New Bioactive Plant Heliannuols from Cultivar Sunflower Leaves[1]

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From an aqueous extract of *Helianthus annuus* L. cvs. SH-222 and VYP leaves, six new heliannuols have been isolated: three 7,10-heliannanes, heliannuols F (**1**), I (**4**), and J (**5**) and three 7,11-heliannanes, heliannuols G (**2**), H (**3**), and K (**6**). Their structure elucidation was based on extensive spectral studies. The phytotoxicity exhibited by compounds **1**−**4** and **6** suggests that these new bioactive sesquiterpenes may be involved in plant defense against dicotyledons.

Terrestrial plants, as well as sponges and other marine organisms, are a source of functionalized aromatic bisabolene sesquiterpenes such as the well-known plant constituent (*R*)-(−)-α-curcumene,[2] which was also isolated from gorgonians in the 1970s.[3,4] Due to their biological activity as antifungals[5] and cytotoxic agents against several cancer cell lines,[6] the total synthesis of various related aromatic bisabolenes has been carried out.[7]

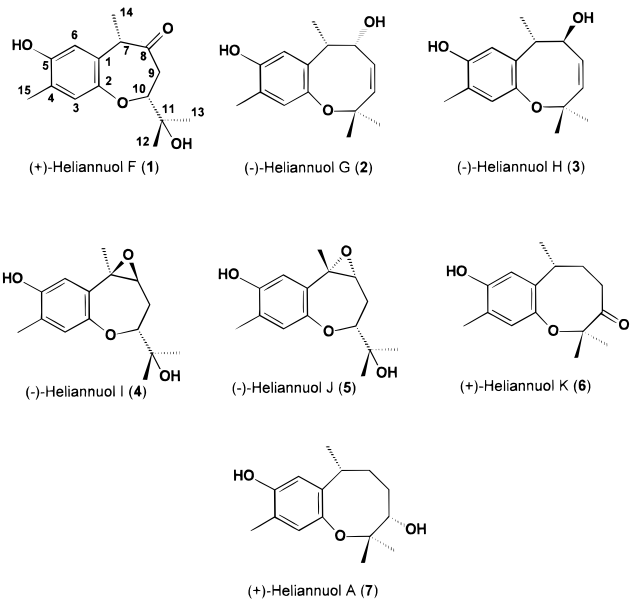
Less well-known are compounds possessing both an aromatic bisabolene moiety and a heterocyclic ring. To date, interest in this novel family of sesquiterpenes has been increased due to the large number of possibilities of cyclization for the heterocyclic ring and the biological activity exhibited as phytotoxic allelochemicals. Representatives of this class of sesquiterpenes are increasing in number, both from terrestrial plants,[8,9] where the first one, (+)-heliannuol A (**7**), a 7,11-heliannane with a benzenoid moiety fused to an eight-membered ether ring, was reported,[10] and from marine sources, where (+)-helianane, the simplest member of this class of 7,11-heliannane, was isolated from the Indo-Pacific sponge *Haliclona fascigera*.[11]

Herein, we report the isolation and structure elucidation of six new heliannuols, constituted by three 7,10-heliannanes (**1**−**3**) and three 7,11-heliannanes (**4**−**6**). Their structure elucidation was based on spectroscopic studies (1D and 2D experiments). The ^{13}C NMR data of heliannuol A (**7**) in $CDCl_3$ at −10 °C were assigned unambiguously using ATP and HETCOR experiments; they are included to establish a correct comparison with the new isolated heliannuols and to correct the previous assignments.[10]

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 of **1**−**4** and **6** 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.[12]

Results and Discussion

Extraction of a fresh-leaf aqueous extract of *H. annuus* L. (Ranunculaceae) cvs. SH-222 and VYP with dichloromethane gave a residue that was fractionated by chromatography on Si gel using hexanes−ethyl acetate mixtures of increasing polarity. Bioassay-guided fractionation of this extract afforded six new heliannuols (**1**−**6**).



Heliannuol F (**1**) showed a HRMS with a molecular ion at *m*/*z* 264.1367, in agreement with the molecular formula $C_{15}H_{20}O_4$. Additional peaks in the EIMS at *m*/*z* 246 $[M - H_2O]^+$ and 231 $[M - H_2O - CH_3]^+$ and an absorption at 3400 cm^{-1} in the IR spectrum, suggested the presence of one hydroxyl group or more. An absorption at 1700 cm^{-1} indicated the presence of a carbonyl group.

Typical signals of heliannuols[8] were observed in the ^1H NMR [H-3 (δ 6.55, s), H-6 (δ 6.59, s), and H-15 (δ 2.16, s)] (Table 1) and ^{13}C NMR [C-3 (δ 111.1, d), C-6 (δ 110.6, d), C-10 (δ 92.1, d), and C-11 (δ 72.3, s)] spectra of heliannuol F (**1**). Two spin systems in the $^1H-^1H$ COSY spectrum of **1** could be distinguished. The first one had two signals, a quartet at δ 3.29 (1H, q) and a doublet at 1.27 (3H, d) assigned to H-7 and H-14, respectively. The second one was formed by three doublets of doublets at δ 4.30 (1H, dd), 2.79 (1H, dd), and 2.19 (1H, dd), assigned to H-10, H-9β, and H-9α, respectively. These data, especially multiplicities and chemical shifts shown by H-7, H-9α, and H-9β in the ^1H NMR spectrum and C-8 (δ 199.2, s) in the ^{13}C NMR spectrum, suggested that the carbonyl group must be placed at C-8. The multiplicities and chemical shifts of the signals assigned to H-12 and H-13 indicated that a hydroxyl group must be attached at C-11. The ^{13}C NMR spectrum [C-10 (δ 92.1), C-11 (δ 72.3)] of heliannuol F indicated that this compound has an ether moiety at C-10, so the structure of **1** was assigned as 8-oxoheliannuol D.

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Table 1. ^{1}H NMR Data of Heliannuols F−K (**1**−**6**)[a]

proton	1	2	3	4	5	6
3	6.55 s	6.56 s	6.57 s	6.55 s	6.53 s	6.71 s
6	6.59 s	6.59 s	6.55 s	6.61 s	6.59 s	6.56 s
7	3.29 q (7.0)	3.43 dq (7.2, 7.2)	3.16 dq (7.9, 7.0)			3.07 ddq (10.5, 10.5, 3.5)
8α		5.09 dd (8.4, 7.4)			3.51 dd (10.0, 6.8)	1.62 dddd (13.5, 6.6, 6.6, 3.5)
8*β*			4.54 ddd (8.4, 7.9, 1.0)	3.62 brd (8.3)		1.95 dddd (13.5, 6.5, 6.5, 3.5)
9α	2.19 dd (14.0, 6.5)			1.90 brdd (12.2, 4.7)	1.95 ddd (12.8, 8.3, 6.8)	2.47 m
		5.79 dd (15.7, 8.4)	5.84 dd (15.5, 8.4)			
9*β*	2.79 dd (14.0, 6.5)			2.27 ddd (12.2, 11.3, 8.3)	2.41 ddd (12.8, 10.0, 7.7)	2.47 m
10	4.30 dd (6.5, 6.5)	5.98 d (15.7)	5.94 dd (15.5, 1.0)	3.79 dd (11.3, 4.7)	4.04 dd (8.3, 7.7)	
12	1.37 s	1.34 s	1.35 s	1.25 s	1.01 s	1.48[b] s
13	1.21 s	1.34 s	1.35 s	1.25 s	1.22 s	1.42[b] s
14	1.27 d (7.0)	1.10 d (7.2)	1.28 d (7.0)	1.68 s	1.62 s	1.28 d (10.5)
15	2.16 s	2.19 s	2.19 s	2.19 s	2.17 s	2.17 s

[a] 399.95 MHz, $CDCl_3$, *J* (in Hz); signal of residual $CHCl_3$, centered at *δ* 7.25 ppm. [b] These values may be interchanged.

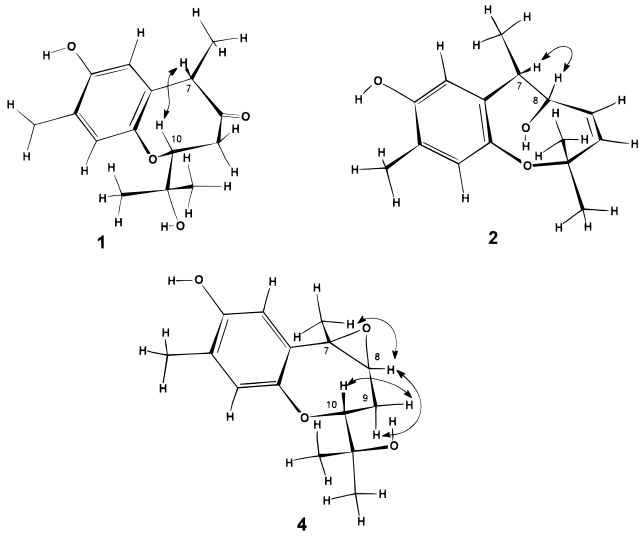

Figure 1. Observed NOEs for the most stable conformers of compounds **1**, **2**, and **4** using PM3 calculations.

The relative stereochemistry was established as 7(*S*) based on the observed NOE effect for the most stable conformer of **1**, using PM3 calculations,[13] between H-7 and H-10 (Figure 1).

Heliannuols G (**2**) and H (**3**) showed spectroscopic characteristics very similar to each other. Their HRMS showed a molecular ion at *m/z* 248.1413 in both cases, according to a molecular formula of $C_{15}H_{20}O_3$. Their EIMS exhibited additional peaks at *m/z* 230 $[M - H_2O]^+$ and 215 $[M - H_2O - CH_3]^+$, which, together with the observed absorptions in their IR spectra at 3415 cm^{-1} (**2**) and 3400 cm^{-1} (**3**), indicated the presence of hydroxyl groups. The ^{1}H NMR spectra of both **2** and **3** showed signals analogous to those observed in previously described heliannuols,[8] namely H-3 [**2** (6.56, s) and **3** (6.57, s)], H-6 [**2** (6.59, s) and **3** (6.55, s)], and H-15 [**2** (2.19, s) and **3** (2.19, s)]. Analysis of their ^{1}H NMR 2D COSY spectra allowed the following correlations to be established: H-14 [**2** (*δ* 1.10, d); **3** (*δ* 1.28, d)] with H-7 [**2** (*δ* 3.43, dq); **3** (*δ* 3.16, dq)], H-7 with H-8 [**2** (*δ* 5.09, dd); **3** (*δ* 4.54, ddd)], H-8 with H-9 [**2** (*δ* 5.79, dd); **3** (*δ* 5.84, dd)], and H-9 with H-10 [**2** (*δ* 5.98, d); **3** (*δ* 5.94, dd)]. The chemical shifts of H-8, H-9, and H-10 suggested the presence of a hydroxyl group, attached to C-8 and a double bond between C-9 and C-10. The appearance of H-12 and H-13 as one singlet that integrated for 6H indicated that the ring closure occurred at the C-11 position.

These data confirmed that **2** and **3** are epimers at the C-8 position. The observed NOE effect between H-7 and H-8 for compound **2** (Figure 1) indicated that both protons had the same orientation, so consequently those compounds were assigned as 5,8α-dihydroxy-9,10-dehydro-7-epihelianane and 5,8*β*-dihydroxy-9,10-dehydro-7-epihelianane, respectively.

Heliannuols I (**4**) and J (**5**) showed very similar spectral patterns. Their HRMS showed molecular ions at *m/z* 264.1361 and 264.1370, respectively, according to the molecular formula $C_{15}H_{20}O_4$. Their EIMS exhibited additional peaks at *m/z* 246 $[M - H_2O]^+$ and 231 $[M - H_2O - CH_3]^+$, which, together with the observed absorptions in their IR spectra at 3411 cm^{-1} (**4**) and 3450 cm^{-1} (**5**), indicated the presence of hydroxyl groups.

Their ^{1}H and ^{13}C NMR spectra suggested heliannuol-type structures: H-6 [*δ* 6.61, s, **4**; *δ* 6.59, s, **5**]; H-3 [*δ* 6.55, s, **4**; *δ* 6.53, s, **5**], and H-15 [*δ* 2.19, s, 3H, **4**; *δ* 2.17, s, 3H, **5**] in their ^{1}H NMR spectra and C-3 (*δ* 111.3, d), C-6 (*δ* 110.9, d), C-10 (*δ* 85.7, d), and C-11 (*δ* 70.1, s) in the ^{13}C NMR spectrum of **4**. ^{1}H NMR 2D COSY spectra showed the following correlations: H-10 [*δ* 3.79, dd (**4**); *δ* 4.04, dd (**5**)] with H-9*β* [*δ* 2.27, (**4**); *δ* 2.41, ddd (**5**)] and H-9α [*δ* 1.90, bdd (**4**); *δ* 1.95, ddd (**5**)]; H-9α and H-9*β* with H-8 [*δ* 3.62, br d (**4**); *δ* 3.51, dd (**5**)].

The singlets for H-14 in both **4** and **5** and the absence of any signals corresponding to H-7 indicated the presence of functionalization at this position in each case. Chemical shifts of H-14 [*δ* 1.68 (**4**), *δ* 1.62 (**5**)] and H-8 [*δ* 3.62 (**4**), *δ* 3.51 (**5**)] and the molecular formula indicated the presence of an oxirane ring between C-7 and C-8. The unusual chemical shifts observed for H-8 could be explained by the presence of an aromatic ring, which can elicit a deshielding effect of 0.5 ppm.[14] The ^{13}C NMR spectrum of **4** indicated that this heliannuol has an ether moiety at C-10[8] (C-10 *δ* 85.7, C-11 *δ* 70.1).

The stereochemistry in **4** and **5** was determined on the basis of NOE experiments. The observed effects are shown in Figure 1 and allowed us to establish that the orientation of H-8 and H-10 must be different in **4**. Consequently, **5** should have the same orientation for H-8 and H-10. Thus, the structure of **4** was assigned as 7*β*,8*β*-epoxyheliannuol D and that of **5** as 7α,8α-epoxyheliannuol D.

Heliannuol K (**6**) was isolated as colorless oil. The HRMS showed a molecular ion at *m/z* 248.1415, consistent with a molecular formula $C_{15}H_{20}O_3$. The ^{1}H NMR spectrum showed signals typical of other heliannuols at *δ* 6.71 (s, H-3), 6.56 (s, H-6), and 2.17 (s, H-15). The COSY ^{1}H NMR spectrum

Table 2. Bioactivity Data of Compounds **1**−**4** and **6**[a]

		lettuce	cress	onion	barley
logran	germination	--	0	0	-
	root length	--	--	--	(−)
	shoot length	-	-	--	(−)
1	germination	-	0	(−)	(+)
	root length	+	-	+	+
	shoot length	(+)	(−)	(+)	+
2	germination	(−)	0	-	0
	root length	0	(−)	+	++
	shoot length	0	(−)	(−)	+
3	germination	-	0	0	0
	root length	+	(−)	+	+
	shoot length	0	0	+	+
4	germination	--	0	-	0
	root length	(+)	(−)	+	+
	shoot length	0	(−)	0	(+)
6	germination	--	(−)	-	0
	root length	0	-	(−)	+
	shoot length	0	-	(−)	+

[a] 0 Not active; (+), (−) Stimulatory or inhibitory values below 20%; '+', '−' Stimulatory or inhibitory values between 20% and 40%; '++', '- -' Stimulatory or inhibitory values higher than 40%.

showed that the signal corresponding to H-7 (δ 3.07; ddq) was correlated with a doublet integration for 3H, which was assigned to H-14 (δ 1.28, d). Additional correlations were found between H-7, H-8α (δ 1.62; dddd), and H-8β (δ 1.95, dddd). Both H-8α and H-8β showed correlations with H-9α (δ 2.47, m) and H-9β (δ 2.47, m). The chemical shifts of H-9α and H-9β, the absence of any signal corresponding to H-10, and the absorption observed in the IR spectrum at 1710 cm^{-1}, allowed a carbonyl group to be assigned at C-10. The appearance of H-12 and H-13 as singlets and their chemical shifts at δ 1.48 and δ 1.42 confirmed that this compound is a heliannuol with an ether function at the C-11 position, and its structure was established as 5-hydroxy-10-oxo-7-epihelianane.

To evaluate the potential of **1**−**4**, **6** 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 these isolates on the germination and growth of selected commercial seeds.[15] The standard target species[12] were the dicotyledonous species, *Lactuca sativa* L. (lettuce) and *Lepidium sativum* L. (cress), and the monocotyledonous species, *Allium cepa* L. (onion) and *Hordeum vulgare* L. (barley).

In general, it was observed that most of the inhibitory effects of **1**−**4** and **6** were apparently for the dicotyledonous species, while stimulatory effects were seen for the monocotyledonous species (Table 2). The most significant effects were those shown by compounds **3**, **4**, and **6** for lettuce germination (inhibitory) and those by **2** over onion root length and **2** and **4** over barley root length (stimulatory). The effects provoked on the germination of lettuce seeds were very intense, and this effect persists with dilution. Indeed, we observed significant values of activity at 10^{-7} M in almost all compounds.

In contrast, the main profile observed over monocotyledonous species were stimulatory. Compound **2** (average of 40%) showed stimulatory effects over the root length of onion. The effects over the growth of barley were provoked both on the root [**2**, average of 50%; **4** and **6**, average of 40%] and shoot length [**2**, average of 40%].

These effects are consistent with those observed in the assays of a water extract of a sunflower cultivar,[16] where inhibitory effects on dicotyledonous species and stimulatory effects on monocotyledonous species were observed. Thus, the phytotoxic allelopathic activity determined suggests that these compounds, especially **3**, **4**, and **6**, may be involved in the cultivar sunflower defense against dicotyledon species.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were made at 399.952 and 100.577 MHz, respectively, on a Varian UNITY - 400 spectrometer with $CDCl_3$ as solvent. The resonance of residual chloroform at δ_H 7.25 and δ_C 77.00 ppm was 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 IR spectra were recorded on a Bio-Rad FTS-7. Optical rotations were determined using a Perkin−Elmer polarimeter (model 241) set on the sodium D line. Column chromatography was performed on Si gel (35−75 mesh), and TLC analysis was carried out using aluminum-packed precoated Si gel plates. For HPLC, LiChrosorb silica 60 was used in the normal-phase mode using a differential refractometer (RI) and a UV detectors, with a Hitachi L-6020A HPLC instrument. All solvents were spectral grade or distilled from glass prior to use.

Plant Material. Leaves of *H. annuus* L. cv. SH-222, commercialized by Semillas Pacífico, and cv. VYP, commercialized by KOYPEJ Córdoba (Spain), were collected in August 1991 and September 1990, respectively, during the third plant-development stage (plants 1.2 m tall with flowers, 1 month before harvest).[16] These materials were provided by Rancho de la Merced, Agricultural Research Station, Junta de Andalucía, Jerez, Spain.

Extraction and Isolation. Fresh leaves from SH-222 (6.0 kg) and VYP (1.7 kg) were soaked with H_2O (plant wt−volume of solvent 1:3) for 24 h at 25 °C in the dark. The filtered H_2O extracts were extracted (8 ×) with 0.5 L of CH_2Cl_2 for each 1.0 L of H_2O, and the combined extracts were dried over Na_2SO_4, and evaporated in vacuo to yield 16.0 g of crude extract. This extract was separated by column chromatography on Si gel using *n*-hexanes−EtOAc mixtures of increasing polarity, to afford 170 × 50 mL fractions, which were reduced to 16 (SH-222) and 30 (VYP) after comparison by TLC. Following bioactive evaluation fractions B and Q (SH-222) and fractions B, D, F, K, L, N, O, Q, and S (VYP) were selected.[16]

Fraction B was chromatographed using Si gel, eluting with hexanes−EtOAc (9:1). The fractions of medium polarity were combined and chromatographed using preparative TLC ($CHCl_3$−MeOH, 99:1). Heliannuol K (**7**) (1 mg) was isolated from HPLC fractions eluted with hexanes−EtOAc (17:3) with a Hibar Si 60 (Merck) 5-μm column (column 1), at a flow rate of 2 mL min^{-1}, and an RI detector.

Fraction Q was chromatographed using Si gel (with N_2 pressure) and eluted with $CHCl_3$−*t*-BuOH (98:2; 2 L), collecting fractions of 50 mL. Fractions 12 and 13 were combined and chromatographed using Si gel and hexanes−EtOAc, 7:3, as eluent, yielding 1 mg of heliannuol F (**1**).

Fractions 17−29 were combined and chromatographed using HPLC column 2 with hexanes−EtOAc (7:3) as eluent, at a flow rate of 3 mL min^{-1}. This afforded heliannuols G (**2**) (1 mg), H (**3**) (1 mg), and I (**4**) (2 mg).

Fraction N (VYP) was chromatographed by using Si gel hexanes−EtOAc (6:4). Fractions were separated repeatedly using preparative TLC with hexane−acetone (7:3) as eluent yielding heliannuols F (1 mg), H (1 mg), and J (1 mg). These compounds were purified using HPLC column 1 with hexane−acetone as eluent at a flow rate of 1.5 mL min^{-1}.

Heliannuol F (1): colorless oil; $[\alpha]^{25}_D$ +15.2° (*c* 0.1, $CHCl_3$); IR (neat, KBr) ν_{max} 3400 (OH), 1720 (carbonyl group), 1609 (C=C aromatic), 1260 (ether) cm^{-1}; ^{1}H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$) δ 129.7 (s, C-1), 151.0 (s, C-2), 111.1 (d, C-3), 118.2 (s, C-4), 148.5 (s, C-5), 110.6 (d, C-6), 44.4 (d, C-7[a]), 199.2 (s, C-8), 43.7 (t, C-9[a]), 92.1 (d, C-10), 72.3 (s, C-11), 27.7 (q, C-12), 29.7 (q, C-13), 21.9 (q, C-14), 16.1 (q, C-15) ([a] these values may be interchanged); EIMS, *m*/*z* 264 $[M]^+$ (4), 246 $[M - H_2O]^+$ (5), 231 $[M - H_2O - CH_3]^+$ (6); HREIMS *m*/*z* calcd for $C_{15}H_{20}O_4$ 264.1362, found 264.1367.

Heliannuol G (2): colorless oil; $[\alpha]^{25}_D$ −6.2° (*c* 0.1, $CHCl_3$); IR (neat, KBr) ν_{max} 3415 (hydroxyl group), 1690 (aromatic), 1173 (ether) cm^{-1}; ^1H NMR data, see Table 1; EIMS, *m/z* 248 $[M]^+$ (57), 230 $[M - H_2O]^+$ (13), 215 $[M - H_2O - CH_3]^+$ (70); HREIMS calcd for $C_{15}H_{20}O_3$ 248.1412, found 248.1413.

Heliannuol H (3): colorless oil; $[\alpha]^{25}_D$ −16.1° (*c* 0.2, $CHCl_3$); IR (neat, KBr) ν_{max}: 3400 (hydroxyl group), 1600 (aromatic), 1265 (ether) cm^{-1}; ^1H NMR data, see Table 1; EIMS, *m/z* 248 $[M]^+$ (42), 230 $[M - H_2O]^+$ (20), 215 $[M - H_2O - CH_3]^+$ (100); HREIMS calcd for $C_{15}H_{20}O_3$ 248.2312, found 248.1413.

Heliannuol I (4): colorless oil; $[\alpha]^{25}_D$ −22.3° (*c* 0.1, $CHCl_3$); IR (neat, KBr) ν_{max}: 3411 (hydroxyl group), 1109 (ether) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR ($CDCl_3$) δ 127.0 (s, C-1), 152.8 (s, C-2), 111.3 (d, C-3[a]), 124.0 (s, C-4), 148.1 (s, C-5), 110.9 (d, C-6[a]), 51.6 (s, C-7), 51.4 (d, C-8), 34.0 (t, C-9), 85.7 (d, C-10), 70.1 (s, C-11), 24.5 (q, C-12[b]), 24.4 (q, C-13[b]), 27.9 (q, C-14), 16.1 (q, C-15), ([a,b] these values may be interchanged); EIMS, *m/z* 264 $[M]^+$ (100), 247 $[M - H_2O]^+$ (8), 231 $[M - H_2O - CH_3]^+$ (38), 175 $[M - C_5H_9O_2]^+$ (73); HREIMS calcd for $C_{15}H_{20}O_4$ 264.1362 found 264.1361.

Heliannuol J (5): colorless oil; $[\alpha]^{25}_D$ −38.4° (*c* 0.1, $CHCl_3$); IR (neat, KBr) ν_{max}: 3450 (hydroxyl group) cm^{-1}; ^1H NMR data, see Table 1; EIMS, *m/z* 264 $[M]^+$ (73), 246 $[M - H_2O]^+$ (6), 231 $[M - H_2O - CH_3]^+$ (31); HREIMS calcd for $C_{15}H_{20}O_4$ 264.1362, found 264.1370.

Heliannuol K (6): colorless oil; $[\alpha]^{25}_D$ +90.0° (*c* 0.1, $CHCl_3$); IR (neat, KBr) ν_{max}: 3400 (hydroxyl group), 1710 (carbonyl group), 1209 (ether) cm^{-1}; ^1H NMR data, see Table 1; EIMS, *m/z* 248.1415 $[M]^+$ (27), 191 $[M - C_4H_9]^+$, 177 $[M - CH(CH_3)_2C/O]^+$; HREIMS *m/z* calcd for $C_{15}H_{20}O_4$ 248.1412, found 248.1415.

Heliannuol A (7): ^{13}C NMR ($CDCl_3$, −10 °C) δ 137.9 (s, C-1), 150.7 (s, C-2), 126.6 (d, C-3), 121.3 (s, C-4), 145.9 (s, C-5), 111.4 (d, C-6), 29.3 (d, C-7), 36.4 (t, C-8), 30.2 (t, C-9), 78.4 (d, C-10), 82.2 (s, C-11), 29.4 (q, C-12), 29.4 (q, C-13), 28.8 (d, C-14), 15.8 (q, C-15).

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

The bioassay consisted of germinating 25 seeds for 5 days (three for germination and two for root and shoot growth) for lettuce and onion, 3 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 control solution, except for barley (5 mL). In the case of barley five seeds were used for 4 days. Test solutions (10^{-4} M) were prepared as initial solution. Test solutions (10^{-5}−10^{-9} M) were obtained by diluting the previous solution. Parallel controls consisted of deionized H_2O. There were three replicates, except for barley (19 replicates), of each treatment, with parallel controls. The number of seeds per replicate and the time and temperature of germination were chosen in agreement with a number of preliminary experiments, by varying the number of seeds, the volume of test solution per dish, and the incubation period. All the pH values were adjusted to 6.0 before the bioassay using MES (2-[*N*-morpholino]ethanesulfonic acid, 10 mM).

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

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References and Notes

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