	=	=	=	=	=
	root length	=	=	=	=	=	=	=
	shoot length	=	=	=	=	=	=	=

[a] 0, not active; (+) and (−), stimulatory or inhibitory values <20%; + and −, stimulatory or inhibitory values between 20 and 40%; ++ and =, stimulatory or inhibitory values >40%.

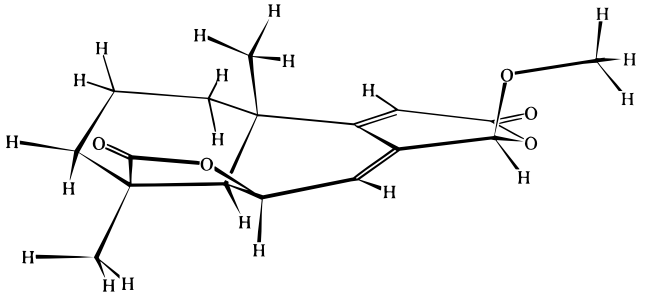

Figure 5. More stable conformer of **11** found by using PM3 calculation.

and tomato (Figures 2 and 3; Table 1), whereas cress is much more affected (Figure 3; Table 1). With regard to monocots, differences between Logran and compounds **9**−**11** are much more striking, especially in wheat and barley (*H. vulgare*, **11**: germination, shoot, and root, 10^{-4} M, −100%), these compounds presenting higher activities than Logran. They also present good dose−response curves, maintaining their activity even at 10^{-6} M in cress and tomato, where root is almost totally inhibited at 10^{-5} M.

On the basis of these results, compounds **9**−**11** are good candidates as herbicide templates with potential use as leads for a new generation of natural agrochemicals.

ACKNOWLEDGMENT

We thank FITÓ S.A. for providing seeds for the bioassay.

LITERATURE CITED

Barrero, A. F.; Sánchez, J. F.; Elmerabet, J.; Jiménez-González, D.; Macías, F. A.; Simonet, A. M. Enantioespecific syntheses of the potent bioactives Nagilactone F and of the mould metabolite LL-Z1271α. An evaluation of their allelopathic potential. *Tetrahedron* **1999**, *55*, 7289−7304.

Ellestad, G. A.; Evans, R. H.; Kunstmann, M. P.; Lancaster, J. E.; Morton, G. O. Structure and chemistry of antibiotic LL-Z1271α, an antifungal Carbon-17 terpene. *J. Am. Chem. Soc.* **1970**, *92*, 5483−5489.

Fischer, N. H. *The Science of Allelopathy*; Putman, A. R., Tang, C. S., Eds.; Wiley: New York, 1986.

Hayashi, Y.; Matsumoto, T.; Tashiro, T. Antitumor activity of norditerpenoid dilactones in *Podocarpus* plants: structure−activity relationship on in vitro cytotoxicity against Yoshida sarcoma. *Gann* **1979**, *70*, 365−369.

Macías, F. A.; Galindo, J. C. G.; Massanet, G. M. Potential allelopathic activity of several sesquiterpene lactone models. *Phytochemistry* **1992**, *31*, 1969−1977.

Macías, F. A.; Galindo, J. C. G.; Massanet, G. M.; Rodríguez-Luis, F.; Zubía, E. Allelochemicals from *Pilocarpus goudotianus* leaves. *J. Chem. Ecol.* **1993**, *19*, 1371−1379.

Macías, F. A.; Simonet, A. M.; Galindo, J. C. G. Bioactive steroids and triterpenes from *Melilotus messanensis* and their allelopathic potential. *J. Chem. Ecol.* **1997**, *23*, 1781−1803.

Macías, F. A.; Simonet, A. M.; Galindo, J. C. G.; Castellano, D. Bioactive phenolics and polar compounds from *Melilotus messanensis*. *Phytochemistry* **1999a**, *50*, 35−46.

Macías, F. A.; Galindo, Juan C. G.; Castellano, D.; Velasco, Raúl F. Sesquiterpene lactones with potential use as natural herbicide models (I): *trans-trans* germacranolides. *J. Agric. Food Chem.* **1999b**, *47*, 4407−4414.

Macías, F. A.; Castellano, D.; Molinillo, J. M. G. In the search for a standard bioassay for allelopathic studies of phytotoxicity. Selection of standard target species (STS). *J. Agric. Food Chem.* **2000**, *48*, 2512−2521.

Zhang, M.; Ying, B. P.; Kubo, I. Nagilactones from *Podocarpus nagi* and their effects on the feeding and growth of tobacco budworm. *J. Nat. Prod.* **1992**, *55*, 1057−1062.

Received for review April 5, 1999. Revised manuscript received February 11, 2000. Accepted April 26, 2000. This research is supported by the Dirección General de Investigación Científica y Técnica, Spain (DGICYT; Projects PB-95-1192 and PB95-1231). We thank the Spanish Ministerio de Educación y Cultura for the grant provided to D.J.G.

JF990321Y