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Bioactive terpenoids from sunflower leaves cv. Peredovick®☆

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Abstract

The CH_2Cl_2 extract of dried leaves of *Helianthus annuus* L. cv. Peredovick® has yielded, in addition to the known sesquiterpene lactones annuolide E and leptocarpin, and the sesquiterpenes heliannuols A, C, D, F, G, H, I, the new bisnorsesquiterpene, annuionone E, and the new sesquiterpenes heliannuol L, helibisabonol A and helibisabonol B. Structural elucidation was based on extensive spectral (one and two-dimensional NMR experiments) and theoretical studies. The sesquiterpenes heliannuol A and helibisabonol A and the sesquiterpene lactone leptocarpin inhibited the growth of etiolated wheat coleoptiles.

Keywords: *Helianthus annuus* L.; Compositae; Cultivar; Sunflower; Sesquiterpenes; Annuionone E; Heliannuol L; Helibisabonols A, B; Allelopathy; Coleoptile; Bioassay

1. Introduction

Chemical studies of *Helianthus annuus* L. have shown that this species is a rich source of terpenoids, particularly sesquiterpenoids (Spring et al., 1981; Macías et al., 1994, 1996) with a wide spectrum of biological activities including potential allelopathy (Spring et al., 1991; Ghisalberti, 1997; Macías et al., 1999b,c, 2000a,b). We have continued our systematic studies of the allelopathic activity of different varieties of *Helianthus annuus* L. by analyzing *H. annuus* L. cv. Peredovick® CH_2Cl_2 dry leaf extract.

Herein, we report the isolation and structural elucidation of four new terpenoids, the bisnorsesquiterpene (**1**), the 7,11-heliannane (**2**) and two sesquiterpenes (**3**, **4**) (Fig. 1), in addition to the known sesquiterpene lactones annuolide E and leptocarpin; the 7,10-heliannanes heliannuols C, D, F, and I; and the 7,11-heliannanes heliannuols A, G and H.

Bioactivity of the isolated compounds was tested (excepting heliannuol L due to the low amount obtained) using the etiolated wheat coleoptiles bioassay (Hancock et al., 1964) in a range of 10^{-4}–10^{-6} M. Helibisabonol A (**3**) was also tested at 10^{-3} M.

2. Results and discussion

The CH_2Cl_2 dry leaf extract of *Helianthus annuus* L cv. Peredovick® was chromatographed on a silica gel column (Flash chromatography) using hexane-acetone mixtures of increasing polarity. Medium polar fractions yielded 13 compounds: the sesquiterpene lactones annuolide E (Macías et al., 1993a) and leptocarpin (Rolando et al., 1979; Delgado et al., 1984); the bisnorsesquiterpene annuionone E (**1**); heliannuols A, C, D, F, G, H, I (Macías et al., 1993b, 1994, 1999a) and L (**2**); and the sesquiterpenes helibisabonol A (**3**) and helibisabonol B (**4**). The compounds **1–4** are first reported in the literature (Fig. 1). Spectroscopic data of the known compounds were identical to those previously reported.

Annuionone E (**1**) was isolated as colourless oil. Its IR spectrum showed the presence of a hydroxyl group

☆ Part 16 in the series "Allelopathic studies in cultivar species"; for part 15 see Macías et al., 2000b.

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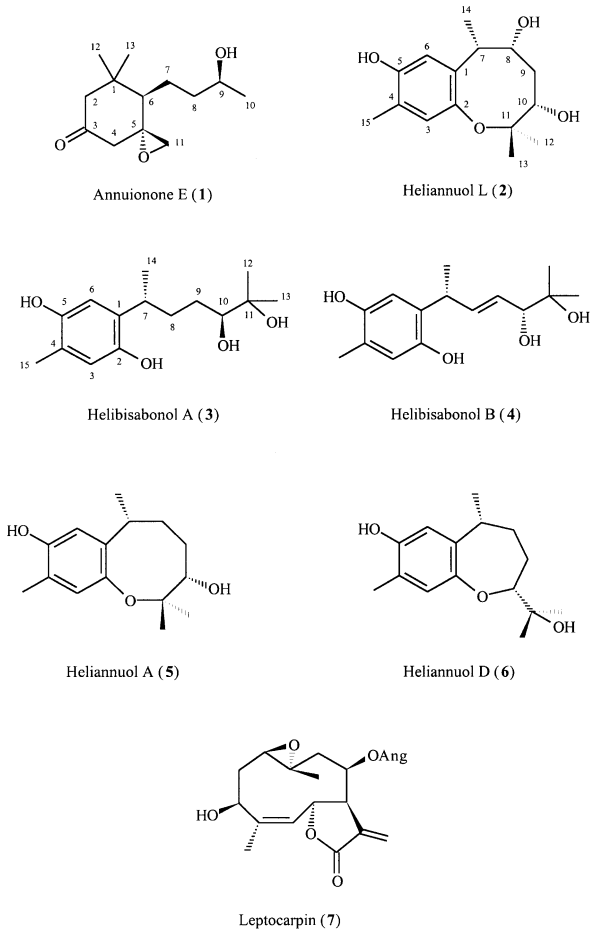

Fig. 1. Terpenoids from *Helianthus annuus*, cv. Peredovick® used in wheat coleoptile bioassay.

(3433 cm^{-1}) and a carbonyl group (1680 cm^{-1}). The molecular ion at *m/z* 226.1598 in the HREIMS spectrum along with the ^{13}C NMR data (Table 2) was in good agreement with the molecular formula $C_{13}H_{22}O_3$ (calculated mass 226.1569). The ^1H NMR 2D COSY spectrum showed two series of correlations: the first one connects signal at δ, 2.38 (H-4, *dd*) with those at δ 2.21 (H-4′, *dd*) and δ 3.56 (H-11′, *dd*); H-11′ is connected with H-11(δ 3.63, *d*). In the second set of correlations H-9 (δ 3.82, *ddq*) was coupled with H-8 (δ 1.68; *m*), H-8′ (δ 1.64, *m*), and H-10 (δ 1.23, *d*); H-8 and H-8′ were coupled with H-7 (δ 1.75, *m*) and H-7′ (δ 1.35, *m*), and these with H-6 (δ 1.62, *dd*). These correlations, along with the presence of three methyl groups at δ 1.23 (*d*, $J_{10,9}=6.2$ Hz; H-10), δ 1.31 (*s*) and δ 1.07 (*s*) (H-12, H-13), and chemical shifts assigned to H-2, H-2′, H-4, H-4′ and H-9 allow us to propose an ionane skeleton with a carbonyl group at C-3 (δ 209.6, *s*) and a hydroxyl group at C-9 (δ 67.9, *d*).

On the other hand, the lack of any signal for H-5 in the ^1H NMR spectrum (Table 1), the chemical shifts of H-11 and H-11′ signals, those for C-11 (δ 78.3) and C-5 (δ 83.5), and the molecular formula $C_{13}H_{22}O_3$ suggested

Table 1
^1H NMR data of compounds **1–4**[a]

H	1[b]	2[b]	3[c]	4[c]
2	2.33 *d*			
2′	2.36 *d*			
3		6.77 *s*	6.51	6.52
4	2.38 *dd*			
4′	2.21 *dd*			
6	1.62 *dd*	6.56 *s*	6.59	6.49
7	1.75 *m*	3.49 *dq*	3.06 *ddq*	3.70 *dq*
7′	1.35 *m*			
8	1.68 *m*	4.32 *ddd*	1.74 *dddd*	5.81 *dd*
8′	1.64 *m*		1.55 *dddd*	
9	3.82 *ddq*	2.35 *ddd*	1.45 *dddd*	5.46 *dd*
9′		1.62 *ddd*	1.23 *m*	
10	1.23 *d*	3.70 *brdd*	3.66 *dd*	3.78 *d*
11	3.63 *d*			
11′	3.56 *dd*			
12	1.31[d] *s*	1.15	1.04[d]	1.07[d]
13	1.07[d] *s*	1.13	1.03[d]	1.02[d]
14		1.21 *d*	1.09	1.26
15		2.18 *s*	2.04	2.1

J (Hz) 1: 2–2′ = 17.0; 4–4′ = 17.7; 4′-6 = 1.2; 6–7 = 9.9; 6–7′ = 6.5; 9–8 = 6.1; 9–8′ = 6.0; 10–9 = 6.2; 11–11′ = 7.9; 11′-4 = 2.9; 2: 7–8 = 3.5; 7–14 = 7.5; 8–9 = 6.0; 8–9′ = 9.0; 9–9′ = 14.3; 9–10 = 6.1; 9′-10 = 2.5; 3: 7–8 = 7.0; 7–8′ = 7.6; 7–14 = 5.1; 8–8′ = 12.4; 8–9 = 5.5; 8–9′ = 9.8; 8′-9 = 8.7; 8′-9′ = 5.8; 9–9′ = 13.1; 10–9 = 1.4; 10–9′ = 4.9; 4: 7–8 = 6.6; 7–14 = 6.6; 8–9 = 15.5; 9–10 = 7.7.

[a] 399. 952 MHz, $CDCl_3$ signal of residual $CHCl_3$, centered at δ 7.25 and $(CD_3)_2CO$ signal residual $(CH_3)_2CO$ centered at δ 2.04. Multiplicities are not repeated if identical with those in the preceding column.
[b] The spectrum was recorded in $CDCl_3$ as solvent.
[c] The spectrum was recorded in $(CD_3)_2CO$ as solvent.
[d] These values may be interchanged within the same column.

Table 2
^{13}C NMR data of compounds **1**, **3** and **4**[a]

C	1[b]	3[c]	4[c]
1	43.4 *s*	132.1	132.1
2	49.4*[d] *t*	147.5* *s*	147.5*
3	209.6 *s*	117.9 *d*	117.9
4	48.6* *t*	121.9 *s*	121.9
5	83.5 *s*	148.8*	148.8*
6	53.6 *d*	113.3	113.3
7	21.4 *t*	29.8 *d*	28.9
8	38.5 *t*	35.4 *t*	138.2 *d*
9	67.9 *d*	24.4 *t*	127.7 *d*
10	20.7 *q*	79.2 *d*	31.9
11	78.3 *t*	72.4 *s*	72.4
12	24.8** *q*	25.5*	26.3*
13	23.7** *q*	29.0*	26.5*
14		21.1 *q*	20.8
15		15.4 *q*	15.4

[a] 100.577 MHz, signals of $CDCl_3$ and $(CD_3)_2CO$ centered at δ 77.0 and δ 206.0 respectively. Degree of protonation was obtained by APT heteronuclear multipulse programs; multiplicities are not repeated if identical with those in the preceding column.
[b] The spectrum was recorded in $CDCl_3$ as solvent.
[c] The spectrum was recorded in $(CD_3)_2CO$ as solvent.
[d] *, **: these values may be interchanged within the same column.

the presence of an oxirane ring placed at C-5 and C-11, consistent with related 5,11-oxirane bisnorsesquiterpenes (Macías et al., 1998). The relative stereochemistry at C-5 and C-6 was assigned as 5*S**,6*R** based on the long-range COSY correlations observed for H-4 and H-11′ and for H-4′ and H-6. These couplings must be explained by a ′W′ path, as C-7 and C-11 apparently adopt a *trans* configuration.

PM3 semiempirical calculations (Stewart, 1989) showed that all most stable conformers with such a relative stereochemistry had a diaxial orientation for protons H-4′ and H-6. Moreover, protons H-4 and H-11′ should adopt a planar disposition (theoretical ϕ 179.5° for H-4′, C-4, C-5, C-6 and-176.2° for H-6, C-6, C-5, C-4) which are in good agreement with the observed coupling constants according with Karplus's rule (Table 3).

With regard to the chiral centre at C-9, NOE studies did not allow to establish any relative configuration because of the alkylic chain nature and its free rotation status. An *S** relative stereochemistry is proposed based on the similarity of experimental coupling constants and theoretical angles obtained using PM3 calculations for those conformers with such stereochemistry (Table 3).

Heliannuol L (**2**) showed a HREIMS with a molecular ion at *m*/*z* 266.1525 consistent with a molecular formula $C_{15}H_{22}O_4$. Additional peaks at *m*/*z* 248 $[M–H_2O]^+$ and the absorption band at 3400 cm^{-1} in the IR spectrum, suggested the presence of associated hydroxyl groups (Pretsch et al., 2000).

Typical signals of heliannuols (Macías et al., 1994), were observed in the ^1H NMR spectrum [H-3 (δ 6.77, *s*), H-6 (δ 6.56, *s*), and H-15 (δ 2.18, *s*)] (Table 1). The structure was supported by the correlations found in the ^1H NMR 2D COSY spectrum: H-14 (δ 1.21, *d*) with H-7 (δ 3.49, *dq*); H-7 with H-8 (δ 4.32, *ddd*) geminal to a hydroxyl group; H-8 with H-9 (δ 2.35, *ddd*) and H-9′ (δ 1.62, *ddd*); and, finally, H-9 and H-9′ with H-10 (δ 3.70, *brdd*). The multiplicity and chemical shifts of H-12 (δ 1.15, *s*) and H-13 (δ 1.13, *s*) along with the lack of signals corresponding to H-11 is in good agreement with a seven- or eight membered heterocyclic ring heliannuol (Macías et al., 1994). The relative stereochemistry at chiral centres C-7 and C-8 was established as 7*S**,8*R** based on the positive NOE effect observed between H-7 and H-8 signals.

After a non conclusive series of NOE's experiments conducted to establish the stereochemistry at C-10, a conformational study of the four possible isomers (seven- or eight membered ring and *R* or *S* configuration at C-10) was pursued (Fig. 2). The search of the most stable conformation of each isomer was made using GMMX (PCMODEL, 2000) and then, refining it by PM3 semiempirical calculations. The dihedral angles obtained for the most stable conformer of each structure compared with the experimental values of coupling constants are presented in Table 3. A clear correspondence can be pointed out between theoretical angles and

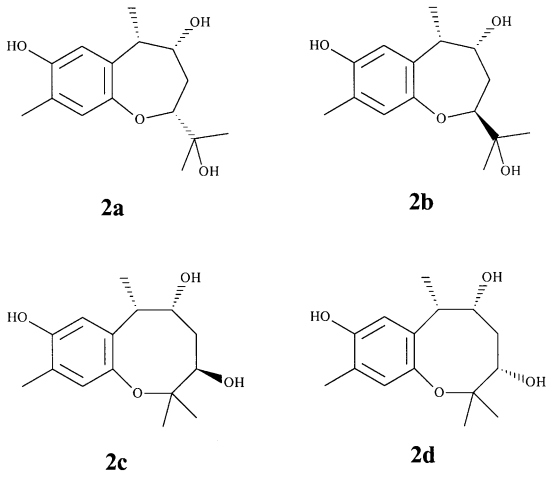

Fig. 2. Different possible isomers of heliannuol L (**2**).

coupling constants of the stereostructure **2d** (*J* (Hz): 7,8 = 1.6; 8,9α = 3.8; 8,9β = 9.6; 9α,10 = 6.2; 9β,10 = 1.2) and the experimental values for **2**. The lack of any conformational equilibrium for the eight member ring, previously observed for the heliannuol A (Macías et al., 1993b) can be explained through the probable existence of hydrogen bonds between the heterocyclic oxygen and the corresponding hydroxyl groups at C-8 and C-10 (Fig. 3), consistent with the typical broad band absorption observed in the IR at 3400 cm^{-1}.

Helibisabonol A (**3**), its ^{13}C NMR spectrum (Table 2) showed six signals assigned to an aromatic ring δ 132.1 (C-1), δ 147.5* (C-2), δ 117.9 (C-3), δ 121.9 (C-4), δ 148.8* (C-5), and δ 113.3 (C-6). Additional two signals

Table 3
Observed coupling constants vs ϕ obtained for the most stable conformers of compounds **1**–**4**

	Protons	Observed *J* (Hz)	ϕ (Theoretical)			
(**1**) Annuionona E	6–7	9.9	137.4°			
	6–7′	6.5	91.5°			
	8–9	6.1	93.6°			
	8′–9	6.0	87.6°			
	4–11′	2.9	174.7°			
			2a	**2b**	**2c**	**2d**
(**2**) Heliannuol L	7–8	3.5	80.5°	73.7°	66.5°	61.6°
	8–9α	6	60.4°	162.2°	51.2°	112.3°
	8–9β	9	53.5°	46.4°	165.2°	3.2°
	9α–10	6.1	75.7°	44.8°	166.9°	44.9°
	9β–10	2.5	37.9°	160.2°	55.0°	69.1°
(**3**) Helibisabonol A	7–8	7.0	70.7°			
	7–8′	7.6	173.7°			
	9–10	1.4	73.8°			
	9′–10	4.9	40.5°			
(**4**) Helibisabonol B	7–8	6.6	52.8			
	9–10	7.7	31.2			

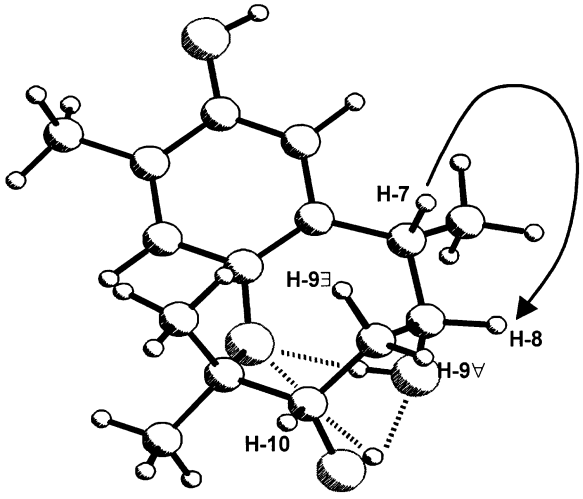

Fig. 3. NOE effects for heliannuol L (**2**), on the minimum energy conformer obtained with theoretical PM3 calculations.

were in good agreement with aliphatic carbons attached to oxygen, δ 79.2 (C-10) and δ 72.4 (C-11).

The HREIMS of **3** showed a molecular ion at *m*/*z* 268.1671 in agreement with the molecular formula $C_{15}H_{24}O_4$ (calculated mass, 268.1675). An additional peak at *m*/*z* 251.1626 $[M–OH]^+$ and the absorption band in the IR spectrum at 3364 cm^{-1} (OH), suggested the presence of a hydroxyl group.

The ^1H NMR recorded in CD_3OD (Table 1), showed signals at δ 6.59, δ 6.51 (1H each, *s*, H-6 and H-3), corresponding to an 1,2,4,5-tetrasubstituted benzene ring, an aromatic methyl group δ 2.04 (3H, *s*, H-15) and two methyl groups located at an oxygenated quaternary carbon (δ 1.04, 3H, *s* and δ 1.03, 3H, *s*; H-12 and H-13).

Structure **3** was further supported by correlations found in the ^1H NMR 2D COSY spectrum, which showed the following series of connectivities in the alkane side chain: the signal of H-10 (δ 3.66, *dd*) was coupled with H-9 (δ 1.45; *dddd*) and with H-9′ (δ 1.23, *m*); H-9 and H-9′ were coupled with H-8 (δ 1.74; *dddd*) and H-8′ (δ 1.55, *dddd*); both H-8 and H-8′ were coupled with the benzylic proton H-7 (δ 3.06; *ddq*), and the latter with the methyl group H-14 (δ 1,09; 3H, *d*).

In order to propose the stereochemistry at the chiral centres C-7 and C-10, a conformational study using semiempirical calculations (PM3) of the two possible diasteroisomers of this compound (7*R**,10*S** and 7*R**,10*R**) was achieved. The comparison between the theoretical dihedral angles in each most stable conformation and the experimental coupling constants (Table 3) let us to propose the relative stereochemistry 7*R**,10*S** for the compound **3**.

Helibisabonol B (**4**), the most significant differences with respect to **3** were found in the ^1H NMR (Table 1), which showed two signals assigned to a vinylic system with a *trans* disposition (δ 5.81, *dd*, $J_{7,8}=6.6$, $J_{8,9}=15.5$ Hz; H-8; δ 5.46, *dd*, $J_{9,10}=7.7$, $J_{9,8}=15.5$ Hz, H-9). The presence of such a double bond induced a downfield shift of the signal corresponding to H-10 (δ 3.78) and H-7 (δ 3.70) in comparison with those of **3** (H-10, δ 3.66 and H-7, δ 3.06).

The relative stereochemistry of the chiral centres C-7 and C-10 is proposed as 7*R**,10*R** based on the comparison between experimental coupling constants of protons H-7 and H-10 with the theoretical values obtained for the most stable conformers of each of the possible isomers 7*R**, 10*R** and 7*R**, 10*S** (Table 3).

2.1. Bioassays

The activity of heliannuols A, C, D (Macías et al., 1994), and G (Macías et al., 1999a) and the sesquiterpene lactones annuolide E (Macías et al., 1993a) and leptocarpin (Macías et al., 1999d) have been previously evaluated in a standard phytotoxic bioassay using several Standard Target Species (STS) (Macías, et al., 2000a,b).

In order to draw a complete picture of the isolated compounds, they were tested (excepting **2** due to the low amount obtained) using the etiolated wheat coleoptiles bioassay (Hancock, et al., 1964) in a range of 10^{-4}–10^{-6} M. Helibisabonol A (**3**) was also tested at 10^{-3} M.

This is a fast bioassay (24 h) and sensitive to a wide range of bioactive substances including plant growth regulators, herbicides (Cutler, 1984), antimicrobials, mycotoxins, and assorted pharmaceuticals (Jacyno and Cutler, 1993).

The growth of etiolated wheat coleoptiles was significantly inhibited ($P<0.01$) 33, 23 and 18% respectively with 10^{-4} M solutions of heliannuol A (**6**), heliannuol D (**7**) and leptocarpin (**5**), while helibisabonol A (**3**) inhibited 27% at 10^{-3} M, all relative to control (11 ± 0.7 mm). The observed activity for leptocarpin (**5**) can be related with the flexibility of this molecule. It has been reported that the different spatial arrangements that the molecule can adopt play an important role in activity (Velasco, 2000), as well as the presence of two electrophilic functions: the α-methylene-γ-lactone moiety and the oxirane ring (Spring and Hager, 1982; Macías et al., 1992).

3. Experimental

3.1. General

^1H NMR and ^{13}C NMR spectra were recorded at 399.952 and 100.577 MHz, respectively, on a Varian UNITY-400 spectrometer and with $CDCl_3$ and $(CD_3)_2CO$ as solvent. The resonances of residual $CHCl_3$ and C_3H_6O at δ_H 7.25 and 2.04 and signals of $CDCl_3$ and $(CD_3)_2CO$ δ_c 77.0 and 206.0, respectively were used as internal reference for ^1H and ^{13}C spectra. Mass

spectra were obtained 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 Silica gel (35–75 mesh), and TLC analyses were carried out using aluminium precoated Silica gel plates. For HPLC, LiChrosorb silica 60 was used in the normal-phase mode with differential refractometer (RI) and UV detectors in a Hitachi L-6020A HPLC instrument. All solvents were spectral grade or distilled from glass prior to use.

3.2. Plant material

Leaves of *H. annuus* L. cv. Peredovick® were collected during the third plant development stage (Macías et al., 1999d) (plants 1.2 m tall with flowers, 1 month before harvest) and were provided by Rancho de la Merced, Agricultural Research Station (CIFA), Junta de Andalucía, Jerez, Spain.

3.3. Extraction and isolation

Dry leaves (1.7 kg) were soaked in CH_2Cl_2 (fresh plant: solvent, 1:3) for 24 h at 25 °C in the dark. The CH_2Cl_2 extract was fractioned by dry flash chromatography on silica gel using *n*-hexane:acetone mixts of increasing polarity, yielding 123×50 ml frs which were reduced to 7 frs. (**A**–**G**) after comparison by TLC. Bioassays of the different fractions (Macías et al., 1999d) with the dicots *Lactuca sativa* and *Lepidium sativum*, and the monocots *Allium cepa* and *Hordeum vulgare* allowed selecting fr. **D** for further study. Fr. **D** was chromatographed using silica gel CC and eluted with *n*-hexane-acetone mixts of increasing polarity. After separation using HPLC with a LiChrosorb silica 60 column compounds annuolide E (2.1 mg), leptocarpin (4.5 mg), annuoinone E (**1**) (5.6 mg), heliannuol A (3.3 mg), heliannuol C (2.0 mg), heliannuol D (2.3 mg), heliannuol F (1.8 mg), heliannuol G (1.6 mg), heliannuol H (1.7 mg), heliannuol I (3.4 mg), heliannuol L (**2**) (1.0 mg), helibisabonol A (**3**) (3.2 mg) and helibisabonol B (**4**) (3.1 mg) were obtained.

3.3.1. Annuionone E (*1*)

Colourless oil; $[\alpha]_D^{25}$ +4.2 (*c* 0.1, CH_3OH); IR (neat, KBr) ν_{max} 3433 (OH), 1698 (C=O), 1271 (C–O–C) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* (rel. int.): 226 $[M]^+$ (9), 169 $[M–C_3H_5O]^+$ (19), 97 $[C_6H_9O]^+$ (100); HREIMS *m*/*z* 226.1598 (calc. for $C_{13}O_3H_{22}$, 226.1569).

3.3.2. Heliannuol L (*2*)

Colourless oil; IR (neat KBr) ν_{max} 3400 (OH), 1109 (C–O–C) cm^{-1}; ^1H NMR data, see Table 1; EIMS *m*/*z* (rel. int.): 266 $[M]^+$ (25), 248 $[M–H_2O]^+$ (4), 233 $[M–H_2O–CH_3]^+$ (32); HREIMS *m*/*z* 266.1525 (calc. for $C_{15}H_{22}O_4$, 266.1518).

3.3.3. Helibisabonol A (*3*)

Colourless oil; $[\alpha]_D^{25}$ −44.9 (*c* 0.1, CH_3COCH_3); IR (neat, KBr) ν_{max} 3364 (OH), 2926 (C–H, Ar), 1656 (C–C, Ar) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* (rel. int.): 268 $[M]^+$ (28), 250 $[M–H_2O]^+$ (17), 151 $[M–C_6H_{13}O_2]^+$ (60); HREIMS *m*/*z* 268.1671 (calc. for $C_{15}H_{24}O_4$ 268.1675).

3.3.4. Helibisabonol B (*4*)

Colourless oil; $[\alpha]_D^{25}$ −7.2 (*c* 0.1, CH_3COCH_3); IR (neat, KBr) ν_{max} 3354 (OH), 2969 (C–H, Ar), 1654 (C–C, Ar) cm^{-1}; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m*/*z* (rel. int.): 266 $[M]^+$ (16), 248 $[M–H_2O]^+$ (11), 151 $[M–C_6H_{11}O_2]^+$ (40); HREIMS *m*/*z* 266.1508 (calc. for $C_{15}H_{22}O_4$, 266.1518).

3.4. Coleoptiles bioassay

Wheat seeds (*Triticum aestivum* L. cv. Duro) were sown in 15 cm diameter Petri dishes moisted with water and grown in the dark at 22±1 °C for 3 days (Hancock et al., 1964). The roots and caryopsis were removed from the shoots. The latter were placed in a Van der Weij guillotine and the apical 2 mm were cut off and discarded. The next 4 mm of the coleoptiles were removed and used for bioassay. All manipulations were performed under a green safelight (Nitsch and Nitsch, 1956). Compounds were predisolved in DMSO and diluted to the final bioassay concentration with a maximum of 0.1% DMSO. Parallel controls were also run.

Crude extracts, fractions, or pure compounds to be assayed for biological activity were added to test tubes. The assay was made in duplicate. Phosphate-citrate buffer (2 ml) containing 2% sucrose (Nitsch and Nitsch, 1956) at pH 5.6 was added to each test tube. Following the placement of five coleoptiles in each test tube, the tubes were rotated at 0.25 rpm in a roller tube apparatus for 24 h at 22 °C in the dark. The coleoptiles were measured by digitalization of their images. Data were statistically analysed using the Welch's test (Martín Andrés and Luna del Castillo, 1990). Data are presented as percentage differences from control. Thus, zero represents the control; positive values represent stimulation of the studied parameter, and negative values represent inhibition.

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