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POTENTIAL ALLELOPATHIC ACTIVITY OF NATURAL PLANT HELIANNANES: A PROPOSAL OF ABSOLUTE CONFIGURATION AND NOMENCLATURE[1]

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Abstract—Proposals for the biogenesis and absolute configuration of 12 heliannanes (based on modified Mosher methodology) are presented. The proposal of biogenesis is discussed for both possible origins—terrestrial or marine. Due to the different skeleton types that have been found, a systematic nomenclature based on the different heterocycle ring size is proposed. Phytotoxicity of compounds **5–11** suggests that these new bioactive sesquiterpenes may be involved in sunflower defense against dicotyledon species, and they could be used as natural herbicide templates.

Key Words—Allelopathy, heliannuol, heliannane, standard target species, *Lactuca sativa*, *Lepidium sativum*, *Allium cepa*, *Hordeum vulgare*, *Helianthus annuus.*

INTRODUCTION

Eleven compounds (**1**–**11**) from the novel sesquiterpene family heliannane have been isolated from *Helianthus annuus* (Macías et al., 1993, 1994, 1999a,b), while the simplest member (**12**) of this class of 7,11-heliannane has been obtained from the Indo-Pacific sponge *Haliclona fascigera* (Harrison et al., 1997) (Figure 1). These compounds have a benzenoid moiety fused to an eight-membered ether

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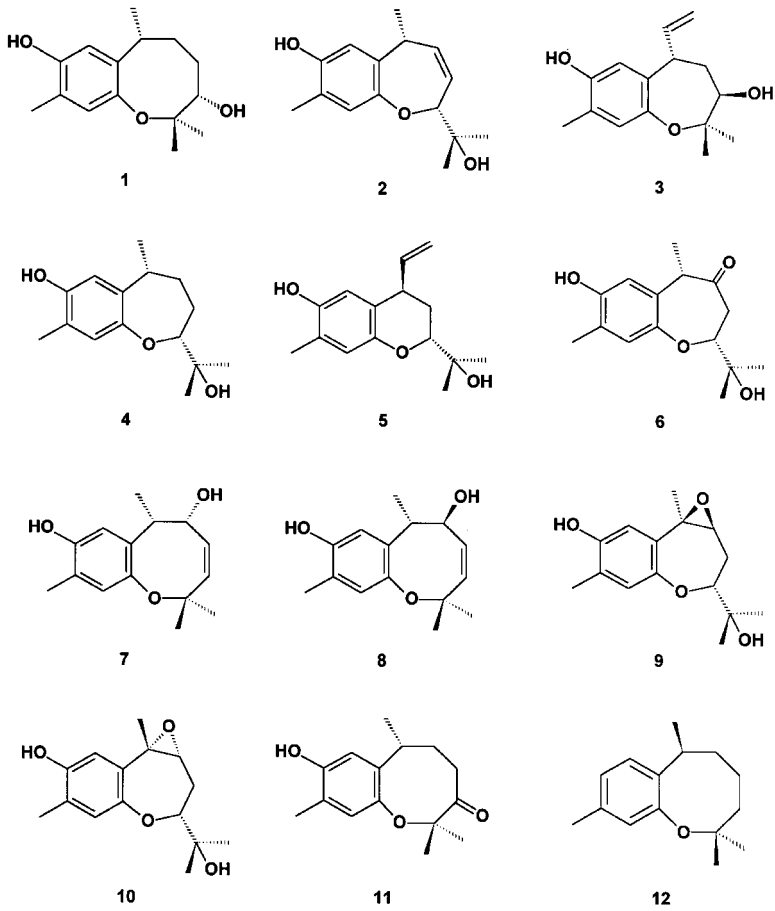

FIG. 1. Isolated heliannanes from *Helianthus annuus* and *Haliclona fascigera.*

ring. Their first synthesis was published recently by connecting directed ortho metalation and olefin metathesis strategies (Stefinovic and Snieckus, 1998). The total synthesis of (±)-heliannuol A (**1**), via intramolecular Julia coupling and intramolecular sulfone ester cyclization, also has been published (Grimm et al., 1994). A general approach to the stereospecific synthesis of this interesting skeleton is in progress in order to obtain information on the structural requirements necessary for bioactivity.

Due to the large number of possible structures that have been found, and in order to establish a clear criterion that can provide single nomenclature rules for future work in this field, a systematic nomenclature based on a bisabolene-

type precursor is proposed. Absolute configuration has been established by using the modified Mosher methodology (Dale and Mosher, 1973; Shi et al., 1998; Ohtani et al., 1989), and it has been corroborated by the asymmetric synthesis of heliannuols (−) D and (+) A (Shishido, 1999).

In order to evaluate the potential phytotoxic allelopathic activity, and consequently the potential use as natural herbicide templates of the isolated heliannuols, we have studied the effect of a series of aqueous solutions at 10^{-3}–10^{-9} M on the root and shoot lengths of *Lactuca sativa* cv. *nigra* and *Lepidium sativum* seedlings (dicotyledons) and *Allium cepa* and *Hordeum vulgare* (monocotyledons) as standard target species (STS) (Macías et al., 1999c, 2000).

METHODS AND MATERIALS

^{1}H NMR spectra were made at 399.952 MHz on a Varian Unity-400 spectrometer with $CDCl_3$ as solvent.

Plant Material Collection, Extraction, and Isolation Procedures. Leaves of *H. annuus* L. cv. SH-222 commercialized by Semillas Pacifico and cv. VYP commercialized by Koype (Spain) were collected in August 1991 and September 1990, respectively, during the third plant development stage (1.2-m-tall flowering plants, one month before harvest). Plant material was provided by Rancho de la Merced, Agricultural Research Station, Junta de Andalucía, Jerez, Spain (Macías et al., 1999d).

*Synthesis of (R)-MTPA ester (**1a**).* Compound **1** (3 mg) was treated with CH_2Cl_2 solutions of dicyclohexylcarbodiimide (13 mg in 0.6 ml), *N*,*N*-dimethylaminopyridine (1.5 mg in 0.25 ml) and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (12 mg in 0.5 ml), and the mixture was stirred at room temperature for 24 hr. After evaporation of the solvent under reduced pressure, the residue was chromatographed by HPLC with an analytical silica gel column (5 μm) and hexane–EtOAc (9 : 1) as eluent, 1 ml/min flow rate, and UV detector. This process afforded 1.5 mg of the (*R*)-MTPA ester **1a**; ^{1}H NMR ($CDCl_3$, −10°C, selected data) δ 3.36 (ddq, 1H, *J* = 7.0, 7.0, 1.0 Hz, H-7β), 2.11 (dddd, 1H, *J* = 12.5, 7.5, 7.0, 1.5 Hz, H-8α), 1.16 (dddd, 1H, *J* = 12.5, 8.0, 1.0, 1.0 Hz, H-8β), 1.48 (dddd, 1H, *J* = 13.2, 8.0, 7.4, 1.5 Hz, H-9α), 1.39 (dddd, 1H, *J* = 13.2, 7.5, 1.0, 1.0 Hz, H-9β), 4.92 (dd, 1H, *J* = 7.4, 1.0 Hz, H-10β), 1.30 (s, 3H, H-12), 1.22 (s, 3H, H-13).

*Synthesis of the (*S*)-MTPA Ester (**1b**).* Treatment of **1** (3.5 mg) with CH_2Cl_2 solutions of dicyclohexylcarbodiimide (15 mg in 0.6 ml), *N*,*N*-dimethylaminopyridine (1.75 mg in 0.25 ml), and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (14 mg in 0.5 ml), as described above, yielded the (*S*)-MTPA ester **1b** (1.6 mg); ^{1}H NMR ($CDCl_3$, −10°C, selected data) δ 3.35 (ddq, 1H, *J* = 7.0, 7.0, 1.0 Hz, H-7β), 2.11 (dddd, 1H, *J* = 12.6, 7.4, 7.2, 1.5 Hz,

H-8α), 1.14 (dddd, 1H, J = 12.6, 7.9, 1.1, 1.0 Hz, H-8β), 1.45 (dddd, 1H, J = 13.2, 7.9, 7.4, 1.5 Hz, H-9α), 1.34 (dddd, 1H, J = 13.2, 7.4, 1.1, 1.0 Hz, H-9β), 4.91 (dd, 1H, J = 7.4, 1.0 Hz, H-10β), 1.39 (s, 3H, H-12), 1.17 (s, 3H, H-13).

Lettuce, Cress, Onion, and Barley Seed Germination Bioassay. Seeds of lettuce (*Lactuca sativa* L. cv. Nigra) and barley (*Hordeum vulgar* L.) were obtained from Rancho La Merced, Junta de Andalucía Jerez, Spain. Seeds of cress (*Lepidium sativum*) and onion (*Allium cepa*) were obtained from Fitó S.L. Assay seeds were selected for uniformity of size and all undersized and damaged seeds were discarded.

The bioassay consisted of germinating 25 seeds for five days (three for germination and two for root and shoot growth) for lettuce and onion, three days (one for germination and two for root and shoot growth) for cress, in the dark at 25°C, in 9-cm plastic Petri dishes containing a 10-cm sheet of Whatman No. 1 filter paper and 10 ml of a test or control solution, except for barley (5 ml). In the case of barley, five seeds were used for four days.

Stock solutions at 10^{-4} M were prepared for each compound. The rest of the test solutions (10^{-5}–10^{-9} M) were obtained by successive dilution. Parallel controls consisted of deionized H_2O.

There were three replicates, except for barley (19 replicates), of each treatment, and parallel controls. The number of seeds per replicate, time, and temperature of germination were chosen in agreement with a number of preliminary experiments that varied the number of seeds, volume of test solution per dish, and the incubation period. All pH values were adjusted to 6.0 before the bioassay by using MES (2-[*N*-morpholino]ethanesulfonic acid, 10 mM) (Macías et al., 2000).

Data are presented as percentage differences from control in Figure 6 below and Tables 1 and 2. Thus, zero represents the control, positive values represent stimulation of the studied parameter, and negative values represent inhibition.

Statistical Treatment. The germination and root and shoot length values were tested by Welch's test; differences between the experimental and the control were highly significant with a value of $P \leq 0.01$.

RESULTS

Relationsihps Between Possible Biogenesis Routes for Heliannanes and Proposed Absolute Stereochemistry. A common γ-bisabolene precursor for both terrestrial and marine heliannuol sesquiterpenes has been proposed (Macías et al., 1994; Harrison and Crews, 1997) (Figure 2). If we consider that from marine sources the initial chirality that arises during the conversion of γ-bisabolene to its aromatic derivatives is maintained until the final cyclization, a provisional assignment of 7*R* stereochemistry in (+)-helianane (**12**) might be appropriate.

TABLE 1. GERMINATION AND GROWTH ACTIVITY OF COMPOUNDS **5–11** AND LOGRAN (L) ON DICOTYLEDON SPECIES[a]

	Germination (% difference from control)						Radical length (% difference from control)						Shoot length (% difference from control)					
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M
Lactuca sativa																		
5	−15	−1	−2	−3	−7	−8	15	15	9	16	33[b]	22	−9	−9	−16	0	−6	−9
6		−18	−20	−18	−73[b]	−23[c]		5	3	4	31[b]	24[b]		5	−1	−1	29[c]	−10
7		−4	−18	−7	−12	−16		−2	3	16	17	2		−3	−3	1	−1	−6
8	−7[a]	−36	−47[b]	−43	−14	−20	37[b]	17	22[c]	16	10	7	−13	−7	7	4	6	6
9	−23	−70	−62	−52	−28	−24	14	3	5	8	1	16	−7	5	13	5	0	−9
11		−28	−71	−80	−30	−36		−3	27	−6	−1	6		2	11	2	8	11
L	−86[a]	−64[a]	−63	−1	0	0	−57[b]	−42[b]	−17	4	13[c]	9	−38[c]	−30[c]	5	6	2	9
Lepidium sativum																		
5	2	−1	−8	−4	−11	−1	−7	−6	−7	−8	−11	0	1	−10[b]	−3	−6	−3	9[c]
6		−6[c]	−1[b]	−3[c]	−8[c]	−1[b]		−26[c]	−29[b]	−23[c]	−16[c]	−18[b]		−6	−13[c]	−16[c]	−18	−13[c]
7		−7	−4	−1[c]	0	−4		−7	−15	−14[c]	−7	10		−23[b]	−16[c]	−16[b]	−8	−6
8		−2	−1	0	−3	0		5	−8	−10	−11	−21		6	−5	−7	6	−4
9		−4	0	1	−4	0		−2	−10	−17	−13	−6		−5	2	−10	−18	2
11		−14[b]	−6	−9[c]	−12[c]	−2[b]		−31[b]	−5	−17[c]	−32[c]	−23[b]		−14[b]	−12	−18[c]	−24[b]	−4
L	−2[b]	−3[b]	−1[b]	−3	1	−2	−57[b]	−50[b]	−35[b]	−2	17	4	−25[a]	−8[c]	−12[c]	−6	1	−5

[a]Values are expressed as percentage different from the control and are not significantly different at $P > 0.05$ for Welch's test.
[b]Values significant at $P < 0.01$.
[c]Values significant at $0.01 < P < 0.05$.

TABLE 2. GERMINATION AND GROWTH ACTIVITY OF COMPOUNDS **5–11** AND LOGRAN (L) ON MONOCOTYLEDON SPECIES[a]

	Germination (% difference from control)						Radical length (% difference from control)						Shoot length (% difference from control)					
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M	10^{-9} M
Hordeum vulgare																		
5		3	−5	−8	11	−3		10	−7	1	23	25		27	9	6	18	13
6		21	20	3	5	−8		22	24	19	24	20		22	22	19	29	23
7		9	14	−2	−8	−6		36	45	54	33	52		22	38	44	35	16
8		6	11	0	3	−12		31	28	29	−8	20		24	25	21	8	14
9		−2	−5	0	18	−2		36	36	34	34	38		19	10	7	18	19
11		3	8	14	−2	2		32	45	32	8	48		20	28	17	21	27
L	−65	−8	2	7	−35[c]	17	−47[c]	1	25	1	−4	−7	−19	17	21	9	2	4
Allium cepa																		
5	−25	−30	−18	−23	−4[c]	−21[c]	55	35	41	21	60	43[c]	3	−1	11	10	23[b]	4
6		−7[a]	−7	−9[c]	−9	−16		56[c]	1	34[c]	5	6		18[c]	−12	1	−3	−6
7		−32	−16	−13	−16	−27		68	31	33	30	25		19	−4	2	11	−2
8		0	14[b]	−13	0.25	−5		19	30[c]	8	46[b]	11		2	32[b]	−7	32[a]	−8
9		−27	−13	−7	−14	−21		27	30	53	28	8		0	6	17	2	−9
11		0	−23	−30	−36	−13		−5	−9	−29	8	20		3	−22	−19	8	13
L	5[a]	0.29	9[c]	6	−6	−4	−72[b]	−61[b]	−39[c]	−4	−2	−14	−50[b]	−45[b]	−17[c]	0	−8	−12

[a] Values are expressed as percentage different from the control and are not significantly different at $P > 0.05$ for Welch's test.
[b] Values significant at $P < 0.01$.
[c] Values significant at $0.01 < P < 0.05$.

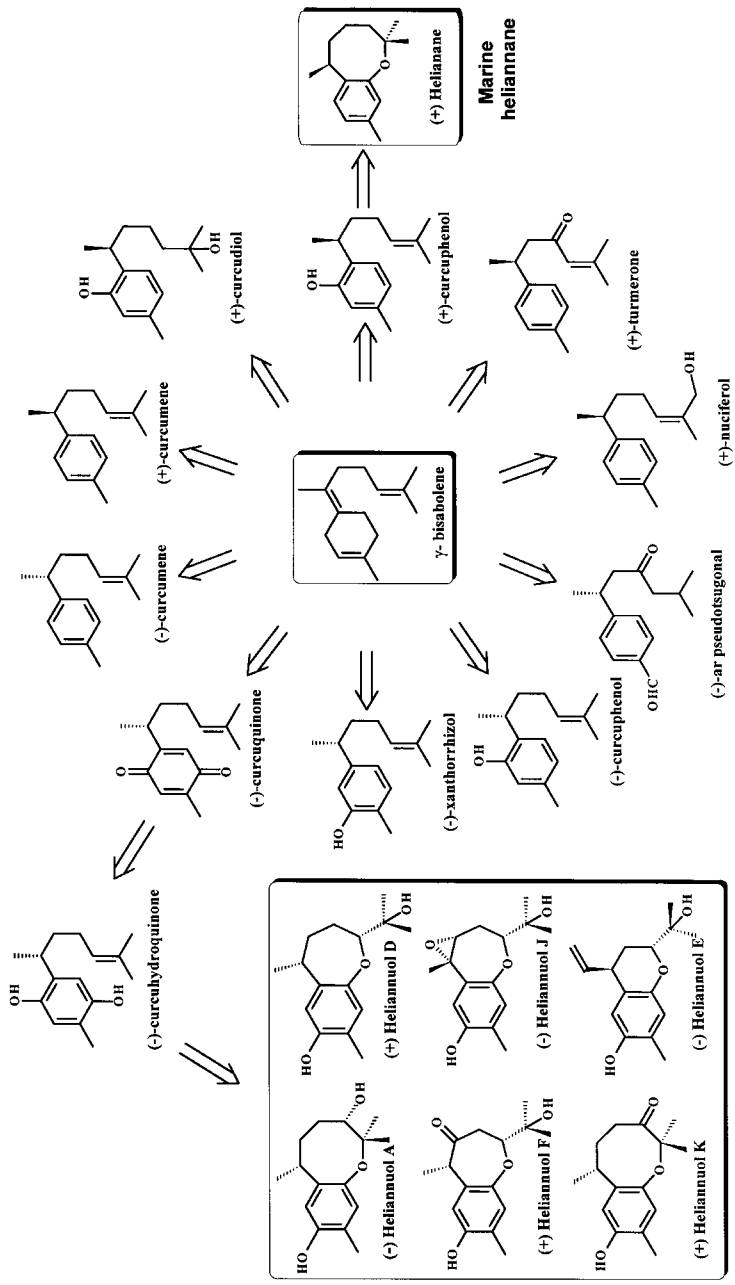

FIG. 2. Probable biogenetic hypothesis for terrestrial plants and marine heliannanes.

However, in terrestrial heliannuols, only (+)-heliannuol K (**11**) has a unique chiral center located at C-7, while the others have at least two. For this reason, it is not possible to assign an absolute stereochemistry based on on biogenetic consideration of the C-7 chiral center formed during conversion to aromatic derivatives. At this point, it is necessary to consider the size of the heterocycle ring and the nature and number of chiral centers.

It is interesting to note that (−)-heliannuol A (**1**) and (+)-heliannuol D (**4**), with structures secured by X-ray analysis, have a common biogenetic precursor (Macías et al., 1993, 1994), different relative stereochemistries ($7S^*$,$10R^*$ for **1** and $7S^*$,$10S^*$ for **4**), and have opposite $[\alpha]_D$ signs, due perhaps to the different nature of the C-10 chiral center.

In view of the information given above, it is not possible to extend the absolute assignation for the whole family of heliannuols, nor for the first four members, heliannuols A–D, based on probable precursors, as has been previously proposed (Harrison and Crews, 1997). This is only applicable for helianane, which has a single chiral center at C-7 and no other functionalization. In all other cases, it is necessary to establish additional bases for absolute assignments.

The analysis of the probable precursors of heliannanes with an established absolute configuration (Figure 2) shows a clear relationship between the observed C_7-Me, and the sign of $[\alpha]$. A positive value can be related to a β-orientation, present in metabolites isolated from terrestrial plants and sponges, whereas a negative value can be related with an α-orientation, present in metabolites isolated from terrestrial plants and soft corals. At least in marine organisms, the chiral center at C_7 emerges along two antipodal directions, one in marine soft coral compounds as 7*S* and another in marine sponge metabolites as 7*R*.

In order to establish the absolute configuration of heliannanes, we used the modified Mosher methodology (Dale and Mosher, 1973; Shi et al., 1998; Ohtani et al., 1989) by carrying out the synthesis of the (*R*)-(−)- and (*S*)-(+)- α-methoxy-α-(trifluoromethyl)phenylacetyl esters of heliannuol A (**1**). Heliannuol A is the most abundant, has a secondary alcohol, and can be easily correlated with other members of the family.

The method requires the assignments of as many proton signals as possible of (*R*) and (*S*)-MTPA esters, obtaining the $\Delta\delta$ (*R*-*S*) values for the protons. Afterwards, protons with positive $\Delta\delta$ should be placed on the right-hand side, and those with negative $\Delta\delta$ on the left-hand side of model A (Figure 3). Finally, PM3 calculations (Stewart, 1989) were used to build a molecular model for the most stable conformer of **1a** and to confirm that all the assigned protons with positive and negative $\Delta\delta$ values are actually found on the right and left sides of the MTPA plane, respectively. Model A thus shows that the correct absolute configuration of compound **1** is 7*R*,10*S*. Following their biogenetic origin (Figure 2), a 7*S*,10*R* absolute stereochemistry for (+)-heliannuol F (**6**), and 7*R*,10*R*

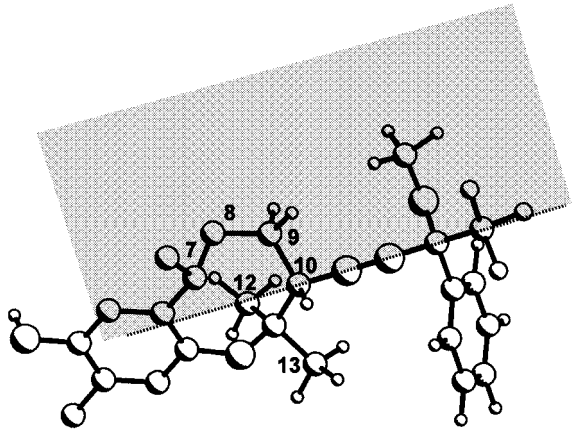

FIG. 3. Model A has been used to establish the absolute configuration of heliannuol A (1) following the modified Mosher methodology for the most stable conformer of their *R*-MTPA ester of heliannuol A (1a) using PM3 calculations.

for (−)-heliannuol B (**2**), 7*R*,8*S*,10*R* for (−)-heliannuol İ (**9**), and 7*S*,8*R*,10*R*) for (−)-heliannuol J (**10**), 7*S*,8*S* stereochemistry for (−)-heliannuol H (**8**), 7*S*,8*R* stereochemistry for (−)-heliannuol G (**7**), 8*S*,10*R* for (−)-heliannuol E (**5**), and 7*R* for (+)-heliannuol K (**11**) might be appropriate. This correlation could not be applied in the case of heliannuol C (**3**) due to the lack of any prefixed chiral center.

Recently, the absolute stereochemistry of (+)-heliannuol A has been established as 7*S*,10*R* and (−)-heliannuol D as 7*S*,10*S* by carrying out their total asymmetric syntheses (Shishido, 1999). These results were shown to be absolutely identical to the natural heliannuols, except for their $[\alpha]_D$. Consequently, the absolute configuration for these two natural heliannuols has been established as 7*R*,10*S* for (−)-heliannuol A (**1**) and 7*R*,10*R* for (+)-heliannuol D (**4**), and corroborates our assignments.

Systematic Nomenclature Proposal. Due to the different skeleton types that have been found, a systematic nomenclature based on the different heterocycle ring sizes is proposed. Members of the heliannane family have been published with four different basic structures. Two different numbering systems have been used, options A and B (Figure 4) (Macías et al., 1993, 1994; Harrison and Crews, 1997). If we consider the possible biogenetic origin from a bisabolane type precursor, it seems more appropriate to use option **A** with numbering as indicated in Figure 4, in accordance with that used for the basic skeleton of bisabolane. (Atta-ur-Rahman and Ahmad, 1992).

Since the terminal methyl groups can rarely be differentiated and the methyl

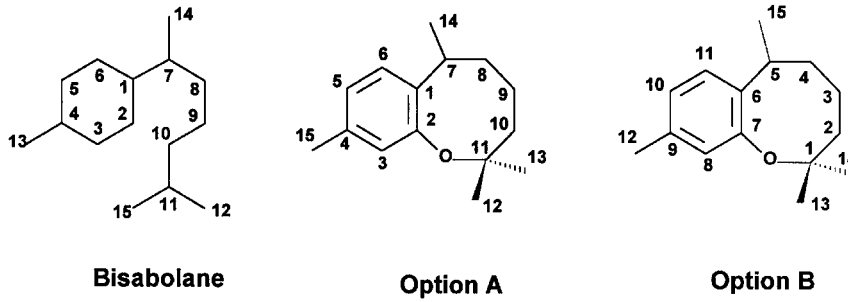

FIG. 4. Options of numbering system for heliannanes based on their biogenetic bisabolane precursor.

group located on the aromatic ring appears well differentiated in the spectroscopy of these compounds, the numbers for carbons 13 and 15 have been interchanged. For these reasons, we assign numbers 12 and 13 to the terminal methyl groups and 15 to the carbon bonded to the benzenic ring. The numbering of those compounds that can be generated via rearrangement with a phenonium ion as intermediate maintains the original number for each carbon. This simplifies the assignment and allows us to propose a simple nomenclature for this kind of natural product.

The different types of structures are related with the different possibilities of cyclization. Indeed, 6, 7, and 8 heterocycle membered rings are possible in the basic structure (Figure 5). It is also necessary to distinguish between the possibility of having the aromatic ring connected to C-7 or to C-8 by rearrangement. In such cases, variations can be indicated easily by two numbers in the compound name, followed by the configuration of the carbon, if necessary. The first one indicates where the aromatic ring is bonded (C-7 or C-8), and the second one indicates where the ether function is located (C-10 or C-11, but C-9 is not discarded in future members of this skeleton). The name of the skeleton should not include any functional indication, and we propose **heliannane**. Additional functionalization may be indicated by the corresponding suffix and/or prefix. In this way, the compounds heretofore isolated (Figure 1) will be systematically named, as indicated in Table 3.

DISCUSSION

Bioassay Data. In order to evaluate the potential of heliannuols as allelopathic agents and to obtain information about the specific requirements needed for bioactivity, we have studied the effects of a series of aqueous solutions at 10^{-4}–10^{-9} M of isolated compounds on the germination and growth of

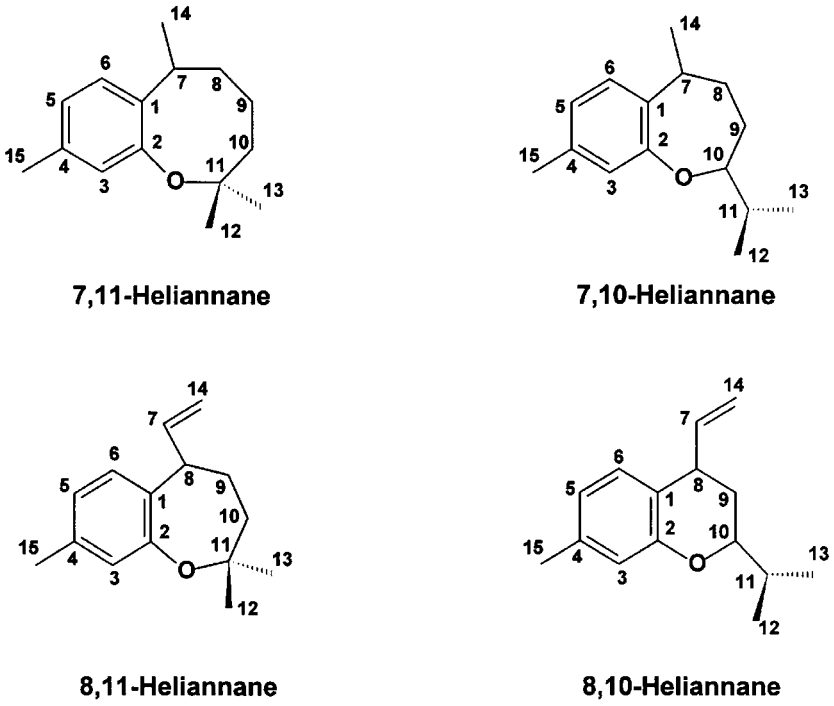

FIG. 5. Different possibilities of cyclization for the heliannane skeleton.

TABLE 3. PROPOSED SYSTEMATIC NOMENCLATURE FOR MEMBERS OF HELIANNANE FAMILY

Trivial name	Systematic name[a]
Helianane (**12**)	7*S*,11-heliannane
Heliannuol A (**1**)	7*R*,11-heliannane-5,10*S*-diol
Heliannuol B (**2**)	7*R*,10*R*-heliann-8(9)-ene-5,11-diol
Heliannuol C (**3**)	8*S**,11-heliann-7(14)-ene-5,10*R**-diol
Heliannuol D (**4**)	7*R*,10*R*-helianane-5,11-diol
Heliannoul E (**5**)	8*R*,10*R*-heliann-7(14)-ene-5,11-diol
Heliannoul F (**6**)	5,11-dihydroxy-7*S*,10*R*-heliannan-8-one
Heliannoul G (**7**)	7*S*,11-heliann-9(10)-ene-5,8*R*-diol
Heliannoul H (**8**)	7*S*,11-heliann-9(10)-ene-5,8*S*-diol
Heliannoul I (**9**)	7*R*,8*S*-epoxy-7,10*R*-heliannane-5,11-diol
Heliannoul J (**10**)	7*S*,8*R*-epoxy-7,10*R*-heliannane-5,11-diol
Heliannoul K (**11**)	5-hydroxy-7*R*,11-heliannan-10-one

[a] * = relative stereochemistry.

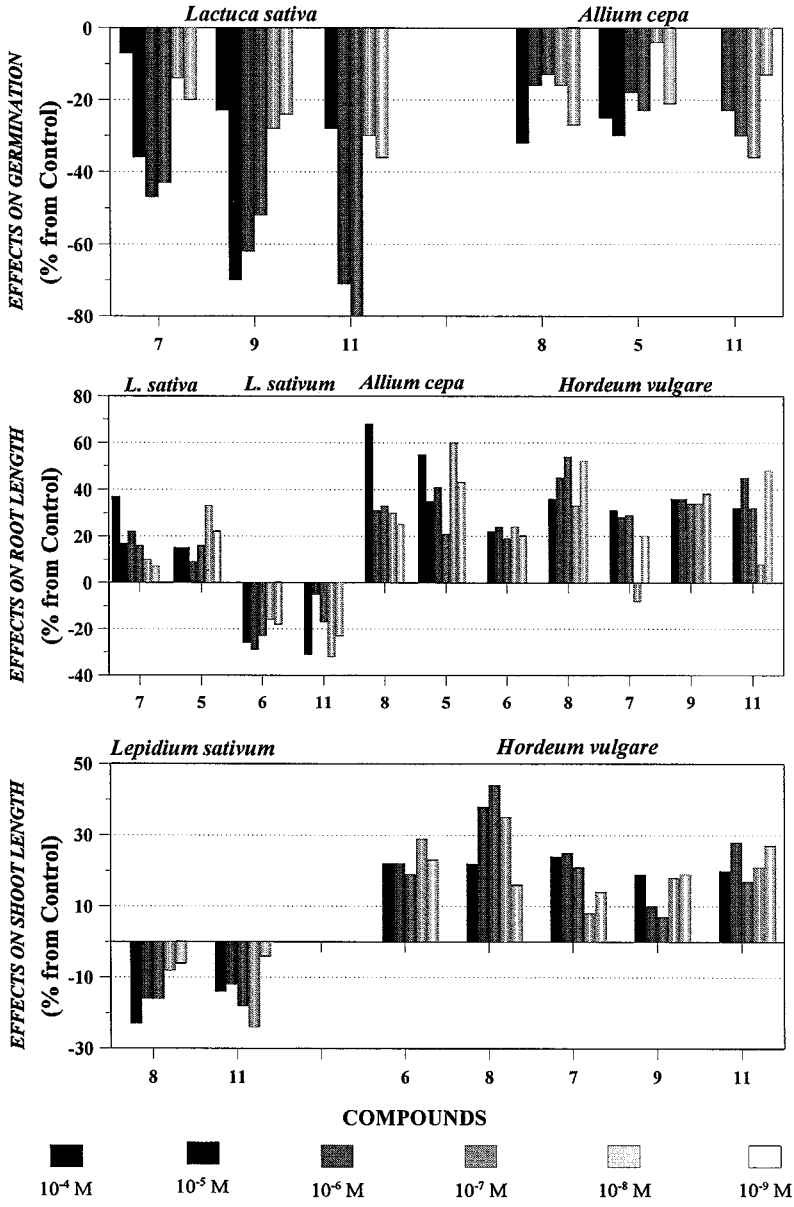

FIG. 6. Selected effects of heliannuols **5–11** over germination and growth of several STS.

selected commercial seeds. The standard target species (STS) were the dicotyledon species, *Lactuca sativa* L. (lettuce) and *Lepidium sativum* L. (cress), and the monocotyledon species, *Allium cepa* L. (onion) and *Hordeum vulgare* L. (barley).

In Figure 6, we show the most significant effects observed. In general, we observe mostly inhibitory effects on the germination of dicotyledon species and stimulatory effects on the growth of monocotyledon species. The greatest activity is shown by compounds **7**, **9**, and **11** on lettuce germination (inhibitory), and by **8** and **5** on onion root length, and by **8** and **9** on barley root length (stimulatory).

Effects on dicotyledon species are inhibitory, except those provoked by **7** and **5** on root length of lettuce. The effects provoked by heliannuols on the germination of lettuce seedlings are intense, and these effects persist with dilution. Indeed, we observed significant values of activity at 10^{-7} M in all compounds that are presented in Figure 6 [**7** (−43%), **9** (−52%), and **11** (80%)]. Although the effects on growth of dicotyledons are not intense, the stimulatory effects of **7** and **5** and the inhibitory effects of **6** and **11** on root length of lettuce are remarkable.

The effects on monocotyledons are different. The main profiles observed are stimulatory, except for the light inhibition observed on germination of onion provoked by **8**, **5**, and **11** (average of −30%). Compound **8** (average of 40%) and **5** (average of 50%) showed stimulatory effects on root length of onion. On the other hand, most of the tested compounds showed a stimulatory profile on barley. These effects are provoked both on roots (**8**, average of 50%; **9** and **11**, average of 40%) and shoot lengths (**8**, average of 40%).

These observations are in accordance with those observed in the assays of water extracts of sunflower (Macías et al., 1999d), where inhibitory effects on dicotyledon species and stimulatory effects on monocotyledon species are observed. Phytotoxic activity, therefore, suggests that these compounds, especially **7**, **9**, and **11**, may be involved in sunflower defense against dicotyledon species, and they could be used as natural herbicide templates.

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