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Bioactive apocarotenoids annuionones F and G: structural revision of annuionones A, B and E ☆

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Abstract

The polar bioactive fractions of *Helianthus annuus* cv. Stella and SH-222 have yielded eight apocarotenoids, two of them isolated for the first time as natural products (annuionones F and G). The isolation of higher amounts of annuionones A and E allowed us to realize a more comprehensive spectroscopical study. We propose a revised structure for annuionone A, B and E based on careful re-analyses of new spectroscopical data.

Keywords: Allelopathic agents; Apocarotenoids; Norsesquiterpenoids; Biological activity; Annuionone

1. Introduction

Many apocarotenoids, compounds with fewer than 40 carbon atoms, but with carotenoid-like structures, are found in plant essential oils and they are often related with flavour of plants, and others are essentially nonvolatile. Synthesis of these compounds appears to occur mainly by catabolism of carotenoids (Parry and Horgan, 1991).

Otherwise, hexylideneallene moiety can be found in certain carotenoids as neoxanthin, mimulaxanthin, furoxanthin, peridinin or paracentrone. This functionalization is maintained in some apocarotenoids as grasshopper ketone (**1**) (Fig. 1), which has been proposed as precursor of the important flavour damascenone (**2**) (Ohlo et al., 1973; Skouroumounis and Sefton, 2000). Its direct progenitors are not completely clarified. One of them, the β-D-glucopyranoside of the allenic triol **3** has been isolated from the leaves of *Premna subscandens* (Sudo et al., 2000) and as its pentaacetate from the leaves of *Lycium halimifolium* (Näf et al., 1990). *In vitro* hydrolysis of **3** has produced the expected damascenone (Skouroumounis et al., 1992; Puglisi et al., 2001). This is the first time that this compound has been isolated as aglycone natural product.

On the other hand, in 1998, we isolated annuionones A (**4**) and B (**5**) from *Helianthus annuus* L. (sunflower), as allelopathic agents (Macías et al., 1998). Then the isolation of annuionone E (**6**) was also reported in 2002 (Macías et al., 2002). Since it was possible to isolate higher amounts of annuionones A and E, a more comprehensive spectroscopical study could be realized. We have reinvestigated the ^{13}C NMR spectrum assignment of **4** and **6** by ^{1}H ^{13}C gHSQC, gHMBC and 2D-INADEQUATE spectroscopy, in addition to the one dimensional 1D-^{1}H NMR spectrum, decoupled ^{13}C NMR

☆ Part 17 in the series "Allelopathic studies in cultivar species". For part 16, see Macías et al. (2002).

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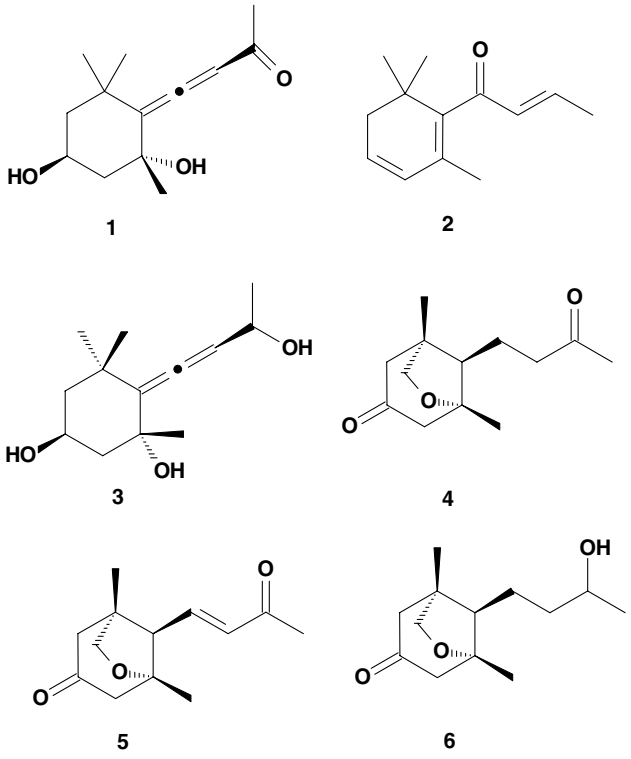

Fig. 1. Apocarotenoids isolated from different sources.

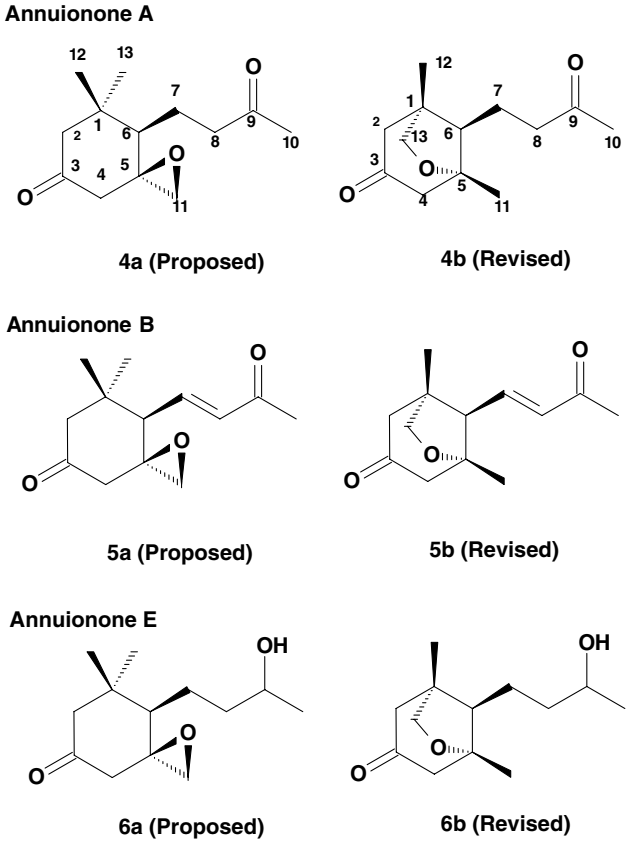

Fig. 2. Isolated annuionones A, B and E and their revised structures.

spectrum, and COSY. These studies suggested the revision of the previously reported structures for these compounds (Fig. 2) to the new structures **4b**–**6b**. These structures are very similar to that of phaseic acid, which is a catabolite from the plant hormone abscisic acid (Todoroki and Hirai, 2002; Zhou et al., 2004). Recently, the synthesis of annuionone A has been performed by Takikawa and coworkers, and confirms this hypothesis for compound **4** (Takikawa et al., 2003).

2. Results and discussion

Fresh leaves of *H. annuus* cv. Stella and SH-222 were extracted with water at room temperature for 24 h. This aqueous extract was extracted with methylene chloride and ethyl acetate. The different fractions obtained were assayed, and those that presented higher bioactivity levels were chromatographed on columns of silica gel using hexane–ethyl acetate mixtures of increasing polarity. The obtained fractions were assayed on etiolated wheat coleoptiles and STS (Macías et al., 2000). The polar bioactive fractions yielded compounds **3**, **4**, **6** (Fig. 1), and **7**–**11** (Fig. 3). The spectroscopic data of **7**–**10** were identical to those previously reported (Gonzalez et al., 1994; Pauli et al., 1990; Pérez et al., 1996).

Annuionone F (**11**) was isolated as colourless oil. Its IR spectrum showed the presence of a carbonyl group (1671 cm^{-1}). The HRMS showed a molecular ion at *m/z* 242.1503 (calcd. = 242.1500), which together with the ^{13}C NMR data (Table 2) were in good agreement with the molecular formula $C_{13}H_{22}O_4$.

The ^1H NMR 2D COSY spectrum showed two correlations series. The first one began with a doublet at δ 3.32 (3H, $J_{2\alpha,2\beta} = 13.8$) assigned to H-2α, which was coupled with H-2β (δ 1.85, *dd*, $J_{2\alpha,2\beta} = 13.8$, $J_{2\beta,4\beta} = 2.0$ Hz), H-2β with H-4β (δ 2.22, *ddd*, $J_{4\alpha,4\beta} = 14.3$, $J_{4\beta,5} = 4.6$, $J_{2\beta,4\beta} = 2.0$ Hz), H-4β with H-4α (δ 2.52, *dd*, $J_{4\alpha,4\beta} = 14.3$, $J_{4\alpha,5} = 12.7$ Hz) and H-5 (δ 2.12, *ddq*, $J_{4\beta,5} = 4.6$, $J_{4\alpha,5} = 12.7$, $J_{5,11} = 6.6$ Hz), and, finally, H-5 with H-11 (δ 0.85, 3H, *d*, $J_{5,11} = 6.6$ Hz). In the second correlation series H-7 (δ 5.70, *d*, $J_{7,8} = 15.5$ Hz) were coupled with H-8 (δ 6.01, *dd*, $J_{7,8} = 15.5$, $J_{8,9} = 6.3$ Hz) which overlaps with H-9 (δ 4.45, *dq*, $J_{8,9} = 6.3$, $J_{9,10} = 6.5$ Hz), and finally H-9 with H-10 (δ 1.33, 3H, *d*, $J_{9,10} = 6.5$ Hz). The unusual chemical shift observed for H-2α could be correlate with the 1,3-diaxial interaction between H-2α and the hydroxyl group placed at C-6, in addition to the potential interaction with the hydroxyl group at C-11.

These correlations, together with the presence of one singlet methyl group at δ 0.78 (H-11), a AB system at δ 3.88 and 3.17 ($J_{13a,13b} = 10.8$ Hz) assigned to H-13, and the chemical shifts assigned to H-2α, H-2β, H-4α, H-4β, H-7, H-8, and H-9 led us to propose an ionane skeleton with a carbonyl groups at position 3, three hydroxyl

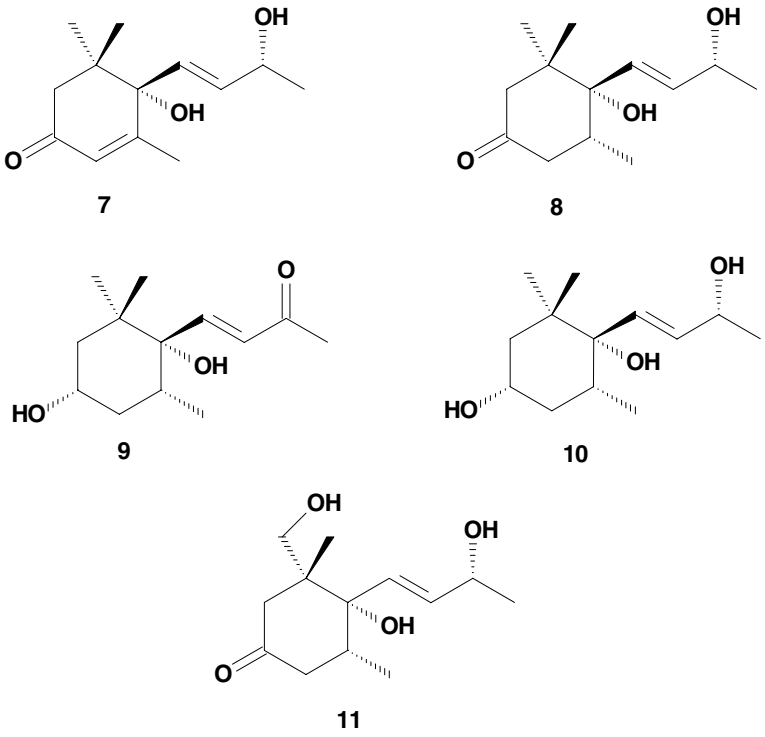

Fig. 3. Isolated apocarotenoids from *Helianthus annuus* cv. Stella and SH-222.

groups at C-6, C-9 and C-13, and a double bond between C-7 and C-8. These data are according with the stereochemistry of the related compounds (3*S*,5*R*,6*S*, 9*R*)-3,6-dihydroxy-5,6-dihydro-β-ionol (Pérez et al., 1996) previously isolated from *Apollonias barbujana* and 4,5-dihydroblumenol A (Gonzalez et al., 1994) isolated for the first time from *Perrottetia multiflora.* The stereochemistry proposed is further supported by the NOEs observed between H-12, H-5 and H-7. This compound has been described for the first time and has been named annuionone F.

Annuionone G (**3**) was isolated as colourless oil. The IR spectrum showed a band at 3430 cm^{-1}, that indicated the presence of hydroxyl groups. The high resolution mass spectra showed a peak at *m/z* 208.1461 $[M - H_2O]^+$ (calc = 208.1463), which together with the ^{13}C NMR data (Table 2) were in good agreement with the molecular formula $C_{13}H_{22}O_3$.

The ^1H NMR 2D COSY spectrum showed a correlation series with protons H-2α, H-2β, H-3, H-4α and H-4β analogous to the previously described compound. A second correlation series was found beginning with H-8 (δ 5.37, *d*, $J_{8,9}$ = 5.8 Hz) that were coupled with H-9 (δ 4.33, *dq*, $J_{8,9}$ = 5.8, $J_{9,10}$ = 6.3 Hz), and finally H-9 with H-10 (δ 1.28, 3H, *d*, $J_{9,10}$ = 6.3 Hz). Additionally, three methyl groups at δ 1.07 (H-12), 1.30 (H-13) and 1.37 (H-11) were observed in the ^1H NMR spectrum.

The ^{13}C NMR spectrum showed three signals corresponding with carbons belonging hydroxyl groups at δ 64.2 (C-3), 72.4 (C-5) and 66.3 (C-9) and three other signals that suggest an allene moiety at δ 117.7 (C-6), 197.7 (C-7) and 99.9 (C-8). This is further supported by the correlation observed between H-8 with C-8 and C-7 in the gHMQC and gHMBC spectra, respectively. These data led us to propose the structure **3** for annuionone G. The stereochemistry of the centres 3, 5 and 8 was in good agreement with the correlation observed on the NOESY spectrum (H-13 with H-3 and H-8; and H-11 with H-12).

Since it was possible to isolate annuionones A and E a more comprehensive spectroscopical study could be realized. We have reinvestigated the ^{13}C NMR spectrum assignment of **4** and **6** by ^1H ^{13}C gHSQC, gHMBC (Table 2) and 2D-INADEQUATE spectroscopy (Fig. 4), in addition to one dimensional 1D-^1H NMR spectrum (Table 1) decoupled ^{13}C NMR spectrum (Table 2) and COSY.

Interpretation of the spectrum of **6** was facilitated by one secure assignments; C-10 (23.7 ppm): This was the only CH_3, linked to a methine carbon (C-9). Starting from this carbon, we were able to assign all of the remaining carbons (Table 2). Also the ^1H ^{13}C gHSQC spectrum distinguished unambiguously C-2 at 48.6 ppm from C-4 at 49.4 ppm.

This fact was also confirmed by the gHMBC spectrum in which the long range correlations of C-13 (δ 78.3 ppm) with the methyl protons of C-12 at δ 0.99 ppm and the protons H-2α and H-2β were observed in addition to the correlation of C-5 with H-13 pro *S*.

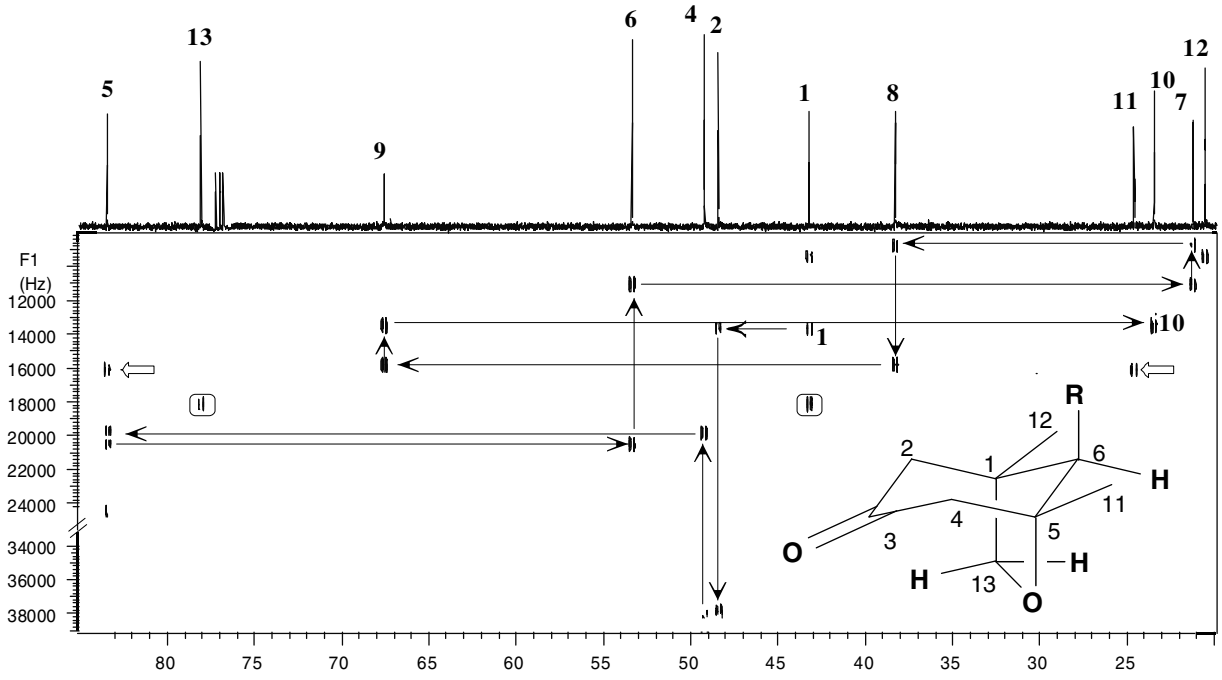

Fig. 4. 2D-INADEQUATE spectrum of annuionone E (**6**) on a 150.831 MHz instrument.

These spectral data allowed elucidating that the structure of **6** has not an epoxide group between C-5 and C-11 (Fig. 2, compound **6a**) but the oxygen atom is connected to the C-13 and C-5 formed a second five members cycle (Fig. 2, compound **6b**). This structure explains the unusual chemical shift values observed for carbons connected to oxygen (δ 83.5 and δ 78.3 ppm) as well as for the protons H-13 pro *S* and H-13 pro *R* (δ 3.52 and δ 3.47 ppm) which were rather big to an epoxide group.

The relative stereochemistry at C-1, C-5 and C-6 was assigned as *1S**,5R**,6R** based on the long range COSY correlations observed for H-2β and H-13 pro *R* and for H-2α and H-6α. These couplings must be explained by a "W" path. The stereochemistry was confirmed by observation of NOE between H-6α and H-13 pro *R*.

The careful re-analyses of new spectral data (Tables 1 and 2) of **4** allows to conclude that the real structure of annuionone A must have a second cycle of five members, like annuionone E, instead of an epoxide group (Fig. 2, compound **4a**).

The relative stereochemistry at C-1, C-5 and C-6 was assigned as *1S**, *5R**, *6R** by observation of NOE between H-6 and H-13 pro *R*.

In conclusion, the new spectral data allowed us to clarify the structures of annuionones A, B and E.

2.1. Bioassay results

The activity of 4,5-dihydroblumenol A (**8**), annuionone A (**4**) (Macías et al., 1998), and annuionone E (**6**) (Macías et al., 2002) have been previously evaluated in a standard phytotoxic bioassay using several standard target species (STS) (Macías et al., 2000).

In order to complete the bioactivity spectrum of the isolated compounds, they were tested (excepting **11** due to the low amount obtained) using the etiolated wheat coleoptile bioassay (Hancock et al., 1964) in a range of 10^{-3}–10^{-6} 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 (Fig. 5) was significantly inhibited ($P < 0.01$) by solutions of **3** (−67%, 10^{-3} M; −41%, 10^{-4} M), **4** (−58%, 10^{-3} M; −56%, 10^{-4} M; −53%, 10^{-5} M), **6** (−65%, 10^{-3} M; −40%, 10^{-4} M), and **7** (−60%, 10^{-3} M; −39%, 10^{-4} M). The activity of **4** and **6** could be related with the presence of the bicyclic ether moiety. An allene function **3** seems to provoke similar effect as well as the presence of unsaturated ketone moiety **7.**

3. Experimental

3.1. General

IR spectra (KBr) were recorded on a Perkin Elmer FT-IR Spectrum 1000, Matton 5020 spectrophotometer. NMR spectra were run on Varian INOVA-400 and Varian INOVA 600 spectrometers. Chemical shifts are given in ppm with respect to residual $CHCl_3$ or $CDCl_3$ signals

Table 1
^{1}H NMR data for compounds **3**, **4**, **6** and **11**[a]

H	Annuionone G (**3**)	Annuionone A (**4**)	Annuionone E (**6**)	Annuionone F (**11**)
2	α 1.91 *ddd* (J = 13.0, 4.3, 2.3 Hz)	α 2.22 *dd* (J = 17.6, 1.1 Hz)	α 2.12 (J = 17.8, 1.2 Hz)	α 3.32 *d* (J = 13.8 Hz)
	β 1.29 *dd* (J = 13.0, 12.7 Hz)	β 2.37 (J = 17.6, 2.7 Hz)	β 2.32 (J = 17.8, 1.8 Hz)	β 1.85 (J = 13.8, 2.0 Hz)
3	4.28 *dddd* (J = 12.7, 4.3, 4.1, 12.5 Hz)			
4	α 2.22 *dd* (J = 4.1, 2.3 Hz)	2.34 *d* (J = 17.6 Hz)	2.24 (J = 17.6 Hz)	α 2.52 *dd* (J = 14.3, 14.3 Hz)
	β 1.37 *ddd* (J = 12.5, 12.5, 12.5 Hz)	2.39 *d* (J = 17.6 Hz)	2.31 (J = 17.6 Hz)	β 2.22 *ddd* (J = 14.3, 4.6, 2.0 Hz)
5				2.12 *m*
6		1.63 *ddd* (J = 8.0, 8.0, 1.1 Hz)	1.58 (J = 6.2, 6.2, 1.2 Hz)	
7		1.64 *dddd* (J = 15.1, 8.1, 8.1, 6.6 Hz)	1.26 (J = 18.0, 11.4, 6.2, 6.0 Hz)	5.70 *d* (J = 15.5 Hz)
		1.83 *dddd* (J = 15.1, 8.1, 8.1, 6.6 Hz)	1.65 (J = 18.0, 6.2, 6.0, 6.0 Hz)	
8	5.37 *d* (J = 5.8 Hz)	2.66 *ddd* (J = 18.0, 8.0, 6.6 Hz)	1.53 *dddd* (J = 18.8, 11.4, 6.1, 6.0 Hz)	6.01 *dd* (J = 15.5, 6.3 Hz)
		2.65 *ddd* (J = 18.0, 8.0, 6.6 Hz)	1.52 *dddd* (J = 18.8, 6.0, 6.1, 6.0 Hz)	
9	4.33 *dq* (J = 6.3, 5.8 Hz)		3.71 *ddq* (J = 6.1, 6.1, 6.1 Hz)	4.45 *dq* (J = 6.3, 6.5 Hz)
10	1.28 *d* (J = 6.3 Hz)	2.18 *s*	1.12 *d* (J = 6.1 Hz)	1.33 (J = 6.5 Hz)
11	1.37 *s*	1.31	1.22	0.85 *d* (J = 6.6 Hz)
12	1.07 *s*	1.07	0.99	0.78
13	1.30 *s*	3.55 *dd* (J = 8.1, 2.7 Hz) pro *R*	3.47 (J = 7.8, 1.8 Hz) pro *R*	3.88 *d* (J = 10.8 Hz)
		3.62 *d* (J = 8.1 Hz) pro *S*	3.52 (J = 7.8 Hz) pro *S*	3.17 (J = 10.8 Hz)

[a] Multiplicites are not repeated if identical with those in the preceding column.

(δ 7.25 and 77.00, respectively). Optical rotations were determined using a Perkin Elmer polarimeter model 241 (on the sodium D line). HRMS were carried out on VG AUTOESPEC mass spectrometer (70 eV).

3.2. Plant material

H. annuus cv. Stella (commercialized by SENASA) and cv. SH-222 (commercialized by Semillas Pacífico) were collected during the third plant development stage (Macías et al., 1999) (plants 1.2 m tall with flowers, 1 month before harvest) and were provide by Rancho de la Merced, Agricultural Research Station (CIFA), Junta de Andalucía, Jerez, Spain.

3.3. Extraction and isolation

Fresh leaves of *H. annuus* cv. Stella (4 kg) were extracted in water (12 l) for 24 h at room temperature in the dark. The aqueous solution was extracted with CH_2Cl_2 and then with EtOAc at room temperature. The solvent of the organic layer was removed by reduced pressure evaporation yielding two extracts of 17.6 g (DCM-A) and 7 g (EtOAc-A), respectively. The plant residue was dried at room temperature and re-extracted with CH_2Cl_2 and methanol yielding after solvent removal afforded 64.0 g (DMC-P) and 69.0 g (MeOH-P), respectively. These extracts were bioassayed with wheat etiolated coleoptiles. DCM-P extract was the most active one. This was chromatographed using hexane–EtOAc mixtures of increasing polarity as eluent over silica gel. Those fractions eluted between hexane–EtOAc 6:4 and 4:6 yielded compounds **6** (23 mg), **4** (5 mg), **8** (10 mg) and **10** (20 mg).

6 kg of fresh leaves of *H. annuus* cv. SH-222 were treated in a similar way. EtOAc-A extract (6.0 g) was chromatographed as above and those fractions eluted between hexane–EtOAc 6:4 and 3:7 yielded compounds **6** (20 mg), **4** (11 mg), **7** (5 mg), **8** (5 mg), **11** (1 mg), **10** (70 mg) and **3** (3 mg). DCM-A extract (16 g) yielded at the same polarity the compounds **6** (31 mg), **4** (2 mg), **8** (40 mg), **10** (68 mg), **9** (32 mg) and **3** (11 mg).

3.3.1. Annuioinone G [(3R,5R,7R)-3,5-dihydroxy-5,6-dihydro-6,7-dehydro-β-ionol] (**3**)

Colourless oil; $[\alpha]_D^{25}$ − 15.5(c 1.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}; 3320 (OH), 1954 (C=C=C). ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; EIMS *m/z* (rel. int.): 226 $[M]^+$, 208 $[M - H_2O]^+$ (28), 107 (100); HREIMS *m/z* 208.1461 $[M - H_2O]^+$ (calc. 208.1463).

3.3.2. Annuioinone F [(1R,5R,6S,9R)-3-oxo-6,13-dihydroxy-5,6-dihydro-β-ionol] (**11**)

Colorless oil; IR ν_{max} (KBr) cm^{-1}; 3400 (OH), 1671 (C=O), 975 (C=C). ^{1}H NMR data, see Table 1; ^{13}C

Table 2
^{13}C NMR data for compounds **3**, **4**, **6** and **11**[a]

C	Annuionone G (**3**)	Annuionone A (**4**)		Annuionone E (**6**)			Annuionone F (**11**)
	$\delta^{13}C$	$\delta^{13}C$	gHMBC	$\delta^{13}C$	gHMBC	^{13}C–^{13}C	$\delta^{13}C$
1	35.2 *s*	43.4	H2, H12, H13	43.4	H2, H6, H12, H13	C2, C12, C13	45.0
2	49.3 *t*	48.6	H6, H12, H13	48.6	H6, H12, H13	C1, C3	46.6
3	64.2 *d*	208.9 *s*	H2, H4	209.6	H2, H4	C2	211.7
4	48.8 *t*	49.3	H11	49.4	H2, H6, H11	C5	45.2
5	72.4 *s*	83.4	H4, H6, H7 H11, H13	83.5	H4, H6, H11, H13	C6, C11	37.0 *d*
6	117.7 *s*	53.1 *d*	H2, H4, H7, H8; H11, H12, H13	53.6	H2, H11, H12, H13	C7	78.3 *s*
7	197.7 *s*	18.6 *t*	H6, H8	21.4	H6, H8	C8	131.5 *d*
8	99.9 *d*	42.6 *t*	H6, H7, H10	38.5	H10	C9	135.7 *d*
9	66.3 *d*	207.4 *s*	H8, H10	67.9 *d*	H8, H10	C10	68.7
10	23.3 *q*	30.0		23.7	H8	C9	24.1
11	31.3 *q*	24.9		24.8	H6, H7	C5	15.1
12	32.3 *q*	20.8		20.7		C1	19.7
13	29.3 *q*	78.3	H2, H12	78.3	H2, H12	C1	70.0

[a] Multiplicites are not repeated if identical with those in the preceding column.

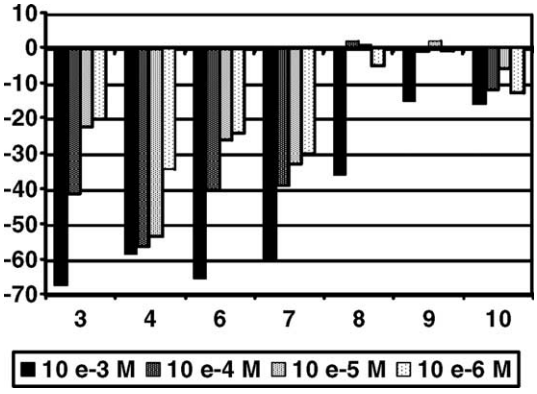

Fig. 5. Bioactivities of compounds in wheat coleoptile bioassay.

NMR data, see Table 2; EIMS *m/z* (rel. int.): 224 $[M - H_2O]^+$ (5), 206 $[M - 2H_2O]^+$ (6); HREIMS *m/z* 242.1503 (calc. for $C_{13}H_{22}O_4$, 242.1500).

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 (three tubes per dilution), 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 analyzed using the Welch's test (Martın 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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References

Cutler, H.G., 1984. Fresh look at the wheat coleoptile bioassay. In: Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America, PGRSA, Boston, MS, USA, pp. 1–9.

González, A.G., Guillermo, J.A., Ravelo, A.G., Jiménez, I.A., Gupta, M.P., 1994. 4,5-Dihydroblumenol A, a new *nor*-isoprenoid from *Perrottetia multiflora*. Journal of Natural Products 57, 400–402.

Hancock, C.R., Barlow, H.W., Lacey, H.J., 1964. The east malling coleoptile straight growth test method. Journal of Experimental Botany 15, 166–176.

Jacyno, J.M., Cutler, H.G., 1993. Detection of herbicidal properties: scope and limitations of the etiolated wheat coleoptile bioassay. PGRSA Quarterly 21, 15–24.

Macías, F.A., Varela, R.M., Torres, A., Oliva, R.M., Molinillo, J.M.G., 1998. Bioactive norsesquiterpenes from *Helianthus*

annuus with potential allelopathic activity. Phytochemistry 48, 631–636.

Macías, F.A., Oliva, R.M., Varela, R.M., Torres, A., Molinillo, J.M.G., 1999. Allelochemicals from sunflowers leaves cv. Peredovick. Phytochemistry 52, 613–621.

Macías, F.A., Castellano, D., Molinillo, J.M.G., 2000. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. Journal of Agricultural and Food Chemistry 48, 2512–2521.

Macías, F.A., Torres, A., Galindo, J.L.G., Varela, R.M., Álvarez, J.A., Molinillo, J.M.G., 2002. Bioactive terpenoids from sunflower leaves cv Peredovick. Phytochemistry 61, 687–692.

Martín Andrés, A., Luna del Castillo, J. de D., 1990. Bioestadística para las Ciencias de la Salud, third ed. Norma, Madrid.

Näf, R., Velluz, A., Thommen, W., 1990. Isolation of a glucosidic precursor of damascenone from *Mycium halimifolium* Mil. Tetrahedron Letters 31, 6521–6522.

Nitsch, J.P., Nitsch, C., 1956. Studies on the growth of coleoptile and first internode sections. A new sensitive, straight-growth test for auxins. Plant Physiology 31, 94–111.

Ohlo, G., Rautenstrauch, V., Schulte-Elte, K.H., 1973. Model reactions for biosynthesis of compounds of damascone series and their synthetic application. Helvetica Chimica Acta 56, 1503–1513.

Parry, A.D., Horgan, R., 1991. Carotenoids metabolism and the biosynthesis of abscisic acid. Phytochemistry 30, 815–821.

Pauli, N., Sequin, U., Walter, A., 1990. Boscialin and boscialin 4′-*O*-glucoside, 2 new compounds isolated from the leaves of *Boscia salicifolia* Oliv. Helvetica Chimica Acta 73, 578–582.

Pérez, C., Trujillo, J., Almonacid, L.N., Trujillo, J., Navarro, E., Alonso, S.J., 1996. Absolute structures of two new C-13-norisoprenoids from *Apollonias barbujana*. Journal of Natural Products 59, 69–72.

Puglisi, C.J., Elsey, G.M., Prager, R.H., Skouroumounis, G.K., Sefton, M.A., 2001. Identification of a precursor to naturally occurring β-damascenone. Tetrahedron Letters 42, 6937–6939.

Skouroumounis, G.K., Massywestropp, R.A., Sefton, M.A., Williams, P.J., 1992. Precursors of damascenone in fruit juices. Tetrahedron Letters 33, 3533–3536.

Skouroumounis, G.K., Sefton, M.A., 2000. Acid-catalyzed hydrolysis of alcohols and their β-D-glucopyranosides. Journal of Agricultural and Food Chemistry 48, 2033–2039.

Sudo, H., Ide, T., Otsuka, H., Hirata, E., Takushi, A., Shinzato, T., Takeda, Y., 2000. Megastigmane, benzyl and phenethyl alcohol glycosides, and 4,4′-dimethoxy-β-truxinic acid catalpol diester from the leaves of *Premna subscandens* Merr. Chemical and Pharmaceutical Bulletin 48, 542–546.

Todoroki, Y., Hirai, N., 2002. Abscisic acid analogs for probing the mechanism of abscisic acid reception and inactivation. Studies in Natural Products Chemistry 27, 321–360.

Takikawa, H., Isono, K., Sasaki, M., Macias, F.A., 2003. Synthesis and structural revision of annuuionone A. Tetrahedron Letters 44, 7023–7025.

Zhou, R., Cutler, A.J., Ambrose, S.J., Galka, M.M., Nelson, K.M., Squires, T.M., Loewen, M.K., Jadhav, A.S., Ross, A.R.S., Taylor, D.C., Abrams, S.R., 2004. A new abscisic acid catabolic pathway. Plant Physiology 134, 361–369.